UNIVERSIDAD DE SANTIAGO DE COMPOSTELA

FACULTAD DE FARMACIA
DEPARTAMENTO DE QUÍMICA ORGÁNICA

"Oxaciclos inspirados en productos naturales: versatilidad químico-farmacológica"

"Oxacycles inspired in natural products: chemico-pharmacological versatility"

Memoria que para optar al grado de Doctora presenta

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Santiago de Compostela, Enero de 2014

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Que la Memoria titulada: "OXACICLOS INSPIRADOS EN PRODUCTOS NATURALES: VERSATILIDAD QUÍMICO-FARMACOLÓGICA", que para optar al grado de Doctora por la Universidad de Santiago de Compostela presenta SALETA VÁZQUEZ RODRÍGUEZ, ha sido realizada bajo nuestra dirección, en el Departamento de Química Orgánica de la Facultad de Farmacia de la Universidad de Santiago de Compostela.

Y considerando que el trabajo constituye tema y labor de Tesis Doctoral, autorizamos su presentación en la Universidad de Santiago de Compostela.

Y para que conste, expedimos el presente certificado en Santiago de Compostela a 8 de Enero de DOS MIL CATORCE

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Agradecimientos
Acknowledgements

"Son más de agradecer las palabras de aliento tras una derrota, que una mano por la espalda tras una gran victoria". Anónimo.

Por eso, la llegada a este puerto sólo ha sido posible gracias a la maravillosa tripulación que me ha ayudado a gobernar mi barco, mientras navegaba por el temido Poseidón, capeando temporales y disfrutando de los días soleados. Y es que yo nací en una burbuja y viví en mi mundo de cristal…

A toda mi tripulación…

A los profesores Eugenio Uriarte y Lourdes Santana, o como siento que debo llamarlos, a Eugenio y Lourdillas, o al "jefe" y a la "jefa", por abrirme las puertas no sólo de su laboratorio, sino también de su amistad. A Eugenio por las mil y una horas "discutiendo, debatiendo, arreglando y destruyendo" este país (y suma y sigue), por su espíritu siempre *deportivo*, por su vitalidad y energía constante, por su capacidad para visualizar la "U.M.P.", y por animarme siempre a volar. A Lourdillas, por las incalculables horas de conversación, hablando de lo divino y lo humano, por su insaciable espíritu aventurero de *Willy Fog* que comparto hasta las entrañas, por el apoyo y ánimo en los momentos duros, por ese carácter único y por ser la reina del "Scifinder" que de tantos apuros nos ha sacado!. A los dos, porque juntos formáis un gran equipo.

A vosotros, Papá y Mamá, que lo sois TODO para mí. Por haberme dado la vida, en más de una ocasión... Por estar a mi lado en los momentos más difíciles, sufriendo conmigo pero manteniendo siempre el barco a flote, y por disfrutar de mis éxitos como si fuesen los vuestros. Porque sin vosotros NADA de lo que soy y seré, sería posible. Porque me habéis dado siempre más de lo que necesitaba e incluso de lo que podíais, anteponiendo siempre a "la nena" antes que a vosotros mismos. Porque vosotros sí que formáis el mejor equipo que conozco… A ti, Mamá, por haberme dado siempre las fuerzas que en muchos momentos me han faltado, por demostrarme con hechos el significado de la palabra "valiente", por luchar como nadie día a día con el único fin de ver una sonrisa en la cara de los tuyos y por la generosidad y el altruismo infinito que demuestras en todos los aspectos de la vida. A ti, Papá, por haberme levantado del suelo con garra y decisión, por enseñarme a descubrir no sólo lo mejor de las personas siempre, sino también "*el lado bueno de las cosas*"; por tener siempre presente que todo es "*cuestión de principios*", por hacerme ver qué es lo importante y por inculcarme los mejores valores que existen. Por tu capacidad de diálogo y análisis, acompañada siempre del amor infinito que me transmites constantemente y por

haberme dedicado tantas y tantas horas, aguantando mis "al borde del abismo"… A los dos, porque siempre trabajáis de la mano y porque es imposible que existan padres que se entreguen más a sus hijos que vosotros.

A mi hermano Marcos, que desde que tengo uso de razón ha sido un referente personal y profesional. Gracias a él he aprendido a vivir y disfrutar de una de las cosas que siempre hace que todos los males desaparezcan: el mar. Por su capacidad indestructible para escalar paredes como las de "El Capitán", y franquear los obstáculos más difíciles con arte y pericia. Por los sueños, inquietudes y experiencias compartidas; por la determinación que presenta a la hora de tomar decisiones, y porque me demuestra siempre que el que quiere puede. *"Do or do not, there is no try"*

A mis abuelos (en memoria) Mercedes, Rafael, Alicia y Pepe. Ellos han sido siempre, sin saberlo, el motor de que me haya sumergido en esta aventura. Porque lo han dado todo, literalmente, por un futuro mejor para nosotros.

A mi pilar número dos: "*Villachunguita*" a.k.a. Laura, Reyes y Ana. A todas ellas como uno, porque cuando estamos juntas funcionamos como tal. Por todas las vivencias compartidas (las que se pueden contar y las que no!). Por los viajes, la "calceta", las mañanas, tardes y noches y por el "al matadero con castañuelas!". Por las "broncas", los consejos, los llantos y las risas, las merecidas y las irracionales. Porque sin duda alguna, ellas han sufrido conmigo no sólo este viaje rumbo a todavía no sé dónde, sino todas mis idas y venidas a todos los niveles, estando SIEMPRE al pie del cañón. Por ver, escuchar y sentir conmigo. Por SER y ESTAR. A Laura por transmitirme su fuerza para superar hasta el mayor de los obstáculos, siempre con una sonrisa. A Reyes por su capacidad para "desnudar mis entrañas" sin necesidad de contarlas y por ser la gestora de mis sentimientos. A Ana por su vitalidad y predisposición constante para tender la mano y enseñarme el significado de "yonqui de la felicidad". A Villachunguita por darme lo que necesito, por estar siempre a mi lado, y por enseñarme y demostrarme cada día que son únicas.

A mis tíos Tito, Luchi, Fernando, Fernanda, Nena, Pili y, Tante y Abdón (en memoria). Unas palabras especiales se merecen Tito y Luchi, por ser mis segundos padres desde que tengo uso de razón, y por tratarme como una hija en todos los aspectos. A Marina y Jose, que ellos saben que para mí son también mi familia, por preocuparse por cada uno de mis pasos, seguirlos de cerca, y haber hecho que sacase mi espíritu "revolucionario".

A mis primos Hugo, Jorge y Adri, porque han hecho posible que viviese la infancia más feliz que un niño puede desear. Por compartir ilusiones, preocupaciones y sueños, y por descubrir juntos el significado de la palabra "crecer".

A mis *frikímicos* Lucía, Dani, Diego, Iria, Lara, Olalla y Ainara, por compartir no sólo las aulas, sino también la vida del día a día durante la carrera, y grandes responsables de que la universidad haya pasado por mí, y no yo por ella. A Ainara por su predisposición para "la rampa". A Olalla por su sentido del humor y su buena voluntad para la amistad a pesar de "las piedras del camino". A Lara por compartir las inquietudes y preocupaciones científicas y seguir de cerca mi camino durante el doctorado estando siempre "ahí". A Diego por estar siempre dispuesto a compartir cualquier instante, con un buen "danone". A Iria por transmitirme su ilusión por las pequeñas cosas, y por ser la bondad en persona. A Dani por compartir lo que con pocos he compartido. Y a Lucía, mi cuca, por estar SIEMPRE a mi lado de forma incondicional, con razón o sin ella, proporcionándome la calma (o la euforia!) en el momento oportuno.

A la mafia del 5ºC, mis otras compañeras de batallas universitarias, Adri "Spunyyyyy", Marta, Cris, y Elena, por compartir altruistamente una de las mejores etapas de mi vida y formar parte de historias inolvidables grabadas a fuego. "¡¿Quién-me-ha-robado-la-mochilaaaaaa?!". A Marta (de la Toweeeeerrr!!), por ser la compañía y amiga perfecta en nuestros "zulos".

A "Juanchu", por su cariño infinito, las conversaciones hasta altas horas de la madrugada (chilena o española), y por las cañas que se van de las manos. Gracias por aparecer.

A los niños de Santiago Jano, Nacho, Juli, Fraga, Rilo y Rebo. Unas palabras en especial para Fraga, por compartir inquietudes y parte de nuestra vida en el "exilio" en Newcastle. Y por supuesto, a Jano, por todo lo que él ya sabe…

A los limensianos en general como "clan", ya sea el de los mayores, los medianos, o los pequeños de una saga que encierra mucho más que la pasión por el mar y un rincón especial que representa mi pequeño paraíso (42º15' N, 8º48' O). Sois tantos que espero que os deis todos por aludidos! Pero en especial a todos aquellos que comparten las eternas noches de verano alrededor de una hoguera de "piñas con sal" o un "baño", desafiando ciclogénesis explosivas, temperaturas extremas y "chuzos como pelotas". Porque la alianza de Eolo y Poseidón es el mejor matrimonio para nosotros. Un huequecito especial tienen: Víctor, el Gurú, porque fue el primero que me metió en el agua cuando era una enana, entre una jauría de machos alpha dominantes, y me puso encima de una tabla, tratándome siempre como la "protegida" pero "dándome caña". A Iaguiño, Nanus y Ra, por las charlas y delirios compartidos en tantas y tantas noches de verano. A Laura, Carmen, Erea y Sara, por las incalculables horas viendo más allá, por los bautizos de "Charlie" y de "Harry", y porque siempre están en el paraíso. A los hermanos Rivas, Alex y Du, por sus ideas, inventos y esa

actitud incansable de revolucionar el mundo. A Alex “2”, quién en cierto modo me ha inculcado el espíritu insaciable de recorrer mundo. A Gus, por ser amigo en momentos clave. A los hermanos Montouto Escudeiro (y familia), en especial a Samu, por enseñarme, soportarme y compartir momentos inolvidables; y a Dieguito “Cu”, porque pasó de ser “el enano”, a ser amigo, confidente y compañero de batallas varias, en mayúsculas. A la familia Ortega Villalón, por el apoyo y cariño transmitido a lo largo de los años, en especial, a Lúa, por su amistad de tantos y tantos años que tanto me ha aportado.

A mis compañeros de laboratorio: Al equipo Sardo de Silvia, Giulio y Ricci, que ha estado al pie del cañón (“aquí no duerme ni dios!”). A Javi, Dolo, Santi, Chachi, Edu, André y Tati, por soportarme día a día entre reactivos y disolventes y mantenerse con una sonrisa ante mis altos y bajos. Un rincón especial me lo reservo para Elías, que supo entenderme desde el minuto dos (en el minuto uno todavía tenía dudas de si mordías…!!!) y para María, por ser amiga y compañera de viajes, por apoyarme y escucharme entre cafés y congresos, y porque desde luego, sin duda alguna, esta Tesis no habría sido posible sin su ayuda. GRACIAS. A Xosé, por tener ese carisma entrañable y la predisposición indiscutible para echar una mano en cualquier cosa y en cualquier momento.

Una mención se merecen los “niños” que han pasado por nuestro laboratorio: Pablo, Andrea (Dr. Bucci), Miguel, Veronika, Carla, Mannuel, Jose, João, Francesco, Nicolo, María, Andrea, Rita y Gabriel, que de algún modo han contribuido al desarrollo de este trabajo, o al menos, a hacerlo mucho más ameno.

A todos aquellos profesores e investigadores que han empleado un poco de su tiempo en transmitirme tanto los conocimientos, como la pasión y motivación por esta especie en peligro de extinción que es la ciencia. A Roger Griffin, Ian Hardcastle y Bernard Golding, de la University of Newcastle upon-Tyne, por iniciarme en este mundo. A Antonio Figueras y Beatriz Novoa, del Instituto de Investigaciones Marinas-CSIC de Vigo por acogerme y sumergirme entre peces y PCRs. A Marcus Tius, de la University of Hawai’i at Manoa, por abrirme las puertas de su grupo durante mi estancia y enseñarme los secretos de la química sintética.

A los profesores e investigadores que han contribuido de forma activa al desarrollo de esta tesis gracias a su incesante colaboración, especialmente a Fernanda Borges de la Universidade do Porto, Karl-Norbert Klotz de la Universität Wurzbürg, Claudio Olea-Azar de la Universidad de Chile, Giovanna Delogu de la Università degli Studi di Cagliari y a Dolores Viña, Ángeles Muñoz e Ysabel Santos, de la Universidad de Santiago de Compostela.

A la tripulación del IIM-CSIC: Ana Mato, Iván, Pep, Sonia, Laura, Ariana, Rubén, Jorge y María, quienes me han mostrado de cerca el maravilloso mundo de la inmunología y la genómica de organismos marinos. Una mención especial se merece Marimar, quién se preocupó y me enseñó todo lo que sé a nivel científico de la genética de "peixes e bivalvos" con una paciencia infinita a la par que un cariño muy típico del "Morrazo".

A la compañía insaciable de Villachunguita: Mago (villachunguito… con lo que eso implica! jeje), Laura Barrera, Jaime, Cris, Nanes, Flo, Sabela y Rubi, por las cenas que se van de las manos, las fiestas temáticas, las churrascadas y picnics contra viento y marea, y por compartir su alegría.

A los "bomberitos" Mario *Richards* "rock star", porque aunque no lo crea, sé que está ahí (pero paga tus deudas con *"Globetrotter"*!) y a David "cuñaaaaaoooo", porque sin quererlo, pasó de ser "el de Laura", a ser un amigo.

A Amelia "gunilla" por los "saraos", el "von biiiiissssssmmmaaarrrkkk!", las charlas, el cariño y los "buboletos" hechos con tanto amor!. Ya sabes que eres de la *Jet Set!*

A los vecinos, y casi familia, Fuji, Nanda, Pili y Sergio, que me han visto crecer.

A los "niños" de Hispanidad, Álvaro "chiquipulpi", Zapa, Carmen, María Porto, Sonia y Marina, porque me acogieron como una más en una etapa de transición.

Al verdadero *Team T*, el gran descubrimiento de la última temporada, que apareció en un momento crítico y me inyectó una dosis peligrosa de "ganas de comerme el mundo". A Evelyn, por su apoyo constante, por su capacidad de análisis y por esa inocencia y corazón infinito que mezclada con su afán de superación la hacen única. A Fer, por ser "una mente maravillosa" y transmitir la calma y sosiego a cualquier persona que esté a menos de dos metros de él. Y a Diego, por el cariño, el apoyo y la preocupación incesantes, por las "bombas" que siempre sacan una sonrisa, y por las confidencias compartidas ("*nos vemos en las trincheras de Vietnam…que quiero saber a qué huele el Napalm*…").

To my *Ohana* "always Alo)(a spirit": Philip, Peter, Anais, Sandra, Félix, Lucho, Daniela, Paviça, Patrick, Florian, Silvia "italian", Silvia "swiss", Johannes, Marcelo, Charito and Bruno "Abuelo". Special thanks to Johannes, for taking care of the friendship even from the distance and who always tries harder to set a reunion. To Philip, because he was a key support every time I was in Hawai'i. To "Abueeeeelooooo", because of all the shooting stars we dreamt of. To Anais, for being my "training leader" during my PhD stay in Hawai'i, but overall, for her friendship. To Patrick, because he appeared at the right time and at the right moment and he showed me the magnificent world of the "alternative music".

To all my Aussie friends, Karina, Fe, Justin, Gabriel, Marie, Nicola, Giorgia, the twins Edu and Marcelo, Mori, Edu “dudu” and Zack for sharing one of the most amazing and adventurous experiences of my life during my time in Australia. Day by day, in Byron Bay… you know… *“a couple of good ones and no worries mate!”*

To my “Italo-Geordie” friends Francesco and Elisa for being my main support in Newcastle. Special thanks to Francesco, who was already a known member among my family, and serendipity, put us together.

Y desde los orígenes, no puedo olvidarme del Martín Códax, ese lugar que pretendía ser sólo un colegio pero que siempre fue especial para todos los que pasábamos por sus aulas. Porque siempre ha sido un lugar diferente, o como muchos de mis amigos denominan, “el colegio rojo y raro ese al que fuiste”. Gracias a Eloy, gran culpable de que hoy en día sea científica y de que cada vez que alzo la vista hacia el cielo, no sólo vea estrellas, sino también galaxias, y divague sobre el significado de la teoría de cuerdas. A Fernando, quién ha sido capaz de transmitirme su pasión por la Historia y el Arte (¿a quién no se le ha pasado ser historiador tras un curso con él?). A Félix (en memoria), que me inculcó otra de mis grandes pasiones, la música. A Ydalinda, Pepa, Jaime y Teté.

Y, como dirían los anglosajones, “last but not least”, a los perriños: Rocky, Pinta, Naish, Atreyu, Gandalf, Maui y Nemo, porque con su simple presencia, me alegran el día y están a mi lado, siendo la mejor compañía.

¿Sabías que…?

- El estómago, sin su capa de mucosidad, que contiene bicarbonato en su superficie para neutralizar el ácido clorhídrico estomacal, se digeriría a sí mismo, ya que el pH del estómago es de 1.5.
- La ciruela kakadu australiana contiene 100 veces más vitamina C que una naranja.
- Cada vaca doméstica emite aproximadamente 150 Kgs de metano al año.
- La Luciferina es la molécula quimioluminiscente responsable de que las luciérnagas emitan luz.
- La capsaicina es el compuesto responsable de que los pimientos de Padrón piquen.
- La serotonina, la dopamina y la norepinefrina son las moléculas responsables de la "química del amor", y un desajuste en sus niveles provoca el llamado "colocón del amante".
- La dosis letal media de la cafeína en humanos es de unos 200 mg por Kg de masa corporal, o lo que equivaldría a unas 30 tazas de café.
- Una molécula de cloro lanzada a la atmósfera destruye unas diez mil moléculas de ozono.
- La farmacéutica Bayer comercializó legalmente la heroína durante 15 años, ya que inicialmente se empleaba como antitusivo y como un sustituto no adictivo de la morfina.
- Un litro de agua de mar contiene más de 35 gramos de sales, fundamentalmente en forma de cloruros y sulfatos. Por eso la salinidad de los océanos es de unos 35 gramos por litro. Sin embargo, en el mar muerto es de 370 gramos por litro, lo que impide que haya vida, a excepción de algunas bacterias extremófilas.
- La mayor mina de oro se encuentra en el mar, pero todavía no existe un sistema rentable para separar las doradas partículas en suspensión.
- Existen tres lagos en el volcán Kelimutu, en Indonesia, que no sólo tienen colores diferentes (turquesa, café y azul oscuro casi negro) sino que siguen cambiando de color a lo largo del tiempo debido a la oxidación de los elementos presentes en ellos (bario, cobre, azufre y arsénico) producida por la actividad volcánica.
- Unos 10 segundos después del Big Bang, la temperatura del universo era de unos mil millones de grados kelvin. A día de hoy, la superficie del planeta más caliente del sistema solar es la de Venus, a unos 470ºC

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A mis padres.

En memoria de mis abuelos.

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Y mientras las olas se estrellaban en las rocas, el mar que me miraba me decía: "Cuando siento dudas respecto a mi poder para destruir las rocas, me volteo a ver las grandes playas de arena..."

Jackselins Arteaga

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Resumen

En la presente Memoria se describe una selección del trabajo realizado de diseño, síntesis y estudio preliminar de compuestos oxacíclicos con interés farmacológico inspirados en productos naturales. De la amplia quimioteca preparada, se presenta más en detalle los resultados de aquellos compuestos que hasta el momento han sido publicados o están en fase de publicación. La mayoría de los compuestos presentados son híbridos con núcleo cumarínico sobre el cual se incorpora el esqueleto de estilbeno o de chalcona en 3 y 8 de la cumarina, o bien con modificaciones en los anillos aromáticos de los mismos. Se presentan también metodologías sintéticas para la preparación de derivados de la rocaglamida. Respecto de los híbridos cumarina-chalcona, se presentan 4 series principales de compuestos sintetizados, en las que se han realizado modificaciones sencillas como la introducción de sustituyentes de distinta naturaleza, en distinto número y en diferentes posiciones de modo que nos fuese posible obtener una relación estructura-actividad para cada serie y para cada diana estudiada. Las metodologías y rutas sintéticas empleadas han sido seleccionadas de manera que nos permitiesen acceder a las estructuras diseñadas de la forma más sencilla, eficiente, directa y versátil posible en la cantidad y con la pureza necesaria para la realización de los estudios estructurales y biológicos. Hasta el presente, fueron realizados estudios preliminares de series seleccionadas de los compuestos sintetizados como inhibidores enzimáticos (MAO-A y MAO-B), ligandos de receptores de adenosina, antioxidantes, antiparasitarios y antibacterianos.

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Summary

In the present dissertation a selection of works on the design, synthesis and preliminary studies of oxacyclic compounds with pharmacological interest inspired in natural products is described. From the extended prepared compound library, more in detail results are presented for those compounds that have been published so far or are in publication process. Most of the compounds presented are hybrid compounds with a coumarinic nucleus, in which the stilbene or chalcone scaffolds are incorporated in positions 3 or 8 of the coumarin. Synthetic methodologies for the preparation of rocaglamide derivatives are also presented. Four main series of synthesized coumarin-chalcone compounds are shown, each of them having undergone simple modifications such as the introduction of substituents of different nature, in different number and positions. Those modifications made possible to establish structure-activity relationships for each series and for each studied target. The methodologies and the synthetic routes employed have been selected in order to access to the designed structures in the most simple, efficient, versatile and direct way, in the amount and with the purity required to carry out the biological studies. To date, preliminary studies on selected series of the synthesized compounds were carried out as enzymatic inhibitors (MAO-A and MAO-B), adenosine receptors ligands, as antioxidants, as antiparasitic and as antibacterial agents.

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AChE	Acetilcolinesterasa
AMPA	Receptores de ácido α-amino-3-hidroxi-5-metil-isoxazolpropiónico
AR	Receptor de adenosina
BAEC	Células endoteliales de aorta bovina
BACE-1	Beta secretasa 1
CHO	Células de ovario de hámster chino
CL	Compuesto líder
CoA	Coenzima A
DNA	Ácido desoxirribonucleico
EA	Enfermedad de Alzheimer
FDC	Combinación a dosis fija
GABA	Ácido γ-aminobutírico
GPCR	Receptor acoplado a proteína G
HIV	Virus de la inmunodeficiencia humana
HPLC	High Performance Liquid Chromatography
IC_{50}	Concentración de compuesto, que induce una reducción del crecimiento celular del 50%
MAO	Monoaminooxidasa
MS	Espectroscopía de masas
MTDL	Ligandos dirigidos a multi-dianas
NMR	Resonancia Magnética Nuclear
ORAC	Capacidad de absorción de radicales oxígeno
PAL	Fenilalanina amonioliasa
QSAR	Relación estructura-actividad cuantitativa
ROS	Especies radicalarias de oxígeno
SAR	Relación estructura-actividad
t.a.	Temperatura ambiente
UV	ultravioleta

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Introduction

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1. Productos naturales y desarrollo de fármacos

Desde los primeros tiempos, el ser humano ha acudido a la naturaleza para obtener sustancias que, o bien le ayudasen a paliar el dolor o los síntomas de sus enfermedades, o bien le facilitasen la obtención de alimentos (veneno para la caza) y sus relaciones sociales y religiosas (estimulantes y alucinógenos). Esta circunstancia ha permitido disponer de una amplia información, que sometida a la observación y al estudio crítico, ha dado lugar al planteamiento de ideas que, debidamente desarrolladas, han llevado a la obtención de nuevos medicamentos. Esta historia previa es la causa de que en la actualidad el modelo de aproximadamente la mitad de los productos farmacéuticos de que se dispone sean de origen natural. Estos modelos, obtenidos a partir de un organismo vivo, han sufrido diferentes modificaciones en el laboratorio, encaminadas a la obtención de moléculas más potentes y menos tóxicas que las originales, las cuales en realidad no habían sido diseñadas para un tratamiento específico.

Hace alrededor de unos 200 años, un joven farmacéutico de 21 años llamado Friedrich Sertürner, aisló uno de los primeros compuestos puros farmacológicamente activos procedente de una planta: la morfina, un alcaloide presente en el opio obtenido de las cápsulas de la adormidera *Papaver somniferum*.[1] Fue en este momento en el que empezó a dejarse atrás la época de las "pociones" y se inició una era en la que los compuestos activos de las plantas podían ser purificados, estudiados y administrados en dosis precisas que no variaban según la fuente o la edad del material.[2] La investigación farmacéutica se expandió después de las segunda Guerra Mundial para incluir la detección masiva de microorganismos para nuevos antibióticos debido al descubrimiento de la penicilina. En 1990, alrededor del 80% de los fármacos eran productos naturales o análogos inspirados en ellos. Antibióticos (p. ej.: la penicilina, la tetraciclina, la eritromicina), antiparasitarios (p. ej. la avermectina), antimaláricos (p. ej.: la quinina, la artemisinina), agentes de control de lípidos (p. ej.: lovastatina y análogos), inmunosupresores para trasplantes de órganos (p. ej.: ciclosporina, rapamicinas) y medicamentos contra el cáncer (p. ej.: taxol, doxorrubicina) revolucionaron la medicina. La esperanza de vida en buena parte del mundo ha aumentado desde entonces de unos 40 años a principios del siglo XX a más de 77 años en la actualidad.

La elucidación estructural, que eclosionó a principios del siglo XX con el desarrollo de nuevas técnicas y conocimientos, permitió el desarrollo de técnicas de síntesis como estrategia para el descubrimiento de nuevos fármacos. Empezaron a jugar un papel importante los mecanismos de acción de los fármacos, así como la modificación estructural partiendo de un compuesto altamente activo, o compuesto líder, de modo que ambos aspectos pasaron a ser objeto de estudio.[3] El crecimiento ha sido exponencial y áreas como la Biología, la Fisiología, la Biología Molecular, la Genética y la Química pasaron a coordinarse con un objetivo claro: aumentar la actividad de los fármacos, disminuyendo su toxicidad y efectos adversos. El desarrollo de técnicas teóricas como el QSAR, el *docking* o la química combinatoria, entre otras, han añadido herramientas adicionales que permiten el diseño racional de fármacos y la producción a gran escala.[4]

Aunque la expansión de la química sintética medicinal en la década de 1990 hizo que el porcentaje de nuevos medicamentos basados en productos naturales disminuyese hasta ~ 50% (Figura 1),[5] entre los años 2005-2010 han sido aprobados 19 fármacos basados en compuestos naturales, 10 de los cuales son considerados productos naturales semisintéticos.[6]

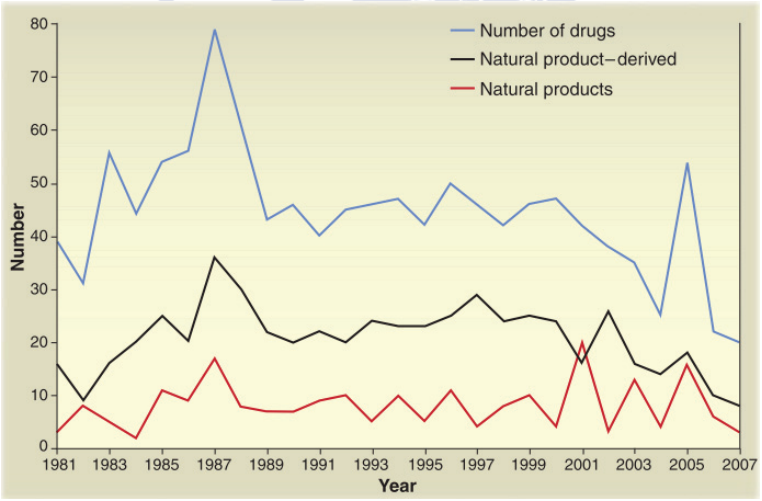

Figura 1. Número de fármacos aprobados en los EEUU entre 1981-2007

Tal es el caso de algunos fármacos aprobados en los Estados Unidos en ese período (Figura 2) [7], entre ellos estructuras polipeptídicas como la exenatida (hipoglicemiante oral) aprobado por la FDA en 2005. En el mismo año se aprueba el primer fármaco de origen marino, el analgésico ziconotida de estructura peptídica comercializado como Prialt® y dos años más tarde se introduce en el mercado el primer antineoplásico también de origen marino, descubierto en el tunicado colonial *Ecteinascidia turbinata*, la trabectedina (Yondelis®) designado en 2007 fármaco para el tratamiento de sarcomas blandos y cáncer de ovario, y desarrollado en nuestro país por la compañía española PharmaMar del grupo Zeltia.[8]

Y más relacionados con nuestro trabajo, queremos hacer referencia al origen natural no solo de los prototipos de estructura flavonoide como podrían considerarse las cumarinas y chalconas que serán extensamente consideradas en las páginas siguientes, sino a estructuras oxigenadas más complejas. Tal es el caso de las flavaglinas, la familia de productos naturales aislados especies del género *Alglaia* a la que pertenece rocaglamida con potencial actividad anticáncer, neuroprotectora,[9] y antimalárica además del interés agroquímico como pesticidas de algunos de sus derivados.[10]

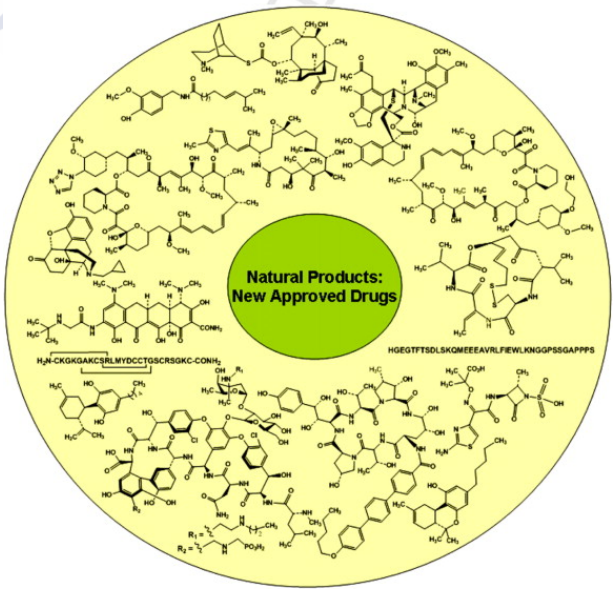

Figura 2.Fármacos aprobados durante el período 2005-2010.

Queda patente por tanto, que la naturaleza ha sido la fuente principal de gran número de fármacos utilizados como tales en terapéutica o bien proporcionando cabezas de serie o compuestos líder a partir de los cuales y mediante modificaciones estructurales se han introducido múltiples compuestos en el arsenal terapéutico. La abundante diversidad de esqueletos de productos naturales se acopla con el "diseño útil" generalmente para proporcionar una mejora del perfil farmacológico del producto natural.

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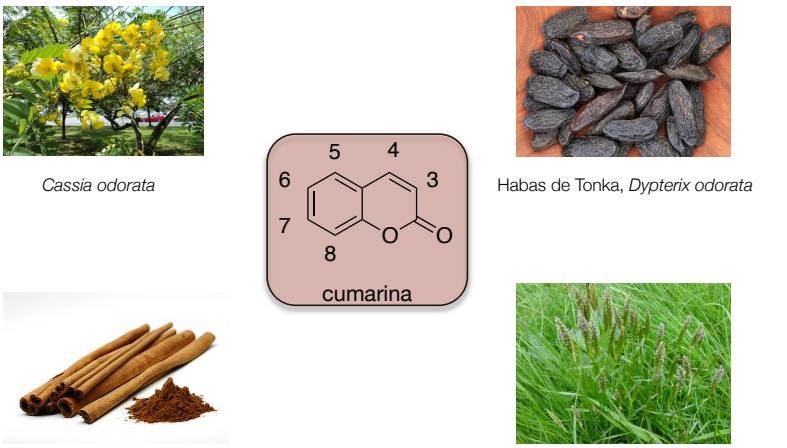
2. Las cumarinas

2.1. Origen de las cumarinas

Las cumarinas constituyen un grupo de compuestos naturales ampliamente encontrados en plantas y otros microorganismos. Las cumarinas pertenecen a la familia de las lactonas, por poseer un sistema 1-benzopiran-2-ona, que corresponde a la benzo-α-pirona (2H-1-benzopiran-2-ona) y cuya nomenclatura sistemática fue establecida por la IUPAC.[11] El nombre de esta familia química deriva del *Coumarouna odorata Aube* (*Dypterix odorata*),[12] planta de la cual fue aislada por primera vez el miembro más simple de esta clase, la propia cumarina.[13] Plantas como la Aspérula, la hierba dulce y el trébol dulce, en particular, se conocen por su olor dulce, que a su vez se debe a su alto contenido de esta sustancia. Es por este motivo por el que este metabolito secundario producido ampliamente en una gran variedad de plantas y aceites esenciales ha sido utilizado como fragancia de productos alimenticios y cosméticos (Figua 3).



Cassia odorata

Habas de Tonka, *Dypterix odorata*

Canela, procedente de *Cinnamomum verum*

Gramas de olor, *Anthoxanthum odoratum*

Figura 3. Estructura de la cumarina y fuentes naturales en las que se encuentra.

Además del aislamiento de cumarinas procedentes de cientos de especies de plantas y otros microorganismos, cabe destacar los derivados de origen sintético, que aumentan significativamente el número de estructuras de cumarina conocidos hasta hoy,[14] así como la complejidad de las mismas.[15] La diversidad estructural de los compuestos derivados de las cumarinas permite su clasificación en cumarinas simples, furocumarinas, piranocumarinas, biscumarinas, cumarinolignanos, triscumarinas, etc. (Figura 4).

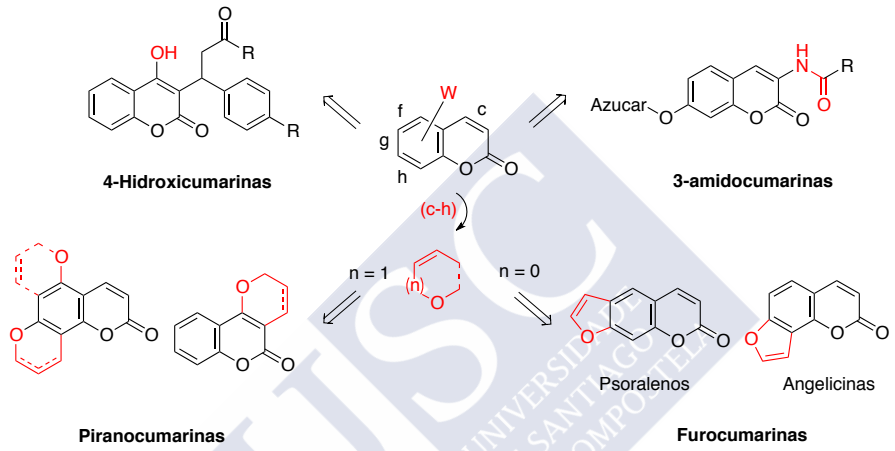


Figura 4. Cumarina, como núcleo central para la obtención de derivados de interés farmacológico

Además, las cumarinas simples más importantes, pueden ser divididas a su vez en función de sus sustituyentes más característicos (Figura 5).[16] Según el tipo de sustituyentes que posean dichas cumarinas, la finalidad para la que pueden ser utilizadas varía: aditivos alimentarios, perfumes, cosméticos, productos agroquímicos o fármacos, entre otros.[17]

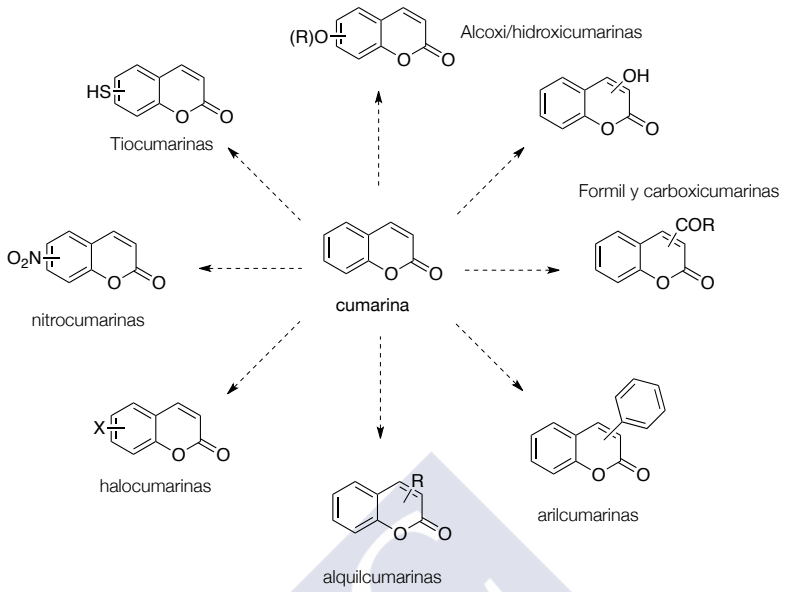


Figura 5. Cumarina, como núcleo central para la obtención de cumarinas simples

Teniendo en cuenta el avance tecnológico que ha permitido facilitar la determinación de estructuras químicas, uno se ve obligado a preguntarse cuál es el factor que despierta interés en los compuestos de esta familia. Entre varios que podrían ser enumerados, se hará hincapié en uno, que sin duda es de gran importancia: la diversidad de actividades biológicas que estos compuestos exhiben. Prácticamente desde el inicio del estudio de las cumarinas, la atención de su investigación se centró en la posibilidad de su actividad terapéutica.[18]

2.2 Biosíntesis de cumarinas

La biosíntesis de las cumarinas empieza con la L-fenilalanina como precursor en plantas como la *Melilotus alba*, (Figura 6), conocida coloquialmente como trébol de olor. La L-fenilalanina es convertida en ácido *trans*-cinámico mediante la acción de la fenilalanina amonioliasa (PAL, de sus siglas en inglés), y posteriormente convertida a ácido *orto*-cumárico mediante la acción de la ácido cinámico *orto*-hidroxilasa. La *orto* hidroxilación de ácidos cinámicos es un paso crucial para la formación de cumarinas. Los ácido *orto*-cinámicos sufren posteriormente un cambio de la configuración de la cadena lateral, transformándose del isómero *trans*, al isómero *cis*, menos estable.

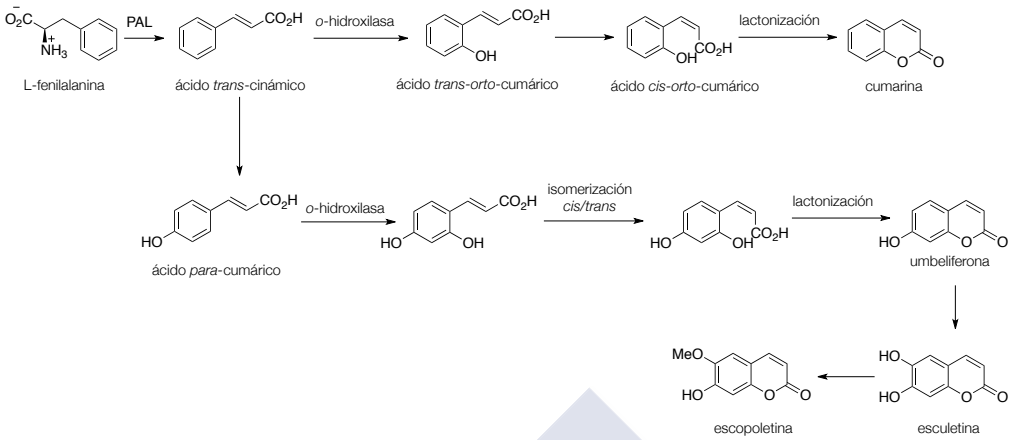


Figura 6.Biosíntesis de cumarinas simples encontradas en plantas.

Mientras que la isomerización *cis/trans* sería desfavorable en el caso de un solo doble enlace aislado, en el caso de los ácidos cinámicos el sistema altamente conjugado permite que este proceso ocurra fácilmente, y que la radiación ultravioleta, como por ejemplo la luz del sol, sea suficiente para producir mezclas en equilibrio. Por último, tiene lugar una lactonización, que da lugar a el esqueleto final de cumarina. Tanto la isomerización *cis/trans*, como la lactonización, son procesos que en la naturaleza están mediados por enzimas, y la luz no es necesaria para la biosíntesis de las cumarinas. Aunque el ácido cinámico sirve como precursor directo de la propia cumarina, la mayoría del resto de las cumarinas simples derivan del ácido *para*-cumárico y de la umbeliferona. Los grupos hidroxilo adicionales presentes en otras cumarinas se suelen añadir a la molécula una vez que el núcleo de la cumarina haya sido construido.

A pesar de que la cumarina en sí se encuentra en plantas como la anteriormente mencionada *Melilotus alba* o trébol de olor, hay evidencias de que las plantas realmente contienen los glucósidos de el *trans* y *cis* ácido *orto*-cumárico, y que la cumarina sólo se libera como resultado de una hidrólisis enzimática y lactonización mediante el daño del tejido de la planta durante los procesos de cosecha.

2.3. Síntesis de cumarinas

Por otra parte, la síntesis de cumarinas y de sus derivados es un área de gran y permanente interés por el gran número de compuestos de esta familia y el amplio espectro de actividades biológicas que presentan. En otros casos, el esqueleto cumarínico es utilizado como intermedio en la síntesis de compuestos más complejos[19] tales como piranocumarinas,[20] furocumarinas o 2-acil-resorcinoles.[21,22]

Si estudiamos los procedimientos sintéticos para la preparación de cumarinas, nos encontramos con una amplia variedad de reacciones clásicas que permiten obtener el núcleo cumarínico.[23,24] Entre estos procedimientos clásicos, destacan las reacciones de Perkin,[25] Pechman[26] o Knoevenagel,[27] aunque otros procedimientos como la reacción de Wittig,[28] Kostanechi-Robisnson o Reformatsky[29] han sido también convenientemente aplicados para la síntesis de estos heterociclos (Figura 7). Sin embargo, es necesario adecuarlos a síntesis específicas y los rendimientos no siempre son satisfactorios,[30] haciendo que el desarrollo de nuevos y fiables métodos de alto rendimiento para la síntesis de las cumarinas sea un tema importante.

Entre estas reacciones clásicas, la que ha sido más utilizada es la reacción de Pechmann debido a la sencillez y disponibilidad de los materiales de partida, es decir, fenoles y β-cetoésteres en presencia de agentes ácidos, así como por los satisfactorios rendimientos obtenidos en ciertos casos.[31,12] El uso de diversos reactivos, tales como H_2SO_4, P_2O_5, $FeCl_3$, $ZnCl_2$, $POCl_3$, $AlCl_3$, PPA, HCl, ácido fosfórico, ácido trifluoroacético, montmorillonita y otras arcillas está bien documentada en la literatura.[32]

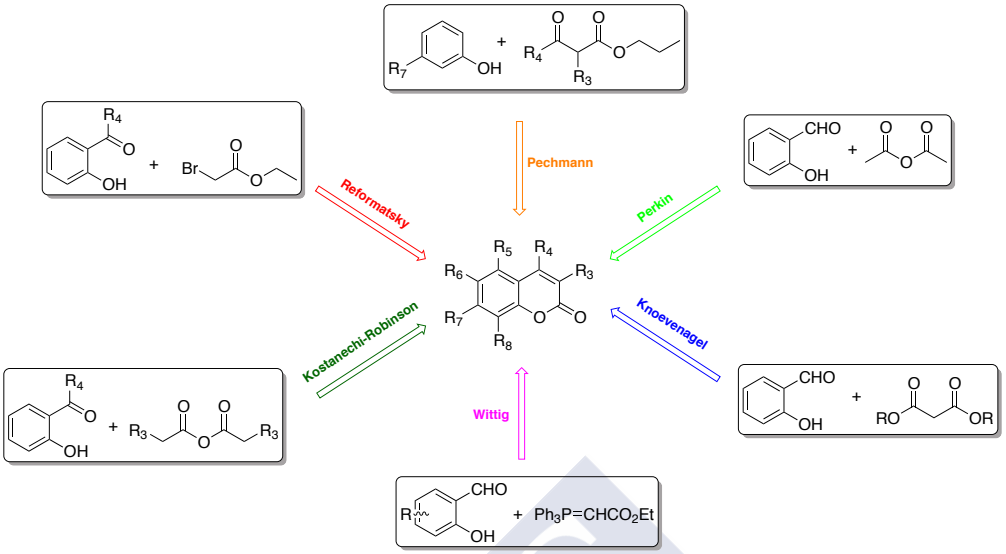


Figura 7. Reacciones clásicas para la síntesis de cumarinas.

La reacción original de Pechmann se lleva a cabo a temperatura elevada. Como se emplean condiciones fuertemente ácidas, la reacción produce aguas residuales ácidas dañinas. Además, las condiciones severas implican limitaciones en los reactivos posibles o requieren la aplicación de los grupos protectores. Varias mejoras han sugerido el empleo de ácidos de Brönsted (libres, o con soporte sólido): HCl, HCl/CH_3CO_2H, HCl/CH_3CH_2OH, H_3PO_4,[33] disoluciones acuosas de líquidos iónicos,[34] resinas de intercambio iónico,[35] así como ácidos de Lewis, tales como $AlCl_3$, $ZnCl_2$[33] o $InCl_3$.[36]

A pesar de la importancia de las cumarinas y de la relevancia tecnológica de su producción, el mecanismo subyacente es poco conocido. Anteriormente se habían propuesto dos mecanismos diferentes para esta reacción[33, 37] que han sido posteriormente adaptados aunque con ligeras modificaciones.

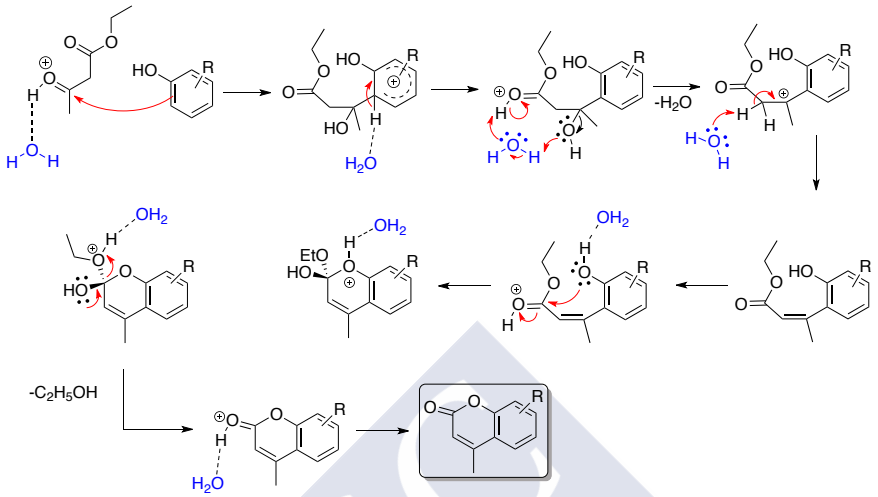


Figura 8. Mecanismo de la reacción de Pechmann propuesto por Robertson *et al.*

El mecanismo propuesto por Robertson et al. (Figura 8) empieza por un ataque electrofílico por el grupo β-carbonílico del acetoacetato de etilo al anillo aromático. Ellos se apoyan en esta propuesta mediante el aislamiento del ácido cinámico como intermedio de reacción, y convierten los ácidos cinámicos en cumarinas.[33,37] Recientemente, también ha sido observado otro intermedio (2,4-dihidroxi-etil-*trans*-*β*-cinamato de metilo), apoyando esta secuencia.[38]

Por otro lado, la propuesta mecanística de Ahmed y Desai (Figura 9), tiene como característica la esterificación como primer paso, seguido por un ataque electrofílico para cerrar el anillo.[33] Este mecanismo también tiene en cuenta la posible formación de la cromona como producto secundario, asumiendo que también puede tener lugar una migración de Fries después del paso de la esterificación.

Ambas propuestas mecanísticas han sido afirmadas como “el mecanismo de la reacción” en la literatura. Algunos artículos se prefieren el mecanismo propuesto por Robertson,[39] otros se han referido al mecanismo de Ahmed y Desai[39,40,35,41] y otros mencionan y aceptan ambos.[33,42]

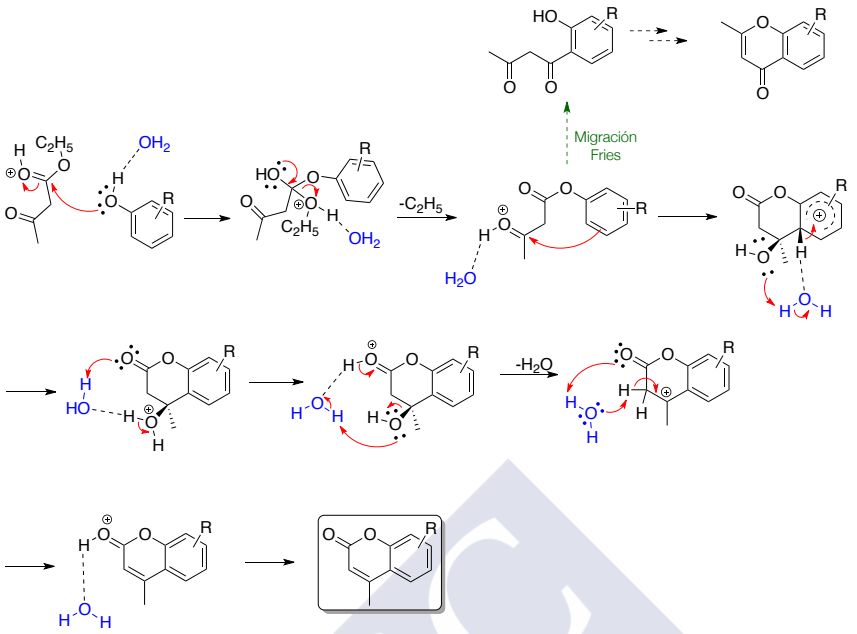


Figura 9. Mecanismo de la reacción de Pechmann propuesto por Ahmed y Dasai.

Otra de las reacciones clásicas empleadas para la síntesis de cumarinas y que destacaremos en esta memoria por ser la metodología elegida para el desarrollo de gran parte de la misma, es la reacción de Knoevenagel.

La reacción de condensación de Knoevenagel es una reacción clásica en química orgánica empleada para la formación de enlaces carbono-carbono.[43] El prototipo de la reacción de Knoevenagel[44,45] es la condensación de un aldehído o una cetona con un éster malónico para generar un éster carboxílico α,β-insaturado. Sin embargo, el término de reacción de Knoevenagel también se utiliza para reacciones análogas en las que un aldehído o una cetona reaccionan con varios tipos de compuestos que poseen un metileno activado, y cuya reacción está mediada por una amina primaria o secundaria que actúa como catalizador en disolventes orgánicos. La reacción de Knoevenagel está considerada como una modificación de la reacción aldólica; la principal diferencia entre ambas aproximaciones es la elevada acidez del hidrógeno activo del metileno cuando se compara con un hidrógeno α-carbonílico.[46] Para evitar que reacciones aldólicas compitan con la reacción de Knoevenagel, el aldehído empleado no debe tener protones en la posición α. Además, con el fin de

mejorar los rendimientos de la reacción, en los últimos años se han desempeñado la reacción en microondas[47,48] o empleado líquidos iónicos.[49]

Posteriormente a la primera descripción de la reacción de Knoevenagel, se introdujeron cambios en las condiciones de reacción y el los reactivos empleados. De este modo, cuando la base empleada es piridina con trazas de piperidina (en lugar de dietilamina) y el ácido malónico es el precursor del enolato, la reacción pasa a llamarse condensación de Doebner o modificación de Doebner.[50,51]

La reacción de Henry,[52,53] también conocida como la reacción de Kamlet,[54] es otra modificación de la condensación de Knoevenagel que utiliza compuestos con un α-nitro metileno activo, y por ello es frecuentemente llamada reacción nitro aldólica. Supone la condensación de nitrometano (u otro nitroalcano) con aldehídos o cetonas. Un resumen esquemático de la reacción de Knoevenagel y y las modificaciones de Doebner y Henry se representa en la Figura 10.

Un amplio número de cumarinas han sido sintetizadas empleando esta metodología, como por ejemplo los ácidos 3-cumarincarboxílicos[55,56] y amidas,[57,58] amino y alquilamino cumarinas,[48] 3-acetil[59] y trifluoroacetil cumarinas,[60] 3-ciano y azidocumarina,[61] aril y arilsulfonamido cumarinas,[62,63] éteres corona 3-benzoilcumarinas[64] y heterociclos puente unidos a cumarinas.[65] Esta estrategia también se ha aplicado para la obtención de derivados de 4-hidroxicumarinas.[66] Bodwell y colaboradores también han presentado la síntesis de 3-aminocumarinas de forma rápida y sencilla empleando la reacción de Knoevenagel.[67] Las cumarinas 3-carboxamidas también has sido obtenidas mediante una reacción de condensación de tipo Knoevenagel, con o sin radiación de microondas.[68,69]

La reacción de Knoevenagel también se ha llevado a cabo con metilenos activados por heterociclos.[70,71] Además, derivados C-glicosílicos de cumarina también han sido sintetizados mediante una reacción de condensación de Knoevenagel en una sola etapa.[72]

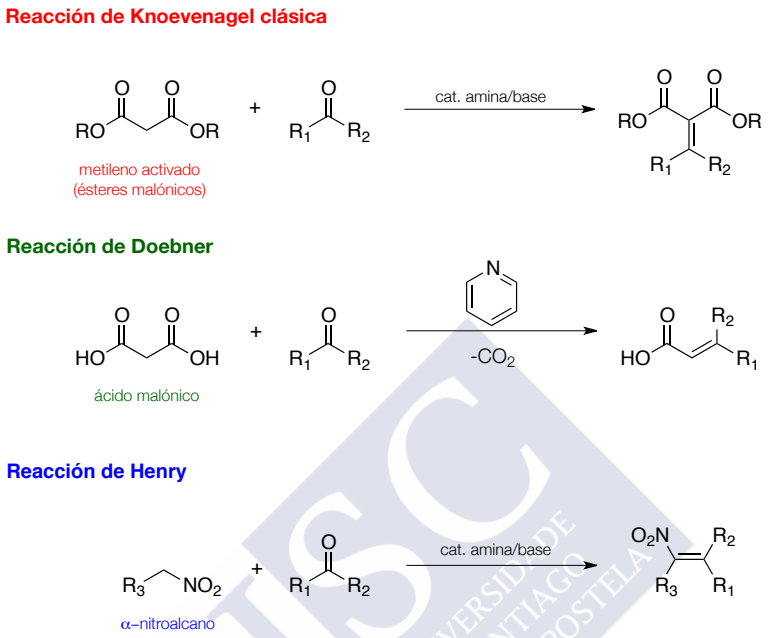


Figura 10. Esquema general de las reacciones de Knoevenagel, Doebner y Henry.

Podemos encontrar en la bibliografía, propuestas de varias modificaciones del método que han tomado una gran importancia. Así, los ácidos 3-cumarincarboxílicos se pueden obtener con elevados rendimientos a partir de los correspondientes *orto*-benzaldehídos y ácidos de Meldrum. [73] La isofraxidina (7-hydroxy-6,8-dimetoxicumarina), por ejemplo, una cumarina ampliamente conocida, ha sido obtenida mediante este rápido procedimiento sintético utilizando un derivado del siringaldehído y el ácido de Meldrum en presencia de ZnO. El ácido 3-cumarincarboxílico obtenido es posteriormente descarboxilado con cobre (Figura 11a).[74] Otro proceso similar pero que emplea una metodología en fase sólida, y en la que el malonato de etilo unido a la resina de Wang y los *orto*-hidroxialdehídos se emplean como materiales de partida, permite obtener también 3-carboxicumarinas sustituidas, tras la descarboxilación térmica de los intermedios (Figura 11b).[75,76]

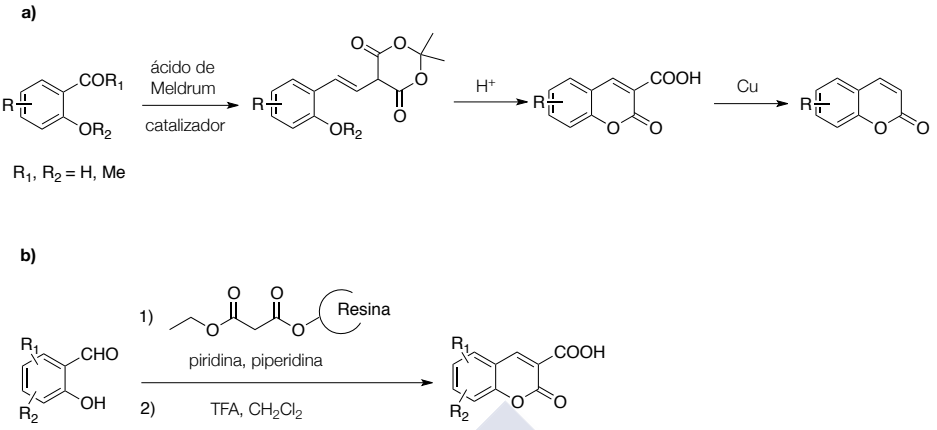


Figura 11. Síntesis de ácidos 3-cumarincarboxílicos mediante a) ácido de Meldrum y b) resinas.

Algunos procedimientos sintéticos también se basan en la ciclación directa de ácidos cinámicos propiamente sustituídos. Estos reactivos están disponibles comercialmente, o bien se obtienen por ciclación de Knoevenagel o mediante la ruptura del anillo de pirona de la cumarina correspondiente.[77] La síntesis de nitrocumarinas,[78] de 3-fenilcumarinas[79] o de 6-acilcumarinas[80] ha sido posible también empleando esta metodología.

Respecto del mecanismo general de la reacción de Knoevenagel (Figura 12), el proceso comienza con la desprotonación del derivado malónico por la piperidina, generando el carbanión correspondiente, resonante con sus correspondientes enolatos. A continuación, se produce el ataque nucleófilo del carbanión sobre el carbono carbonílico del aldehído o cetona que da lugar a la formación de un nuevo enlace carbono-carbono, generando un intermedio que tras pasos de transferencia protónica con la base protonada generan un intermedio aldólico que sufre deshidratación para dar el compuesto final α,β-insaturado.

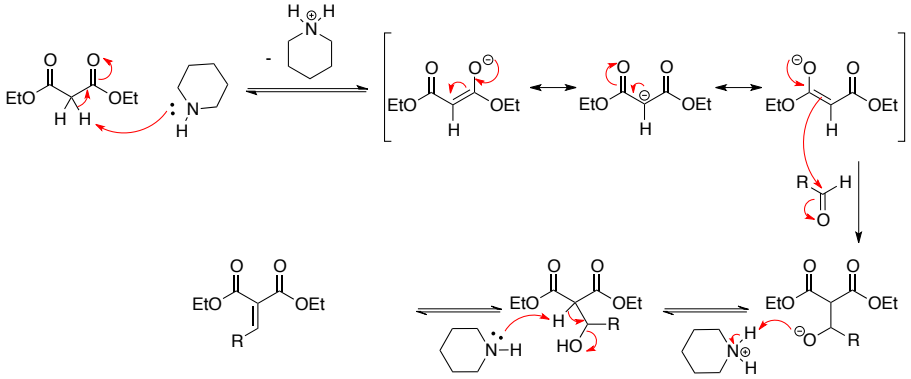


Figura 12. Mecanismo general de la reacción de Knoevenagel.

2.4. Actividad biológica de las cumarinas

El origen del descubrimiento del primer fármaco de estructura cumarínica introducido en terapéutica, está íntimamente relacionado con la biosíntesis y más concretamente con los procesos químicos de fermentación del trébol *Melilotus officinalis* fuente importante de cumarinas. En 1941 Link comprobó que la enfermedad hemorrágica ya detectada en 1920 y conocida como "enfermedad del trébol dulce" que afectaba al ganado, provenía de la ingestión de heno contaminado con dicho trébol y conservado en malas condiciones. La cumarina presente en el proceso de fermentación es oxidada a 4-hidroxicumarina la cual sufre un proceso de condensación con una molécula de formaldehido generado en dicha fermentación para formar el compuesto dímero responsable de dichas hemorragias: el dicumarol, comercializado ya en 1942 (Figura 13).[81]

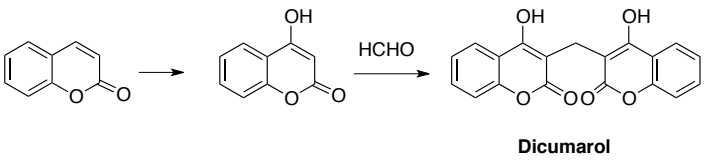


Figura 13. Formación del dicumarol tras procesos de fermentación en trébol *Melilotus officinalis.*

Aparecía así el primer derivado cumarínico con actividad farmacológica, cuyo descubrimiento ha servido de inspiración para el desarrollo de la warfarina, comercializada en España con el nombre de Aldocumar®, o el acenocumarol, comercializado como Sintrom®, ambos empleados en clínica como fármacos anticoagulantes.[82] El carbocromeno, también disponible actualmente, es otro ejemplo de una conocida cumarina con actividad cardioprotectora debido a su acción vasodilatadora e inhibidora de la agregación plaquetaria A partir de este momento, el estudio de los compuestos cumarínicos ha crecido incesantemente de forma que hoy día se conocen estructuras con una gran diversidad estructural, motivo por el cual es también elevada su diversidad fármaco-terapéutica. De este modo, se pueden encontrar cumarinas con propiedades antioxidantes, [83] anti-inflamatorias, [84 , 85] antivirales, [86 , 87 , 88] antimicrobianas, [89 , 90] cardioprotectoras [91 , 92] o como inhibidores enzimáticos,[93,94] entre otras. (Figura 14).[15]

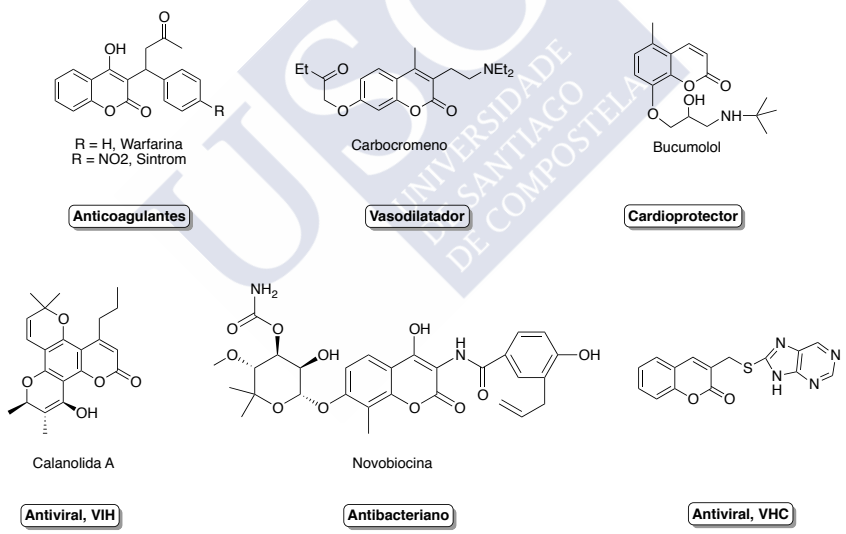
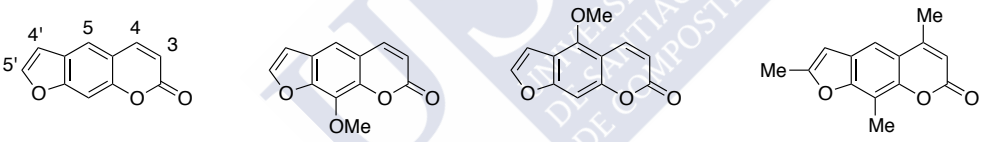


Figura 14. Cumarinas empleadas actualmente como fármacos comerciales.

Además de las cumarinas mencionadas, las furocumarinas o psoralenos, productos tricíclicos de condensación con el anillo de furano, presentan una gran variedad de propiedades farmacológicas derivadas fundamentalmente de su capacidad

de fotorreacción con el ADN.[95] El compuesto de referencia y principal responsable de la aplicación farmacológica de esta familia es la furo[3,2-*g*]cumarina lineal conocida propiamente como psoraleno. [96 , 97] De hecho, ya desde tiempos remotos (aproximadamente 200 A.C.) extractos o partes de plantas que contienen esta molécula eran utilizados en el antiguo Egipto e India para tratar enfermedades hiperproliferativas de la piel.

El descubrimiento de fuentes de luz ultravioleta supuso un significativo avance en el uso de la fototerapia[98] y en 1974 fue introducido el término fotoquimioterapia para describir el uso de radiación ultravioleta junto con un fotosensibilizador.[99] La terapia PUVA (Psoralero + radiación ultravioleta-A)[100] es el ejemplo más conocido de la aplicación de estos compuestos y fue introducido por El Motif, que en 1948 aisló y aplicó el 8-metilpsoraleno (8-MOP) en el tratamiento del vitíligo.[101] En la actualidad, el 8-MOP sigue siendo el derivado más común empleado en la terapia humana, aunque el 5-MOP, el 4,8,5'-trimetilpsoraleno (TMP) o el mismo psoraleno (Figura 15) también son utilizados en el tratamiento de diferentes desórdenes hiperproliferativos de la piel incluyendo el vitíligo, la psoriasis, o la dermatitis atópica.



Psoraleno

8-MOP
8-metóxipsoraleno
xantotoxina

5-MOP
5-metóxipsoraleno
bergapteno

TMP
4,8,5'-trimetilpsoraleno
metoxaleno

Figura 15. Furocumarinas lineales (psoralenos) actualmente empleados para tratar desórdenes hiperproliferativos de la piel.

Por último, además de las actividades farmacológicas mencionadas, queremos referirnos de modo especial a la actividad de las cumarinas como inhibidores enzimáticos, campo en el que podemos encontrar una amplia variedad de estructuras, tanto de cumarinas simples como de sus derivados, que actúan sobre diferentes sistemas enzimáticos. Un especial interés mostraron algunos inhibidores de diferentes enzimas con potencial aplicación en el tratamiento de las enfermedades neurodegenerativas (Figura 16). Entre estas enzimas se encuentra la β-secretasa, generalmente referenciada como BACE (beta-site APP cleaving enzyme), y para cuya

isoforma 1 (BACE-1) se encontraron 3-amidocumarinas con potencial uso en la terapia de la enfermedad de Alzheimer (EA).[73] Otra de las enzimas implicada en la EA y cuyos inhibidores han sido ampliamente estudiados es la acetilcolinesterasa (AChE), para la que también se han encontrado derivados de cumarina, [102,103,] con gran actividad y una potencial aplicación terapéutica.[104,105]

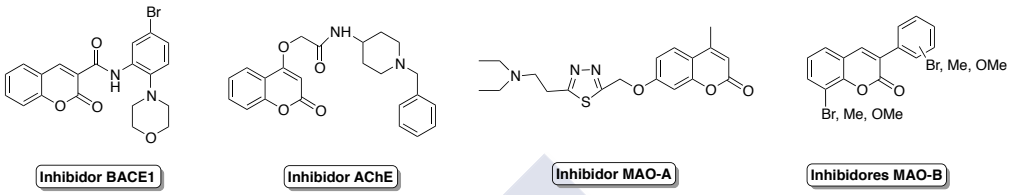


Figura 16. Derivados de cumarina como inhibidores de enzimas implicadas en enfermedades neurodegenerativas.

Por otro lado, es conocido el papel clave que las monoaminoxidasas (MAOs) desempeñan en la inactivación de los neurotransmisores, responsables de algunos trastornos neurológicos.[106, 107] En años recientes, se ha encontrado que algunos análogos cumarínicos presentan una interesante actividad inhibidora de la MAO (IMAO).[108] Esta enzima presenta dos isoformas, MAO-A, cuya regulación puede tener implicaciones terapéuticas para el tratamiento de la depresión[109], y la MAO-B, con implicaciones en la terapia empleada contra el Parkinson.[110] En este campo se profundizará un poco más a lo largo de la discusión de esta memoria, pero cabe destacar que en los últimos años se han encontrado derivados cumarínicos con una actividad inhibidora selectiva de la MAO-B.[111,112,113]

También se han descrito derivados cumarínicos multi-diana que presentan una actividad dual inhibidora de la BACE1 y de la AChE, [114,115] o monoaminoxidasa (MAO) y AChE,[116] todos con interés en la terapia de la EA. Algunos de los compuestos multifuncionales descritos se muestran en la. Muchos son los ejemplos de los compuestos multifuncionales descritos (Figura 17), como ocurre con las estructuras siguientes.

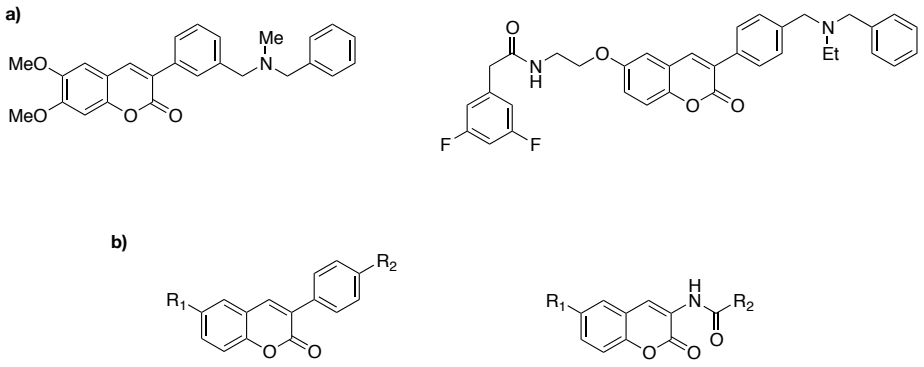


Figura 17. Inhibidores duales de a) AChE y BACE1 y b) AChE y MAO.

Además de los sistemas enzimáticos expuestos, es preciso considerar otros aspectos muy directamente relacionados con la etiopatología de las enfermedades neurodegenerativas. En los últimos años se demostró que en los pacientes de EA aumenta la actividad MAO-B cerebral y este hecho origina un aumento de radicales libres, responsables del estrés oxidativo, muerte celular, y desarrollo de las placas amiloides. En este sentido es una teoría ampliamente aceptada el efecto beneficioso de la terapia antioxidante con dietas ricas en flavonoides, terapia que está recibiendo cada día mayor atención con objeto de detener o retrasar la neurodegeneración.[117] Así pues, es importante considerar el interés de los análogos cumarínicos antes descritos y su potencialidad terapéutica en dichas enfermedades debido a su capacidad antioxidante, aspecto que también estudiaremos en esta Memoria.

3. Las chalconas

3.1. Origen de las chalconas

Las chalconas, que están consideradas como los precursores biosintéticos de otros flavonoides e isoflavonoides, representan una de las mayores clases de compuestos naturales ampliamente distribuída en el reino vegetal (Figura 18). Además, las chalconas también pueden ser precursores sintéticos clave de muchos heterociclos biológicamente importantes, tales como las benzotiacepinas, pirazolinas y las 1,4-dicetonas.

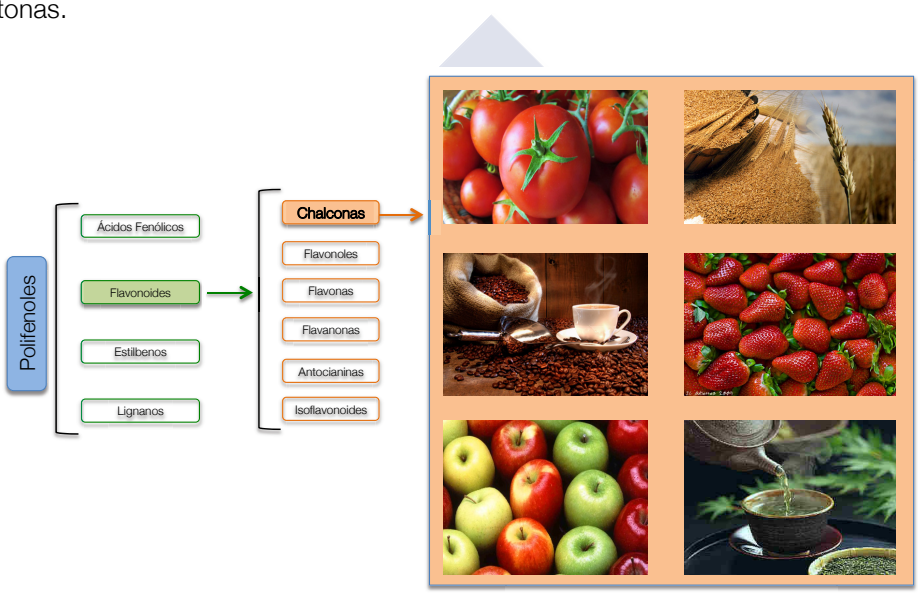


Figura 18. Fuentes naturales y origen biosintético de las chalconas.

Químicamente, los compuestos de la clase de las chalconas poseen un esqueleto estructural común de 1,3-difenil-2-propen-1-ona y son consideradas como flavonoides de cadena abierta, en los cuales los dos anillos aromáticos (anillos A y B) están unidos por un sistema carbonílico α,β-insaturado de tres átomos de carbono. Las chalconas pueden existir en dos formas isoméricas, *cis* y *trans*, de las cuales el isómero *trans* es el considerado más favorable termodinámicamente (Figura 19).

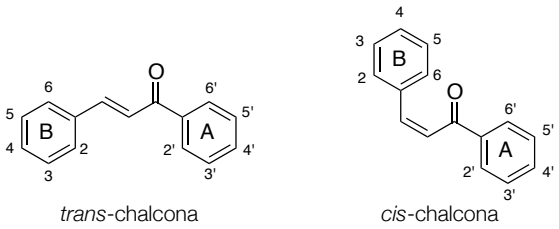


Figura 19. Isómeros *trans* y *cis* del esqueleto básico de chalcona

La presencia del dobles enlaces conjugados en las chalconas, así como la existencia de un sistema π-electrónico completamente deslocalizado en ambos anillos bencénicos, hace que estas moléculas suelan poseer bajos potenciales redox y una elevada probabilidad de que den lugar a reacciones de transferencia electrónica.

Las chalconas de origen natural, así como sus análogos sintéticos, muestran un amplio espectro de actividades biológicas.[118,119] Por esta razón, las chalconas se han convertido en objeto de interés continuo tanto en el mundo académico como en la industria. Se cree que el fragmento cetónico α,β-insaturado es probable que sea el responsable de muchas de las actividades biológicas observadas, ya que la eliminación de esta característica estructural resulta en la pérdida de bioactividad. Con este fragmento intacto, se han sintetizado nuevas moléculas derivadas realizando modificaciones en ambos anillos.[120]

3.2. Biosíntesis de chalconas

Las chalconas actúan como precursores de un vasto número de derivados de flavonoides encontrados en todo el reino vegetal los cuales, en su mayoría, contienen un anillo heterocíclico de 6 miembros, que se forma a partir de ataques nucleófilos de tipo Michael por parte de un grupo fenol sobre la cetona insaturada, dando lugar a flavanonas. Como se muestra el la Figura 20, la biosíntesis de las chalconas empieza con la L-fenilalanina. A continuación, la condensación de la cinamoil-CoA derivada de la L-fenilalanina con tres moléculas de malonil-CoA catalizada por la chalcona sintetasa,

da lugar a la chalcona. La flavanona correspondiente es posteriormente sintetizada por la enzima chalcona isomerasa, que cataliza la reacción de forma estereoespecífica.[121,122]

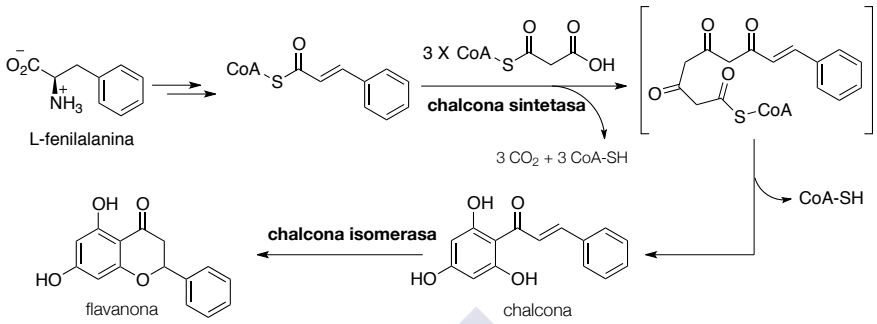


Figura 20. Ruta biosintética general de las chalconas

El continuo y exhaustivo estudio de las distintas rutas biosintéticas (en especial la del ácido shikímico y la del malonato) ha permitido explicar tanto la formación como muchas de las transformaciones metabólicas que experimentan estas moléculas. Se observó que en estos procesos, juegan un papel fundamental enzimas como la chalcona-sintetasa y la chalcona-isomerasa, que se encargan de la biosíntesis y de las trasformaciones biosintéticas de diversificación estructural que experimentan las chalconas naturales, y que conducen a diversos miembros de la familia de los flavonoides. (Figura 21)

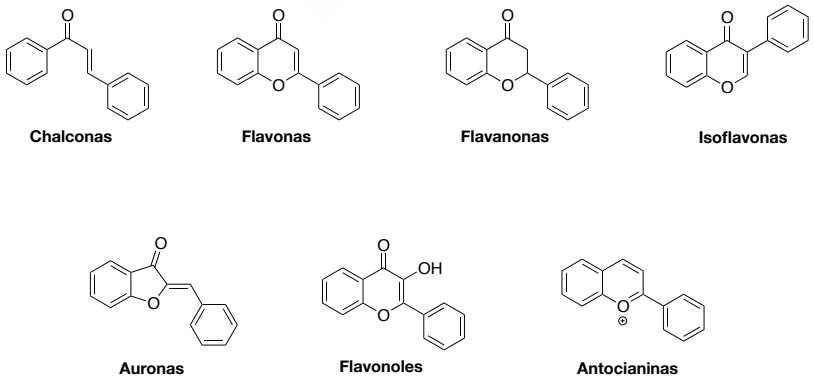


Figura 21. Núcleos estructurales de los principales grupos de flavonoides

3.3. Síntesis de chalconas

Desde el punto de vista preparativo, tradicionalmente las chalconas se obtienen mediante la condensación de Claisen-Schmidt en medio ácido o básico, que actúa como catalizador, bajo condiciones homogéneas.[123,124] (Figura 22). A pesar de tratarse de una de las reacciones más clásicas de la química orgánica, destaca por su sencillez experimental y por su robustez, permitiendo la formación de forma eficiente del doble enlace carbono-carbono independientemente de la complejidad estructural de las moléculas a ensamblar.

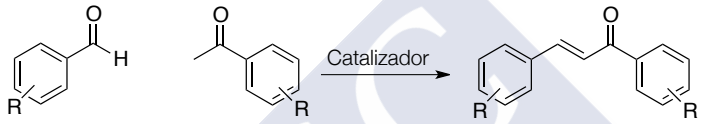


Figura 22. Reacción de Claisen-Schmidt para la síntesis de chalconas.

Esta reacción ha sido ampliamente utilizada no solo para la preparación de chalconas, sino también para la síntesis de flavanonas, derivados del 1,3-diarilpropano y para la síntesis de una nueva familia de macrociclos, todas ellas en un único paso.

En cuanto al mecanismo, la reacción de Claisen-Schmidt pertenece al grupo de las condensaciones aldólicas,[125,126] transformaciones catalíticas que pueden transcurrir a través de dos mecanismos distintos. Dependiendo del tipo de catalizador que se emplee en la reacción, de su naturaleza ácida o básica, la reacción trascurre vía enol o enolato respectivamente (Figura 23).

Cuando lo que se emplea es un catalizador ácido, la etapa inicial en el mecanismo de reacción conlleva la tautomerización de la cetona para formar el enol. Además, el ácido también activa el grupo carbonílico del aldehído mediante su protonación, haciendo altamente electrófilo dicho átomo de carbono. El enol formado actúa como nucleófilo en su carbono-α, permitiéndole atacar al aldehído protonado y conduciendo posteriormente a la formación del aldol, que sufre una deshidratación para dar lugar al compuesto carbonílico insaturado final.

Sin embargo, cuando lo que se emplea es una base moderada, el mecanismo de reacción transcurre vía enolato produciéndose el ataque nucleófilo del mismo al carbono carbonílico del aldehído. El producto resultante es la sal del alcóxido del producto aldólico, que tras dar lugar al aldol, puede sufrir fácilmente una deshidratación para generar el compuesto carbonílico insaturado, en este caso la chalcona.

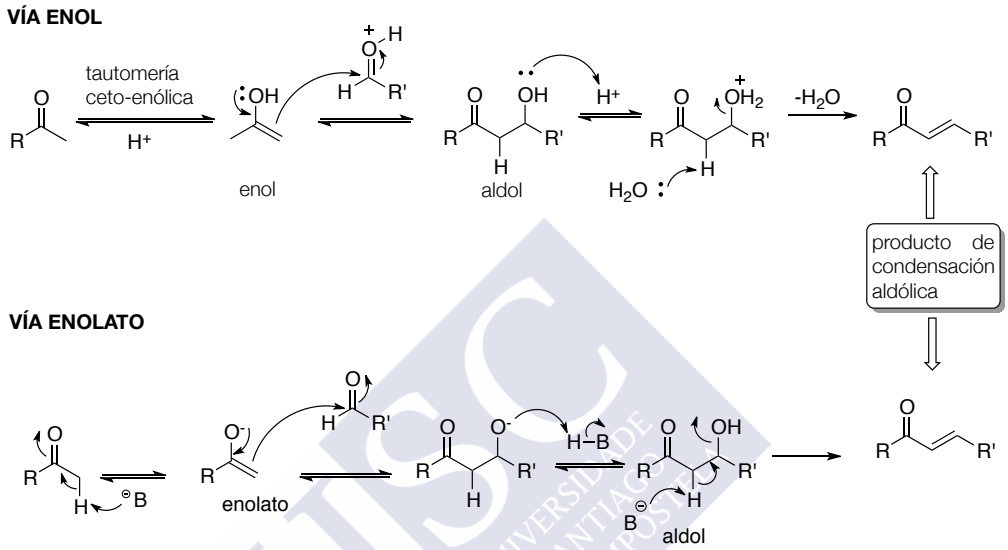


Figura 23. Mecanismos de la reacción de Claisen-Schmidt en condiciones ácidas (via enol) o básicas (vía enolato).

A pesar de que los agentes de catálisis básica para la reacción de Claisen-Schmidt son abundantes, el uso de catalizadores ácidos puede tener la ventaja de evitar que ocurra tanto la reacción competitiva de Cannizzaro del aldehído como otras reacciones laterales secundarias no deseadas cuando existen grupos sensibles a las condiciones básicas.

Además de los catalizadores clásicos, existe un amplio y heterogéneo arsenal de sustancias químicas capaces de servir como catalizadores en esta reacción. Entre los catalizadores más novedosos se encuentran las hidrotalcitas activadas,[127] hidroxipatita,[128] zeolitas,[129] sílica aminopropilada sol-gel,[130] $BiCl_3$,[131] K_2CO_3 anhidro[132] o líquidos iónicos ácidos[133] entre otros.

Aunque como se ha dicho anteriormente la reacción de Claisen-Schmidt es la más utilizada, otras técnicas de renombre también han sido empleadas para la síntesis de chalconas.[134] Así, podemos encontrar métodos como la reacción de acoplamiento de Suzuki,[135] la reacción de cross-coupling directa,[136] la reacción de Heck,[137,138] las reacciones mediante radiación microondas,[139] la acilación de Friedel-Crafts[140] o la olefinación de Julia-Kocienski,[141] representadas de forma esquemática en la Figura 24.

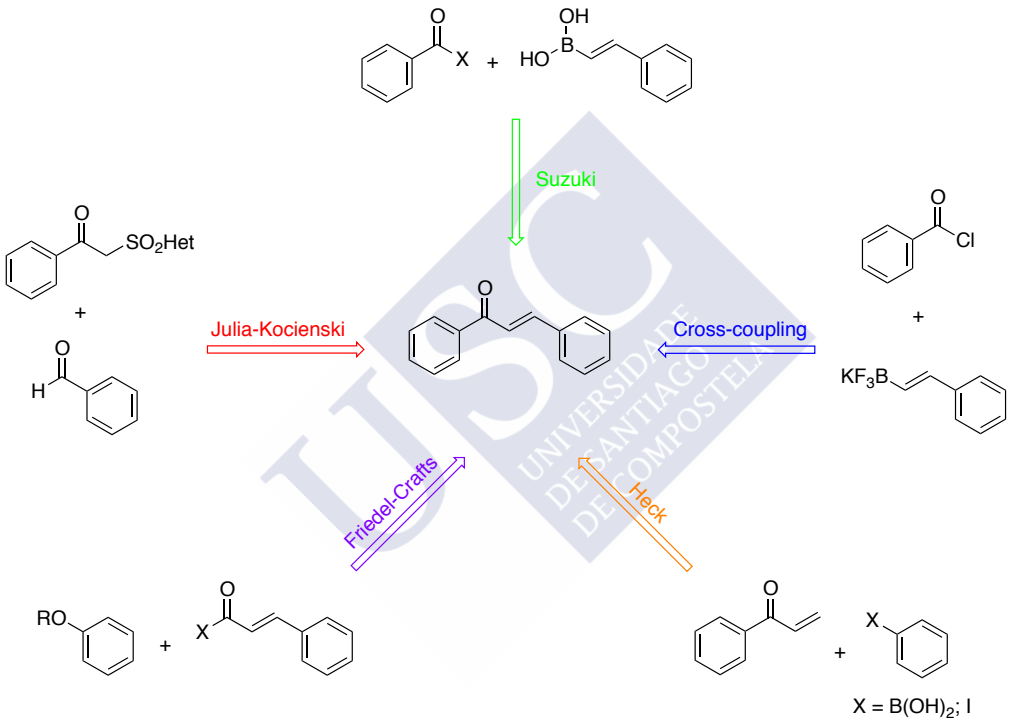


Figura 24. Reacciones clásicas empleadas para la síntesis de chalconas

3.4. Actividad biológica de las chalconas

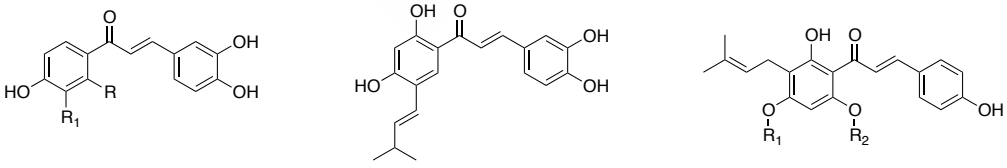
La actividad biológica de las chalconas es tan relevante y variada como la metodología sintética previamente mencionada. De hecho, no muchos patrones estructurales manifiestan un rango tan amplio de actividades farmacológicas. Dimmock y colaboradores realizaron una profunda revisión de las propiedades de las chalconas en 1999.[142] Desde entonces, las chalconas han seguido siendo una de las áreas de

investigación más activas, y prueba de ello son las múltiples revisiones que se pueden encontrar actualmente y en las que se recoge el potencial farmacológico de las mismas.[143,144,145,146,120]

La mayor parte de los estudios farmacológicos realizados sobre esta familia de compuestos orgánicos han estado dirigidos hacia la identificación de nuevos agentes antibacterianos, [147 , 148] antiprotozoarios, [149] antifúngicos, [150] antivirales, [151 , 152] , antineoplásicos[153] o antioxidantes[154] entre otros.

Además de las actividades farmacológicas mencionadas, cabe destacar que estas moléculas también presentan múltiples propiedades derivadas de su capacidad de interacción con biomoléculas. Dentro de este grupo, es de recalcar el potencial de las chalconas como analgésicos, [155] anti-inflamatorios, [156 , 157] antihistamínicos, [158] antimitóticos[159] o inmunomoduladores.[160]

Respecto a la actividad antioxidante, se sabe que las plantas producen una gran variedad de antioxidantes como respuesta al daño molecular que producen las especies reactivas de oxígeno (ROS, por sus siglas en inglés), y que los compuestos fenólicos forman la mayor clase de antioxidantes derivados del mundo vegetal. Entre los múltiples compuestos fenólicos, los flavonoides son quizá el grupo más importante, ya que tienen la capacidad de atrapar radicales libres y prevenir la peroxidación lipídica. [161 , 162] Muchas hidroxichalconas tanto simples como con sustituyentes isoprenílicos en sus anillos fenílicos poseen un elevado potencial antioxidante (Figura 25).[163]



Buteína, R = OH; R_1 = H
Sappanchalcona, R = OCH_3; R_1 = H
Okanina, R = R_1 = OH

Broussochalcona A

Xanthohumol, R_1 = H; R_2 = CH_3
Desmetilcanthohumol, R_1 = R_2 = H

Figura 25. Estructuras químicas de chalconas antioxidantes de origen natural

Así, podemos encontrar 3,4-dihidroxichalconas como la buteína, la sappanchalcona o la okanina, que son antioxidantes particularmente efectivos y en los que la presencia tanto de un grupo hidroxilo en C-2' en el anillo A como del grupo catecol en el anillo B, parecen ser favorables para su actividad anti-radicalaria, mientras que la falta del enlace α,β-insaturado hace que su eficacia disminuya.[164]

Muy estrechamente relacionada con la actividad antioxidante, la actividad anti-inflamatoria es también un campo en el que las chalconas presentan resultados muy prometedores[165,166] si bien igual que ocurre con aquella, es muy difícil establecer una relación estructura-actividad. La inflamación es una respuesta biológica compleja asociada a múltiples condiciones fisiopatológicas. Como respuesta a un estímulo inflamatorio, los macrófagos liberan varias moléculas pro-inflamatorias, entre las que se incluye el radical libre de corto periodo de vida como el óxido nítrico (NO). Una serie de derivados de chalcona con posibles efectos anti-inflamatorios fueron ensayados por Heredia y colaboradores [167] y entre los que encontraron un compuesto significantemente activo como atrapador de aniones superóxido. Además, estudios *in vitro* de dimetilamino-chalconas[168] analizaron la actividad inhibidora en la producción de NO y de los mediadores de la prostaglandina E_2 (PGE2) y se observó que se producía una inhibición de la producción de NO dependiente de la concentración. Además de las dimetil-aminochalconas, 3,4-dihidroxichalconas fluoradas, 3',4',5'-trimetoxichalconas o la 2',4',6'-tris(metoximetoxi)chalcona (TMMC), presentaron potentes efectos anti-inflamatorios (Figura 26).

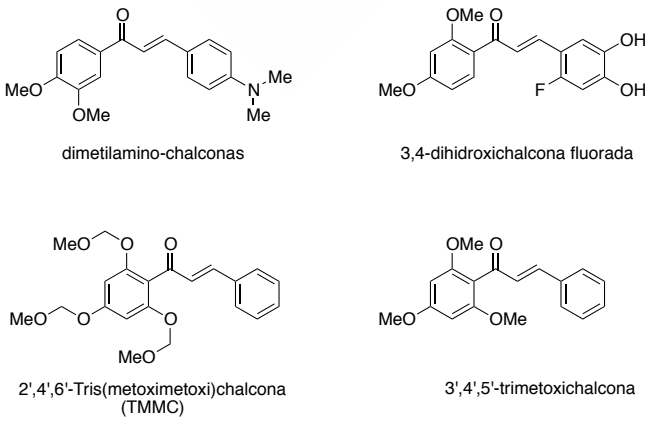


Figura 26. Estructuras químicas de derivados de chalcona con actividad anti-inflamatoria.

Estudios recientes se han centrado en las actividades anti-proliferativa y de reducción tumoral de algunas chalconas, e incluso se ha observado que una ingesta de flavonoides presentes en una dieta vegetal es segura y no está asociada con ningún efecto de salud adverso, de modo que se ha vuelto a generar un elevado interés en esta clase de moléculas.[169] Entre los actuales agentes antitumorales identificados, las chalconas representan una importante clase de moléculas pequeñas útiles en la quimioterapia del cáncer.[170] El Xanthohumol por ejemplo (Figura 25), que es uno de los mayores ingredientes presentes en la cerveza, puede ser potencialmente útil no sólo como antioxidante, sino como agente quimioprotector durante la hiperplasia y carcinogénesis de próstata y decrece su proliferación.[171] Otras chalconas (Figura 27), como algunas chalconas borónicas, chalconas C-metiladas aisladas de la pulpa y las semillas de los frutos de *Syzygium samarangense*, conocida como la manzana de java, bases de Mannich de chalconas heterocíclicas o chalconas aril o aroil sustituidas, representan también compuestos con potencial actividad antitumoral.

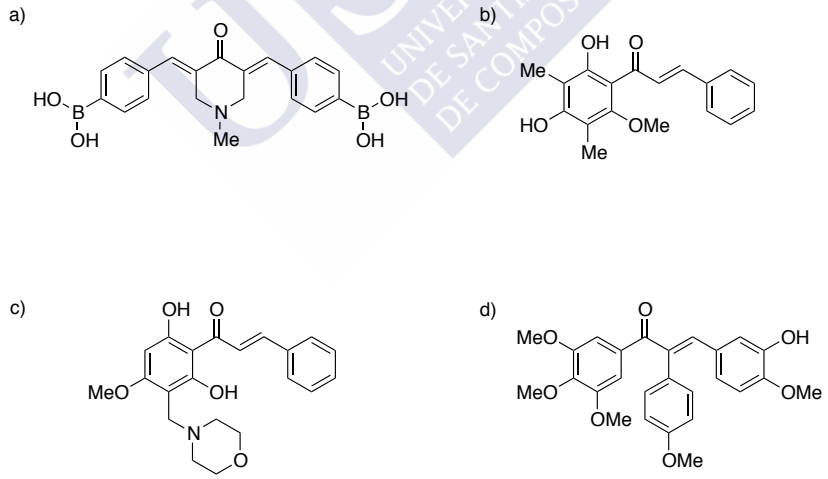


Figura 27. Chalconas con actividad antitumoral. a) chalcona borónica; b) chalcona C-metilada extraída de de los frutos de *Syzygium samarangense*; c) base de Mannich de una chalcona; d) chalcona aril-sustituída.

Respecto a la actividad anti-infecciosa, la estructura de chalcona puede considerarse como un importante cabeza de serie a partir del cual se han obtenidos derivados con actividad muy diversa: antibacteriana,[147] antifúngica,[172] antiviral,[173] antimalárica[174,175] o anti-leishmania entre otras. Algunos ejemplos de chalconas que muestran este tipo de actividades se muestran en la Figura 28.

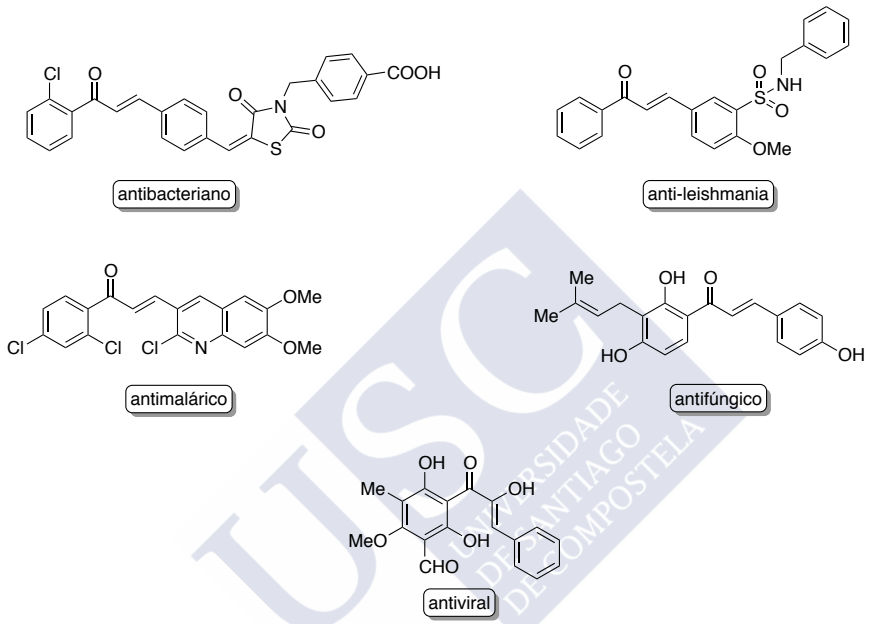


Figura 28. Estructuras químicas de derivados de chalcona con diferentes actividades anti-infecciosas.

Por último, debemos hacer una breve referencia a la actividad inhibidora de ciertos sistemas enzimáticos. Además de la actividad inhibidora de la ciclooxigenasa-2 (COX-2)[176] relacionada con su acción antiinflamatoria ya mencionada, nos referimos a la actividad inhibidora MAO encontrada por primera vez en 2009 para estos prototipos[177], así como para algunas furano-chalconas[178] (Figura 29). En otros casos se da cuenta de dicha actividad en los vinil isósteros,[179] o de la elevada actividad iMAO-B en isoflavonoides estructuralmente próximas.[180] Por último, queremos mencionar la actividad sobre la tirosinasa[181,182] encontrada en determinadas chalconas, muy relacionadas con sus análogos cumarínicos y otros flavonoides naturales y sintéticos, motivo por el cual es muy difícil poder establecer de forma inequívoca y predictiva la

relación estructura–actividad sobre las múltiples dianas farmacológicas sobre las que pueden interaccionar.

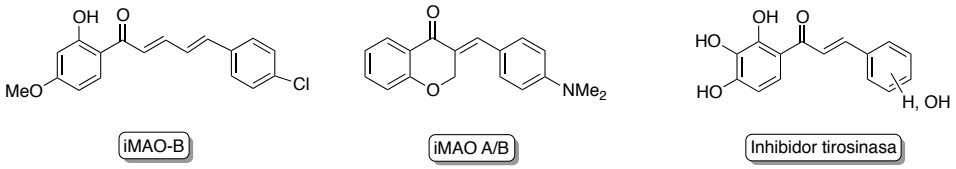


Figura 29. Derivados de chalconas como inhibidores enzimáticos.

Toda esta información justifica que varios autores incluyan a esta familia de compuestos en la lista de estructuras privilegiadas, un concepto enunciado originalmente por Evans[183] y que agrupa a aquellos núcleos que por su alta bioactividad constituyen estructuras de alto interés farmacológico.

4. Los estilbenos

Los estilbenos son polifenoles de bajo peso molecular producidos por plantas como respuesta a una infección tras la exposición a un microorganismo. Se encuentran ampliamente distribuídos en el mundo vegetal y presentan un amplio rango de actividades biológicas tales como antibacterianos, antifúngicos, antitumoral, cardioprotectora e inhibidores de la tirosinasa entre otros.[184,185] Existe un gran interés en su potencial beneficioso para la salud y en su capacidad para mejorar la resistencia en enfermedades en plantas.[186]

Dentro de los estilbenos, el resveratrol[187] es un estilbeno presente en la piel de la uva[188,189] que se origina en respuesta a un daño externo o interno de, por ejemplo, una infección por hongos o exposición a la radiación UV.[190] El interés por este compuesto y sus derivados se incrementó cuando los estudios epidemiológicos establecieron una relación inversa entre el consumo de vino tinto y la incidencia de enfermedades cardiovasculares, además de las propiedades hemostáticas y del aumento del HDL circulante descrita para el etanol.[191,192]

El resveratrol (3,4',5-trihidroxiestilbeno), está formado por dos anillos aromáticos conectados mediante un doble enlace de estireno que hace posible que exista como isómeros *cis*- y *trans*- (Figura 30).[193,194] La forma *trans* del resveratrol, parece ser la responsable de las principales propiedades farmacológicas de esta molécula. Es, hoy en día, un producto comercial común, a partir del que se puede obtener por irradiación UV la forma *cis*.

Figura 30. Isómeros *cis*- y *trans*-resveratrol y fuente natural en la que se encuentra (*Vitis vinífera*)

Por su gran interés farmacológico y su elevado potencia como estructura privilegiada en el campo de la química farmacéutica, el resveratrol ha sido muy ampliamente estudiado en los últimos años.[195, 196] En concreto, destacaremos su actividad inhibidora de la MAO, siendo el *cis*-resveratrol menos efectivo que el *trans*-resveratrol, sobre dicha enzima.[197]

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5. La Rocaglamida

La rocaglamida (Figura 31) es un compuesto de origen natural perteneciente a una clase de moléculas denominadas flavaglinas, nombre que hace alusión a la estructura flavonoide a partir de la que se origina y a la especie en la que está presente. De hecho los procedimientos sintéticos utilizan rutas biomiméticas a partir de las 3-hidroxiflavonas y su cicloadición con esteres cinámicos (Figura 32).[198,199] Fue aislada por primera vez en 1982 por King y colaboradores basándose en su actividad antileucémica.[200] Desde entonces, alrededor de otras 50 flavaglinas como el rocaglaol o el silvestrol han sido encontradas, principalmente por los laboratorios de farmacognosia de Kinghorn, Pezzuto and Proksch[201,202] en las plantas del género *Aglaia* que se encuentran mayoritariamente en los bosques del sudeste asiático y de las islas del Pacífico.

Figura 31. Estructura de la rocaglamida encontrada en la planta *Aglaia elliptifolia*.

King demostró por primera vez que la rocaglamida presentaba una actividad *in vivo* significante en ratones infectados con leucemia linfocítica P388.[203] Desde entonces, la rocaglamida y sus compuestos relacionados, han mostrado actividad citostática y citotóxica frente a diversas líneas celulares tumorales humanas, con valores de IC_{50} en el rango de 1.0-6.0 ng/mL[204,205,206] Sin embargo, otro tipo de actividades han sido encontradas para este tipo de compuestos, que presentan también actividad como insecticidas, anti-fúngicos, anti-inflamatorios o como agentes neuroprotectores.[207,208] Aunque como ya hemos indicado, esta estructura solo está presente en las especies *Alglaia*, estructuras muy próximas son sin embargo mucho

más abundantes. Tal es el caso de la viniferina, un estilbenoide aislado de la *Vitis vinífera* que incorpora también un anillo de benzofurano en su estructura y que puede considerarse dímero del resveratrol a partir del que se biosintetiza (Figura 32) y que como él, tiene propiedades antioxidantes e inhibidoras de la formación de las placas beta amiloides.[209,210]

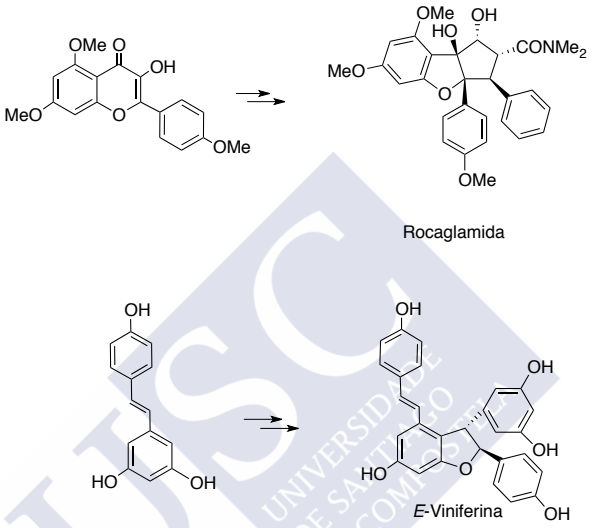

Figura 32. Precursores biosintéticos de algunas flavaglinas.

El grupo de las flavaglinas es un grupo de productos naturales que incorporan en su estructura un esqueleto benzofuránico, motivo por el cual ha merecido la atención de los investigadores que intentaron su síntesis y elucidación estructural, estudio que ha permitido la identificación de otros miembros de la serie así como su potencialidad terapéutica.

Debido a la escasa abundancia natural de este tipo de compuestos la síntesis química, ya sea síntesis total o semisíntesis, es necesaria para que este tipo de compuestos y sus derivados puedan ser empleados como fármacos.

Tanto la complejidad estructural como su importante actividad antitumoral han hecho que este esqueleto sea una diana sintética muy atractiva. La síntesis

estereoselectiva del denso patrón de sustitución de estas dianas presenta un gran reto sintético, ya que esta molécula contiene 5 estereocentros contiguos y grupos arilo en posición *cis* en carbonos adyacentes. En los útimos años, tan sólo contadas síntesis totales han sido descritas, poniendo en evidencia las dificultades asociadas con su síntesis. Una de las reacciones que están cobrando gran importancia para la síntesis de la rocaglamida y sus derivado, así como su aplicación en la síntesis de oxacíclos derivados de compuestos naturales, tiene como base el empleo de la reacción de ciclación Nazarov,[211,212,213].

La reacción de ciclación Nazarov fue originalmente descubierta por Ivan Nikolaevich Nazarov en 1949.[214] La reacción típica de ciclación de Nazarov es el proceso que convierte divinil cetonas tales como **I** (Figura 33) en ciclopentenonas **IV** y/ó **V**.[215] La reacción está catalizada por ácidos de Bronsted o de Lewis. El primer intermedio que se forma es el catión pentadienilo **II**. El confórmero *s-trans/s-trans* **IIB** del catión da lugar a una electrociclación 4π conrotatoria permitida termodinámicamente que da lugar al catión alílico cíclico **III** como segundo intermedio. La pérdida de un protón finaliza la reacción. En ausencia de una corriente de polarización en **III**, el protón se puede eliminar de dos formas, dando lugar a **IV** y **V**.

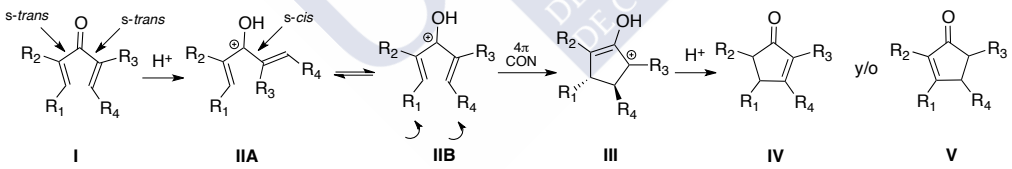

Figura 33. Mecanismo de la ciclación de Nazarov.

Esta reacción ha sido empleada por Frontier y colaboradores como paso clave para la síntesis de (D,L)-rocaglamida, de manera que esta reacción de ciclación es capaz de formar el esqueleto carbonado completo de la rocaglamida,[216] aunque ha sido también empleada para la síntesis de otros compuestos naturales como la (D,L)-terpestacina.[217] A pesar de que esta reacción supone una excelente herramienta para la síntesis total de compuestos naturales, la presencia de múltiples estereocentros en el núcleo de ciclopentenona, supone un reto sintético. Por este motivo, el estudio y

optimización de esta reacción de ciclación que permita controlar la estereoquímica de los estereocentros generados ha supuesto un constante tema de estudio.

En esta Memoria se presentará un estudio para la optimización de la ciclación de Nazarov que permita la unión de dos átomos de carbono a través de un enlace σ de modo que se forme átomos de carbono vecinos con todos sus átomos de carbono cuaternarios y con un buen control de la estereoquímica de la ciclopentenona formada.

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6. Farmacomodulación

6.1. Hibridación molecular y terapia multi-diana.

En base a lo anteriormente mencionado, y teniendo en cuenta la gran variedad de actividades farmacológicas que pueden mostrar los prototipos descritos, es importante considerar el diseño de compuestos multi-diana en los que sobre el esqueleto cumarínico se incorporan grupos farmacóforos distintos con objeto de obtener estructuras híbridas.

La naturaleza multifactorial de gran número de enfermedades y la resistencia a los fármacos antimicrobianos han motivado la aparición de la metodología multi-diana MTDL (multitarget-directed-ligands) en el descubrimiento de fármacos como una clara alternativa a la polifarmacología mediante combinación de varios fármacos FDC (Fixed dose combination). La metodología MTDL permite utilizar una única entidad química que pueda actuar sobre varias dianas terapéuticas. Esta técnica puede tener efectos beneficiosos en varios aspectos farmacológicos tales como el disminuir problemas asociados a dosificación múltiple, interacciones medicamentosas, disminuir o retardar la aparición de resistencias, etc.

Mediante esta técnica de diseño, se han preparado derivados de naturaleza flavonoide multi-diana que presentan una actividad dual inhibidora de la BACE 1/AChE, o bien MAO/AChE, todos con interés en la terapia de la EA.[218] En otros casos productos naturales de estructura flavonoide como la linarina muestran actividad neuroprotectora debido a su capacidad antioxidante y a la vez inhibidora de la AChE (Figura 17).

En otros casos, nuestro grupo de investigación utiliza esta técnica e incrementa las propiedades cardiovasculares de la cumarina mediante la incorporación del esqueleto resveratrol con propiedades también también cardioprotectoras.[237] En el mismo campo destacan híbridos de chalcona, flavona así como cumarinas que incorporan en su estructura el anillo pirimidínico presente en el vasodilatador minoxidilo (Figura 34).[219,220]

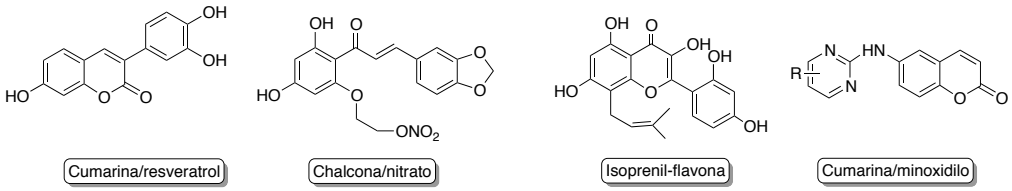

Figura 34. Híbridos multi-diana con actividad cardiovascular.

Por su parte, la elevada morbilidad y mortalidad originada por la infecciones protozoarias demanda cada vez más nuevas estrategias terapéuticas, y en este campo la terapia multidiana se compite cada vez más con la terapia multifármaco[221]. Destacan entre los compuestos más importantes el híbrido chalcona-benzoxaborol, farmacóforos con demostrada actividad frente a *Tripanosoma brucei y Trapanosoma cruzi*,[222] el híbrido chalcona-cloroquina activo frente a *Plasmodium falciparum*.[223] Finalmente, el híbrido cumarina-PABA eficaz antibacteriano[224] o el antiviral híbrido chalcona-anilida inhibidor dual de la integrasa y del receptor de transmenbrana viral CCR5 (C-C quimiocina receptor)[239] de son ejemplos que ilustran la aplicación de la metodología multidiana en el diseño de nuevos prototipos (Figura 35).

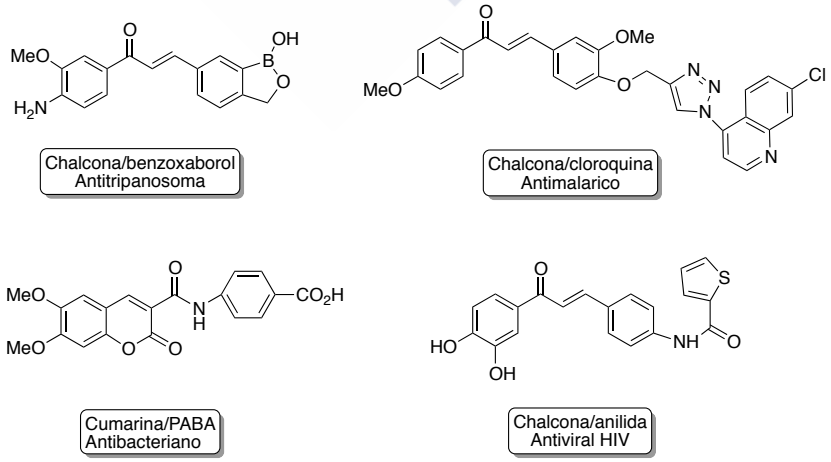

Figura 35. Híbridos multi-diana empleados como antimicrobianos.

6.2. Bioisosterismo.

Fue en 1919 cuando Langmuir,[225] estudiando el comportamiento químico y la reactividad de determinadas sustancias que poseían átomos o grupos con el mismo número de electrones de valencia, por ejemplo, isoelectrónicos, creó el concepto de isosterismo para definir átomos o moléculas orgánicas o inorgánicas que poseían el mismo número y/o la misma distribución de electrones. Años más tarde, en 1932, Erlenmeyer[226] propuso ampliar el término isosterismo, definiendo los isósteros como elementos, moléculas o iones que presentaban el mismo número de electrones en el nivel de valencia. Su contribución incluye la propuesta de que los elementos de la misma columna de la tabla periódica son isósteros entre ellos (por ejemplo, carbono, Silicio y Germanio), y la creación del concepto de anillos electrónicamente equivalentes, que sería posteriormente ampliado al término bioisosterismo de anillos.

El término bioisosterismo fue acuñado por Friedman y Thornber a principios de los años 50. Friedman introdujo el término bioisosterismo para describir el fenómeno observado entre sustancias relacionadas estructuralmente que presentaban propiedades biológicas similares o antagónicos.[227] Más tarde, Thornber propuso ampliar el término de bioisósteres, definiéndolos como subunidades, grupos, o moléculas que poseían propiedades fisicoquímicas de efectos biológicos similares.[228]

A lo largo de los años, se han identificado innumerables relaciones bioisostéricas en compuestos tanto de origen natural como sintético. En la naturaleza, se han identificado muchos ejemplos de isosterismo como una manera de ampliar la quimiodiversidad (Figura 36), siendo notables las relaciones bioisostéricas clásicas que se encuentran entre los aminoácidos esenciales serina y cisteína, o tirosina y cisteína; entre las bases de purina y pirimidina como citosina y uracilo, o adenina y guanina; o entre los ácidos salicílicos y antranílicos, que originaron dos importantes clases de fármacos antiinflamatorios no esteroideos como el ácido acetilsalicílico y los ácidos mefenámicos, respectivamente. Además, también podemos encontrar ejemplos en la naturaleza de la aplicación de bioisosterismo no clásico, tal y como la relación bioisostérica existente entre el ácido γ- aminobutírico (GABA) y el muscimol (Figura 36), o entre los neurotransmisores glutamato y AMPA.

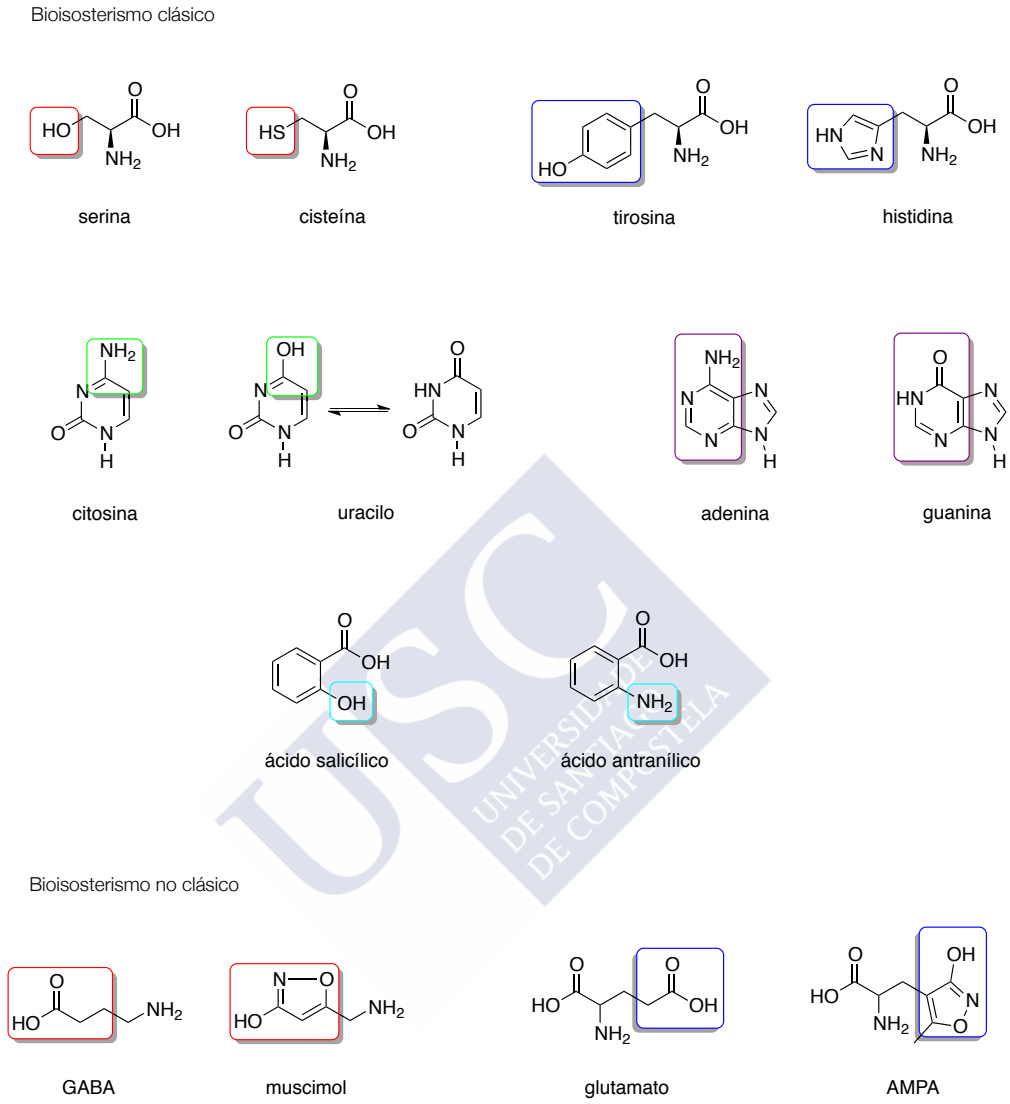

Figura 36. Ejemplos de bioisosterismo clásico y no clásico encontrados en compuestos naturales

Por tanto, el bioisosterismo es una estrategia empleada en química medicinal para el diseño racional de nuevos fármacos, aplicada sobre un compuesto líder (CL) como un proceso especial de modificación.[229] El éxito de esta estrategia para el desarrollo de nuevas sustancias que sean atractivas terapéuticamente ha experimentado un crecimiento significativo en distintas clases terapéuticas, siendo

ampliamente utilizada por la industria farmacéutica para descubrir nuevos análogos de innovaciones terapéuticas comercialmente atractivas, y también como una herramienta muy útil en la modificación molecular.

Existen innumerables razones para emplear el bioisosterismo para diseñar nuevos fármacos, incluyendo la necesidad de mejorar la actividad biológica , ganar selectividad frente a un determinado receptor o subtipo de isoforma enzimática, o incluso para optimizar la farmacocinética que un CL puede presentar.

6.2.1 Vinilogía.

El principio de vinilogía fue formulado por Claisen en 1926[230] al observar la similar acidez de la formil acetona y del ácido acético. Hoy día, la vinilogía es explicada por el efecto mesomérico que puede ser aplicado a otros sistemas conjugados tales como grupos imino y etinil y sistemas aromáticos.

Este principio tiene importantes aplicaciones en el diseño de fármacos si bien es de aplicación cuando la actividad farmacológica dependa de aspectos electrónicos y no esté determinada por la distancia entre los grupos sobre los que se aplica. Frecuentemente, los vinílogos originados son más sensibles a la degradación metabólica como ocurre con los vinílogos de la fenilbutazona[231] (Figura 37). Sin embargo, en otros casos, como el de la acetilcolina, la formación de su vinílogo origina un derivado menos sensible a la acción de la acetilcolinesterasa.[232]

Por su parte, la introducción de un anillo bencénico en la procainamida xantina origina análogos que conservan su actividad anestésica local de la misma.

Más relacionado con nuestro trabajo, debemos mencionar que la aplicación de la vinilogía a la chalcona con actividad inhibidora sobre la MAO-B, origina un biosóstero que conserva dicha actividad.[179]

Así pues y teniendo en cuenta que nuestro trabajo parte de tres farmacóforos: la estructura de chalcona, el anillo cumarínico (su análogo cíclico), y el resveratrol, reconocidos compuestos líder por todo lo expuesto, emplearemos como punto de partida la hibridación estructural cumarina-resveratrol, y posteriormente centraremos el trabajo en los híbridos cumarina-chalcona. Sobre los prototipos generados, haremos

uso de la bioisostería y vinilogía para el diseño y la síntesis algunos compuestos que se presentan en la presente Memoria.

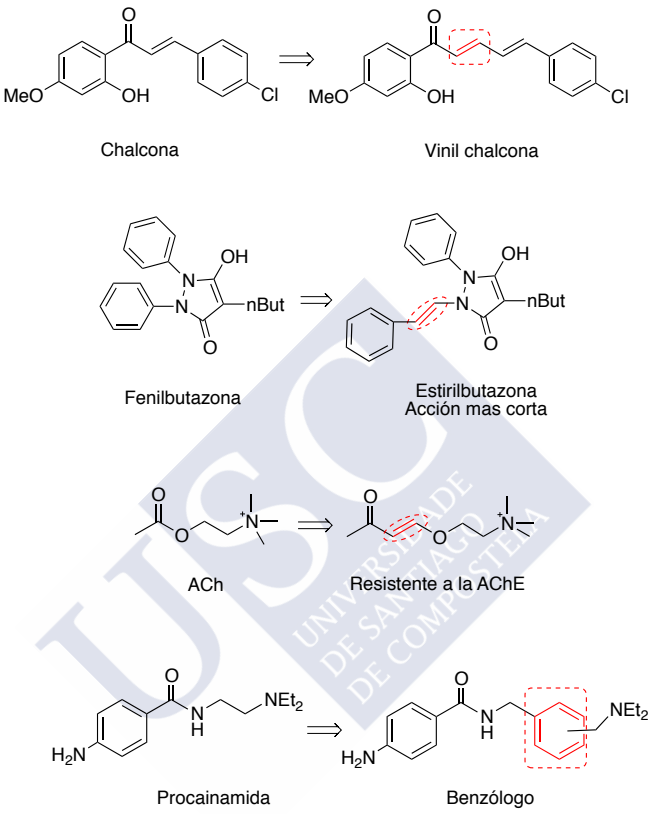

Figura 37. Principio de vinilogía aplicada al diseño de nuevos fármacos.

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1. Antecedentes

Desde hace tiempo, nuestro grupo de investigación ha estado trabajando en el campo de las cumarinas, no sólo buscando nuevas metodologías sintéticas e intentando optimizar sus efectos vasodilatadores y/o cardioprotectores, sino también con el fin de encontrar nuevas aplicaciones en el campo de la química médicinal. Por tanto, el estudio del esqueleto cumarínico[94,82,233,] y sus modificaciones estructurales dirigidas a diferentes actividades farmacológicas[234 , 235 , 236] ha sido una línea de investigación en la que hemos profundizado ampliamente, y en la que se han realizado revisiones recientes.[16,17]

En los últimos tiempos, nuestro grupo de investigación de ha centrado en la preparación de compuestos híbridos cumarina-resveratrol (3-arilcumarinas) en los cuales el anillo cumarínico comparte el anillo bencénico y el doble enlace de la pirona, con el resveratrol, de modo que el resveratrol queda fijado como isómero *trans* (Figura 38).[237] Los estudios de actividad de estos prototipos llevados a cabo por el grupo de Farmacología de la Facultad de Farmacia de la Universidad de Santiago de Compostela, con el que mantenemos una estrecha colaboración, han demostrado que presentan una muy buena actividad vasodilatadora e inhibidora de la agregación plaquetaria.[238]

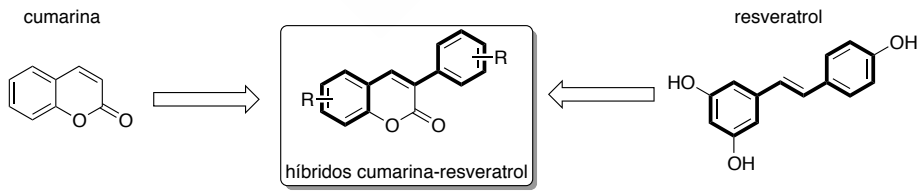

Figura 38. Compuestos híbtridos cumarina-resveratrol sintetizados en nuestro laboratorio.

Estudios más recientes han revelado además que estos prototipos muestran una gran actividad como inhibidores de monoamino oxidasa (MAO)[111,239] presentando además una elevada selectividad por la isoenzima MAO-B, lo que permite que sean

considerados como nuevos prototipos estructurales en el diseño de nuevos inhibidores enzimáticos. Además, la reciente obtención de una patente por parte de nuestro grupo de investigación [240] en la que se reivindica su utilidad en enfermedades neurodegenerativas tales como Parkinson o Alzheimer, corrobora una vez más el interés farmacológico de estos cabeza de serie.

Además de los resultados mencionados, nuestro grupo de investigación ha elaborado una base de datos con la actividad iMAO experimental de más de mil compuestos que ha permitido desarrollar un modelo QSAR[235, 236] capaz de predecir con alta eficacia la actividad iMAO de nuevas moléculas. Los estudios previos de relación estructura-actividad (SAR, por sus siglas en inglés) que se han llevado a cabo sobre las 3-arilcumarinas, han permitido concluir que la posición *para* sustituida resultó ser la más favorable para una buena actividad iMAO, pero sobre todo, resultó ser crucial para la selectividad sobre MAO-B, mientras que los orto derivados presentan una menor actividad que sus correspondientes isómeros *meta* y *para*.

Isósteros de cumarinas como pueden ser las cromonas y sus derivados flavonoides, han estado ampliamente asociados a propiedades antioxidantes. En la (Figura 39) se muestran las estructuras de quercetina y catequina, que nos han servido como inspiración para el diseño de nuevos compuestos y el estudio de propiedades antioxidantes.

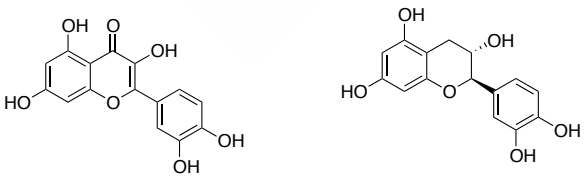

Figura 39. Antioxidantes asociados a estructuras flavonoides.

2. Objetivos

Tomando como punto de partida el descubrimiento de la actividad iMAO de las 3-arilcumarinas, y teniendo en cuenta los resultados obtenidos y la amplia experiencia de nuestro grupo de investigación en el campo de los análogos cumarínicos, inicialmente se planteó ampliar la serie de 3-arilcumarinas trabajando de forma conjunta con la Dra. Maria Matos, quien obtuvo resultados muy prometedores y que se recogen en su Tesis Doctoral.[241] Posteriormente, se planteó el diseño, la síntesis y el estudio de compuestos híbridos en los que se incluyese en lugar del resveratrol, otro compuesto de origen natural, una de las "estructuras privilegiadas" que Evans definió: las chalconas. De este modo, los esqueletos de cumarina y chalcona estarían incluidos en la misma estructura, de forma paralela a los híbridos cumarina-resveratrol (Figura 40).

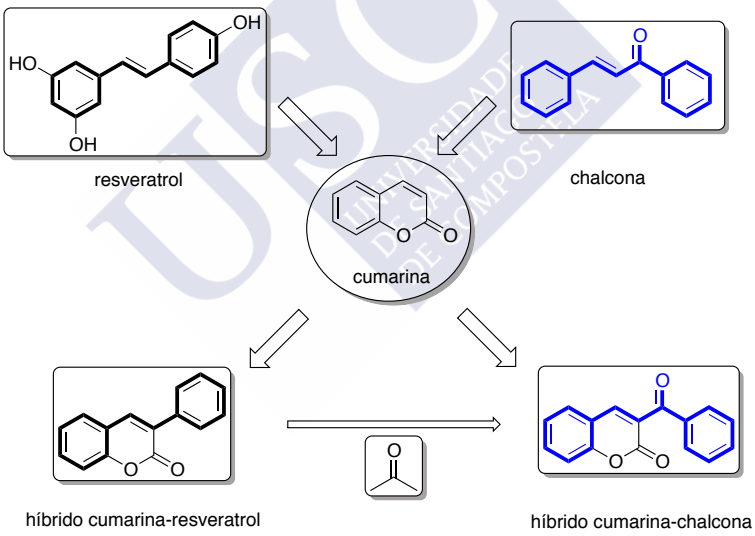

Figura 40. Diseño de híbridos cumarina-resveratrol y cumarina-chalcona

Aunque inicialmente se planteó el diseño y la síntesis de híbridos cumarina-chalcona de tipo 3-benzoilcumarina, la versatilidad estructural de ambos esqueletos de nos permite diseñar diversos tipos estructurales de híbridos. El desarrollo de esta Memoria se centrará en aquellos híbridos en los que realizando sustituciones en las

posiciones 3 y 8 del núcleo cumarínico podamos obtener las diferentes moléculas híbridas cumarina-chalcona y en las que aplicaremos el principio de vinilogía (Figura 41).

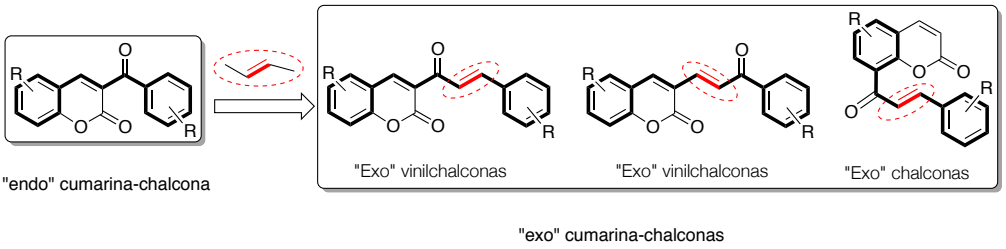

Figura 41. Prototipos estructurales de híbridos cumarina-chalcona diseñados.

Los derivados de la Figura 41 que denominamos "endo" cumarina-chalconas, contienen el doble enlace de la chalcona en la posición 3,4 del anillo cumarínico, quedando englobada dicha estructura en el mismo y fijando así la isomería *trans*. Por otro lado, la introducción del grupo "vinilico" a ambos lados del grupo carbonilo exocíclico origina los híbridos denominados "exo" cumarina-chalconas, que presentan un mayor grado de libertad tanto conformacional como de posible isomería *cis/trans*, ya que ambos farmacóforos comparten únicamente un anillo aromático, ya sea el de la pirona en el caso de las cumarinas 3-sustituídas, o bien un anillo bencénico en el caso de las cumarinas 8-sustituídas.

Se plantea el desarrollo de procedimientos sintéticos sencillos, directos, eficientes y versátiles que nos permitan acceder a este tipo de estructuras en la pureza y cantidad suficiente para realizar los ensayos farmacológicos frente a diferentes dianas. Además, estas rutas sintéticas deben permitirnos introducir sustituyentes de diferente naturaleza en distinto número y posición, de modo que se puedan suplir las múltiples combinaciones de variabilidad estérica, electrónica y/o lipofílica.

Inicialmente, se estudiará el efecto que pudiese presentar la introducción de un grupo carbonilo puente entre la cumarina y el grupo fenilo de las 3-fenilcumarinas (Figura 40), o híbridos cumarina-resveratrol, sobre la actividad farmacológica iMAO observada en los prototipos previos. Sin embargo, sería también un objetivo constante la búsqueda de nuevas dianas farmacológicas así como el análisis de propiedades con

posible aplicación terapéutica. De este modo, entre otros objetivos farmacológicos, se pensó en explorar actividades quimioterápicas de diferente tipo (antibacteriana y antiparasitaria), la interacción con receptores como los de adenosina y las propiedades antioxidantes, entre otras.

Además de la síntesis y el estudio de la actividad biológica de los compuestos sintetizados, o de series seleccionadas de los mismos, se intentará realizar un posterior diseño racional de nuevas series de compuestos, ayudándonos cuando fuese necesario, de herramientas computacionales como QSAR o modelización molecular.. De este modo, los objetivos anteriormente mencionados podrían resumirse en un proceso cíclico de síntesis, actividad biológica y diseño racional (Figura 42).

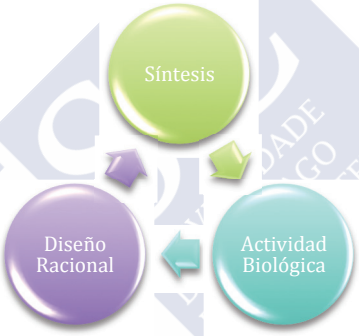

Figura 42. Esquema de trabajo empleado para el diseño racional, síntesis y estudio de los objetivos planteados.

Por último, y dado el interés del grupo en la síntesis de compuestos oxacíclicos inspirados en productos naturales, se planteó el objetivo de desarrollar nuevas metodologías sintéticas basadas en la reacción de ciclación de Nazarov para la obtención de compuestos derivados de productos naturales como la rocaglamida.

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1. Generalidades

En el trabajo que a continuación se presenta en esta Memoria se incluye la descripción de las metodologías sintéticas empleadas para la preparación de series de compuestos seleccionadas, su correspondiente purificación y caracterización estructural, la evaluación biológica frente a diferentes dianas farmacológicas y estudios de modelización molecular. Dado el carácter multidisciplinar que supone trabajar en el campo de la química farmacéutica, es importante mencionar que muchos estudios aquí presentados han sido fruto de incesantes e interesantes colaboraciones con diversos grupos de investigación tanto nacionales como internacionales.

En la Universidad de Santiago de Compostela, los estudios de iMAO han sido realizados por la Profesora Dolores Viña, y los ensayos de la actividad antibacteriana han sido realizados por las Profesoras Ysabel Santos y Ángeles Muñoz. En la Universidad de Santiago de Chile se han llevado a cabo los estudios de la actividad y propiedades antioxidantes a cargo del Profesor Claudio Olea-Azar, y los estudios de la actividad antitripanocida, por el grupo del profesor Juan Diego Maya. Los estudios de citotoxicidad y citoprotección han sido realizados por el grupo del Profesor Frederick Villamena, de la Ohio State University. En la Universidad de Wurzbürg se han realizado los estudios de interacción con receptores de adenosina, en colaboración con el grupo del Profesor Karl-Norbert Klotz. En la University of Hawai'i at Manoa en colaboración con el grupo del Profesor Marcus Tius se ha llevado a cabo el desarrollo y optimización de metodologías sintéticas empleando la reacción de Nazarov para la aplicación en la preparación de compuestos oxacíclicos basados en productos naturales como los derivados de la rocaglamida. Y por último, los estudios de *docking* han sido realizados por Giulio Ferino y Santiago Vilar. Sin todas estas colaboraciones, este trabajo no hubiese sido posible.

La versatilidad de las diferentes vías sintéticas nos ha permitido obtener amplias series de derivados, muchos de los cuales han sido posteriormente estudiados frente a diversas dianas farmacológicas y que nos han permitido establecer interesantes relaciones de estructura y actividad (SAR) y que nos permiten un diseño racional de nuevos fármacos basados en las estructuras de los receptores y en las estructuras de los ligandos de los mismos.

2. Estrategia Sintética

Para la consecución de los objetivos sintéticos anteriormente expuestos, ha sido necesario estudiar la metodología sintética que resultaría más apropiada para la obtención de los distintos tipos de esqueletos planteados. Estos procedimientos, tal y como es preceptivo en estos primeros estudios de diseño de nuevos prototipos, deben ser sencillos, directos, rápidos, eficientes y de gran versatilidad. Hemos buscado las rutas sintéticas más rápidas y cuyos reactivos, preferentemente de bajo coste, se adaptaban mejor a nuestras necesidades. Las reacciones más empleadas se describen brevemente a continuación, y la descripción se realizará en función del tipo de esqueleto híbrido a preparar.

Con el objetivo de disponer de una amplia quimioteca de compuestos que facilite la exploración de las relaciones estructura-actividad, así como el establecimiento de comparaciones con derivados afines previamente obtenidos en nuestro laboratorio, se han preparado series de compuestos en cuya estructura general, para el caso de los híbridos más ampliamente estudiados en esta memoria (híbridos cumarina-chalcona), tendremos tres puntos de variabilidad: el anillo bencénico de la cumarina, y los anillos aromáticos A y B de la chalcona.

2.1. Síntesis de 3-arilcumarinas.

Las 3-arilcumarinas, o híbridos cumarina-resveratrol, han sido las primeras estructuras que han sido objeto de nuestro estudio dada la prometedora actividad encontrada como inhibidores de la MAO. Se amplió la serie de 3-arilcumarinas que estaba siendo sintetizada por la Dra. Maria Matos, quien puso a punto las metodologías sintéticas desarrolladas. Las reacciones empleadas para la síntesis de los distintos derivados fueron la reacción de Perkin,[242,243] la reacción de Perkin modificada[244] y la reacción de Suzuki[245] (Figura 43) y se encuentran amplia y detalladamente descritas en la Tesis Doctoral de la Dra. Maria Matos.[241]

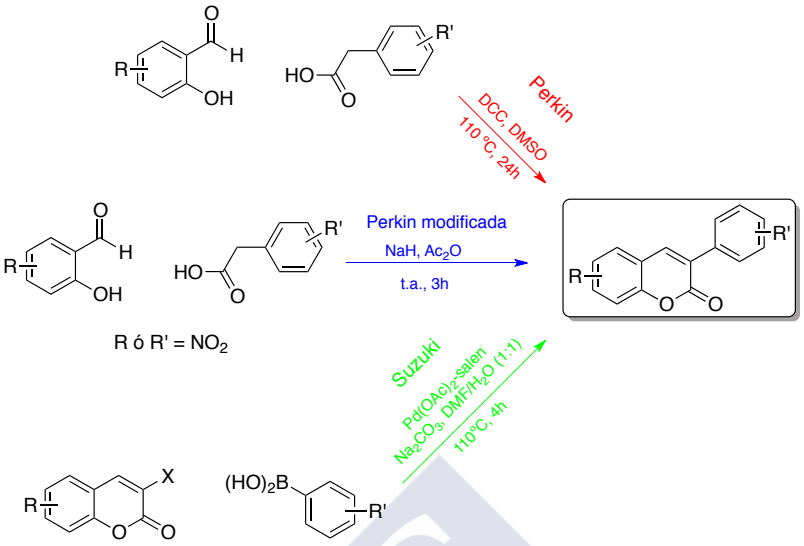


Figura 43. Rutas sintéticas empleadas para la síntesis de 3-arilcumarinas.

2.2. Síntesis de "endo" cumarina-chalconas.

Las "endo" cumarina-chalconas fueron las siguientes estructuras que han sido objeto de nuestro estudio dada su relación estructural con las 3-arilcumarinas previamente sintetizadas en nuestro laboratorio como ya se ha comentado en el apartado de antecedentes. En el diseño de estas estructuras, hemos tenido en cuenta que la sustitución sobre el anillo de cumarina se realizaría en la posición 3. Incorporaríamos un grupo benzoílico en dicha posición, de modo que en la estructura final, el anillo B de la chalcona correspondería al anillo bencénico del núcleo de cumarina, mientras que el anillo A se encontraría como grupo benzoilo en la posición 3. De este modo, la configuración *trans* de la chalcona quedaría fijada a través del enlace α,β-insaturado del anillo de pirona (Figura 44).

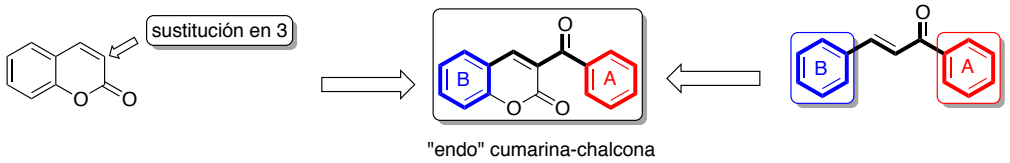


Figura 44. Diseño de híbridos 3-endo cumarina-chalcona.

2.2.1. Síntesis de 3-benzoilcumarinas.

Las 3-benzoilcumarinas han sido las primeras estructuras que nos hemos planteado diseñar. La etapa clave para la obtención de este tipo de compuestos tiene como base la reacción de Knoevenagel[44] entre un salicilaldehído y un β-cetoester aromático. La reacción puede llevarse a cabo en medio ácido o básico, pero en este caso las reacciones fueron llevadas a cabo en medio básico siguiendo la literatura.[246,91] Dada la sencillez y la versatilidad de esta reacción, hemos podido obtener una amplia familia de compuestos con rendimientos satisfactorios de entre el 42 y el 98%, siendo los derivados que contenían anillos heteroaromáticos aquellos que presentaron un menor rendimiento (Figura 45).

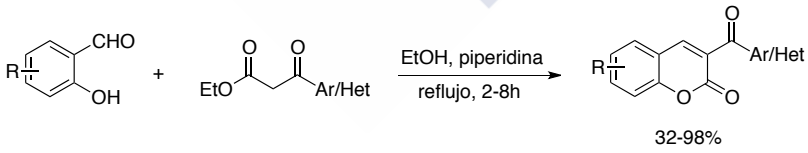


Figura 45. Esquema de reacción para la síntesis de 3-benzoilcumarinas.

Esta síntesis vía reacción de Knoevenagel ha sido llevada a cabo en etanol y en presencia de piperidina, dejando la reacción a reflujo de 2 a 8 horas, dependiendo del tipo de derivado sintetizado. Sólo en el caso de los derivados que contenían grupos hidroxilo y/o grupos amino, fue necesaria la síntesis de los precursores etoxi/metoxilados y/o con grupos nitro que fueron posteriormente derivatizados mediante procesos de hidrólisis y/o reducción. La purificación de todos los compuestos

finales se realizó bien mediante cromatografía en columna o bien mediante recristalización.

La síntesis de los derivados hidroxilados se llevó a cabo en condiciones drásticas, tratando los éteres precursores con BBr_3 en diclorometano (DCM) durante 48 horas a 80 ºC. En el caso de los derivados amino, los compuestos híbridos precursores con grupos nitro fueron tratados con cloruro de estaño dihidrato ($SnCl_2.2H_2O$) en etanol a reflujo durante 3-7 h (Figura 46).

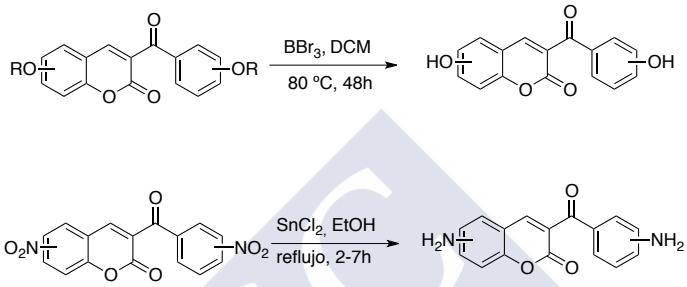


Figura 46. Síntesis de hidroxi/amino-3-benzoilcumarinas.

La caracterización de todos los compuestos obtenidos se ha realizado mediante resonancia magnética nuclear (NMR, por sus siglas en inglés) tanto de protón como de carbono, por espectrometría de masas, análisis elemental y puntos de fusión. La identificación estructural de este tipo de compuestos resulta bastante clara debido a las señales características del anillo cumarínico.

Se realizó también un estudio estructural mediante Rayos X de una cumarina seleccionada de esta serie de compuestos (compuesto **16**, Tabla 1). De este modo el análisis de este compuesto nos ayuda a contribuir a la elucidación de los requisitos estructurales necesarios para entender la planaridad parcial del núcleo de cumarina en este tipo de compuestos, y la torsión del grupo 3-benzoilo respecto a este núcleo.[247]

2.2.2. Síntesis de 3-benzoil-4-hidroxicumarinas.

La presencia en la posición 4 del anillo de la cumarina de un grupo hidroxilo hace que el planteamiento sintético varíe respecto de las 3-benzoilcumarinas no sustituidas en esta posición. Para la síntesis de este tipo de derivados, se siguió una ruta de dos etapas: una primera etapa en la que generamos el éster aromático en la posición 4, y una segunda etapa en la que, mediante una transposición de Fries, dejamos el hidroxilo libre en la posición 4 y el grupo benzoilo se transpone a la posición 3 de la cumarina (Figura 47)

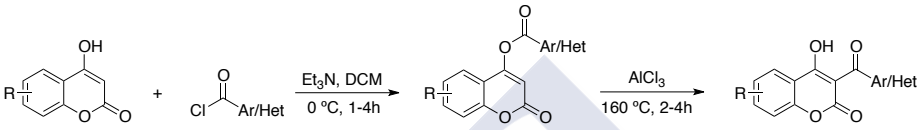


Figura 47. Síntesis de 3-benzoil-4-hidroxicumarinas.

Para la síntesis del éster, se trata la 4-hidroxicumarina propiamente sustituida con el correspondiente cloruro de ácido en diclorometano a 0 ºC en presencia de trietilamina (Et_3N) durante un periodo de entre 1 y 4 horas.[248] La reacción transcurre en la mayoría de los casos con rendimientos casi cuantitativos. En la segunda etapa, el éster obtenido se trata con tricloruro de aluminio ($AlCl_3$), preparando una mezcla triturada de ambos reactivos, y la reacción transcurre a 160 ºC durante un tiempo de entre 2 y 4 horas en ausencia de disolvente.[249] Los rendimientos globales para la obtención de los compuestos finales, teniendo en cuenta ambas etapas, oscilan entre un 42 y un 93%.

2.3. Síntesis de "exo" cumarina-chalconas".

El siguiente objetivo sintético que nos planteamos fue la preparación de híbridos cumarina-chalcona "exo", que como ya se ha comentado anteriormente en el apartado de objetivos, suponía otorgar una mayor grado de libertar conformacional a la molécula. Estudiamos las posiciones 3 y 8 de la cumarina para la incorporación del núcleo de chalcona. De este modo, nos planteamos preparar 3 tipos de derivados "exo": una clase de 8-exo(cumarina-chalconas), y dos clases de 3-exo(cumarina-chalconas) (Figura 48).

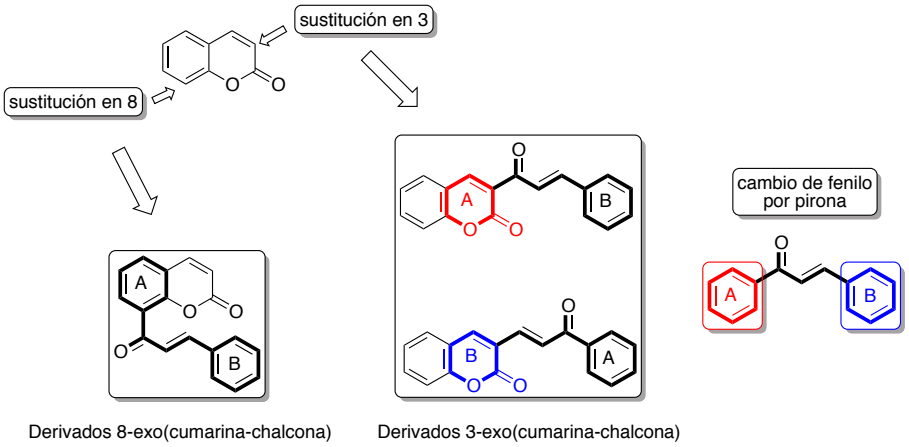


Figura 48. Diseño de híbridos cumarina-chalcona tipo "exo".

Se estudió primeramente la sustitución en la posición 3 del esqueleto cumarínico en la que realizaríamos la sustitución bien del anillo benzílico A, o bien del anillo benzílico B de la chalcona, por el anillo de pirona de la cumarina. Posteriores modificaciones llevarían a la sustitución bioisostérica de ambos anillos benzílicos por anillos heteroaromáticos. En el caso de las 8-exo(cumarina-chalconas), se conservaría la estructura clásica de chalcona, y sólo se harían modificaciones en el anillo B de la chalcona por anillos heteroaromáticos en casos puntuales.

Cabe destacar que en prácticamente la totalidad de los derivados preparados, los isómeros obtenidos para este tipo de compuestos híbridos han sido los isómeros *trans*.

2.3.1. Síntesis de 3-cinamoilcumarinas.

La síntesis de 3-cinamoilcumarinas se ha llevado a cabo siguiendo una ruta en dos etapas en la que el primer paso conlleva la preparación de 3-acetilcumarinas propiamente sustituidas, seguida de la síntesis de los compuestos finales mediante una reacción de Claisen-Schmidt como se muestra en la Figura 49.

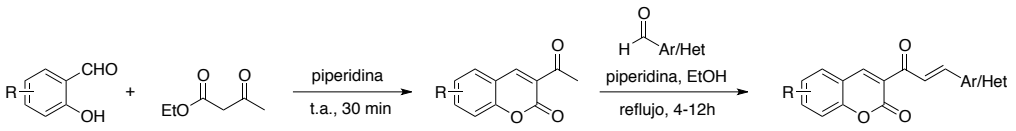


Figura 49. Síntesis de derivados 3-cinamoilcumarina.

Para la síntesis de las 3-acetilcumarinas sustituidas se empleó la reacción de Knoevenagel en la que se trataron el correspondiente salicilaldehído propiamente sustituido y el acetoacetato de etilo con piperidina a temperatura ambiente durante 30 minutos en ausencia de disolvente.[59] Se obtuvieton así las 3-acetilcumarinas con excelentes rendimientos (entre 78 y 94%). A continuación, las 3-acetilcumarinas se mezclaron con los benzaldehídos apropiados y se trataron con piperidina en etanol a reflujo durante 4-12 horas,[250] lo que dio lugar a las 3-cinamoilcumarinas deseadas con rendimientos (entre 58 y 91%).

2.3.2. Síntesis de 3-cinamoil-4-hidroxicumarinas.

Al igual que en el caso de las 3-benzoil-4-hidroxicumarinas, cuando se planteó la síntesis de las 3-cinamoil-4-hidroxicumarinas, hubo que recurrir a una ruta sintética alternativa respecto de las 3-cinamoilcumarinas. Esta ruta también constaría de dos etapas. Una primera etapa en la que en lugar de preparar mediante reacción de Knoevenagel las 3-acetilcumarinas, empleamos 4-hidroxicumarinas sustituidas que darían lugar a las correspondientes 3-acetil-4-hidroxicumarinas, y una segunda etapa en la que sobre las 3-acetil-4-hidroxicumarinas emplearíamos la misma reacción de Claisen-Schmidt que la utilizada en la segunda etapa de la síntesis de 3-cinamoilcumarinas (Figura 50).

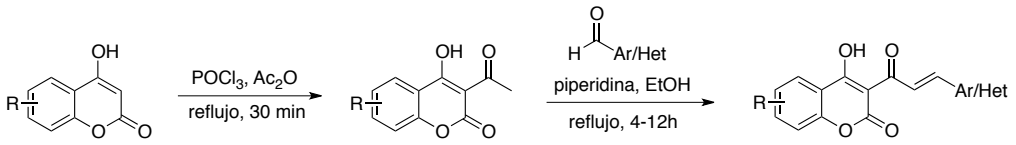


Figura 50. Ruta sintética para la preparación de 3-cinamoil-4-hidroxicumarinas

Para preparar las 3-acetil-4-hidroxicumarinas se trataron las 4-hidroxicumarinas sustituidas con oxicloruro de fósforo ($POCl_3$) en ácido acético glacial (Ac_2O) a reflujo durante 30 minutos,[251] con rendimientos del 72 al 93%. La última etapa del proceso sintético para este tipo de estructuras correspondió a la condensación de Claisen-Schmidt mencionada previamente en la síntesis de 3-cinamoilcumarinas, con rendimientos que oscilan entre 52 y 97 %.

2.3.3. Síntesis de 3-(3-aril-3-oxo-propernil)-4-clorocumarinas.

Para la preparación de 3-(3-aril-3-oxo-propenil)-4-clorocumarinas se ha empleado la reacción de Wittig, ampliamente utilizada para la síntesis de alquenos o compuestos α,β-insaturados, y en la que reaccionan un aldehído o una cetona, con un iluro de fósforo. La síntesis de los compuestos finales consta de dos etapas: una primera etapa en la que se prepara el iluro de fósforo a partir de una α-haloarilcetona, y una segunda etapa en la que se produce el acoplamiento entre el iluro de fósforo y la 3-formilcumarina para formar el oxafosfetano intermedio que se rompe liberando el alqueno final (Figura 51).

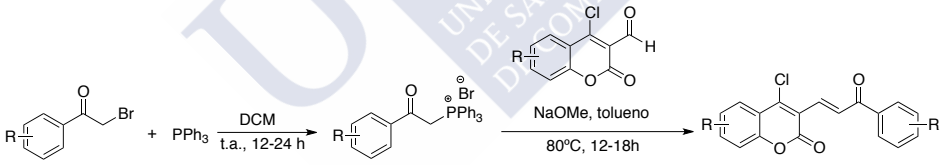


Figura 51. Síntesis de 3-(3-aril-3-oxo-propenil)-4-clorocumarinas.

Para sintetizar el iluro precursor, se disolvieron la α-haloarilcetona y la trifenilfosfina (PPh_3) en diclorometano y la reacción se dejó bajo agitación en atmósfera de argón entre 12 y 24 horas.[252] Tras la elaboración y purificación, se obtuvieron los iluros con rendimientos cuantitativos. A continuación, el iluro obtenido se mezcló con la 3-formil-4-clorocumarina propiamente sustituida en presencia de metóxido sódico (NaOMe) en tolueno, y la mezcla resultante se dejó a 80 ºC durante 12-18 horas.[253] El compuesto final fue aislado y purificado con rendimientos de entre el 61 y el 83%.

2.3.4. Síntesis de 8-cinamoilcumarinas.

Se decidió ampliar la versatilidad estructural del núcleo de cumarina a la sustitución en la posición 8 con el fin de introducir la estructura de chalcona en esta posición, de modo que el anillo A de la chalcona permanecería integrado dentro del esqueleto cumarínico, ya que se compartiría el anillo bencénico de dicha estructura, mientras que el anillo B del esqueleto de chalcona permanecería como anillo bencénico o bien como un anillo heteroaromático.

Preparamos inicialmente el precursor 8-acetil-7-hidroxicumarina en dos pasos, partiendo de la umbeliferona (7-hidroxicumarina). En el primer paso realizaríamos la acetilación del grupo hidroxilo en la posición 7, y a continuación, mediante una transposición de Fries, obtendríamos el precursor 8-acetil-7-hidroxicumarina. La cumarina obtenida se hizo reaccionar con el aril o heteroaril-aldehído correspondiente y mediante una reacción de condensación de Claisen-Schmidt dio lugar a la 8-cinamoilcumarina deseada (Figura 52).

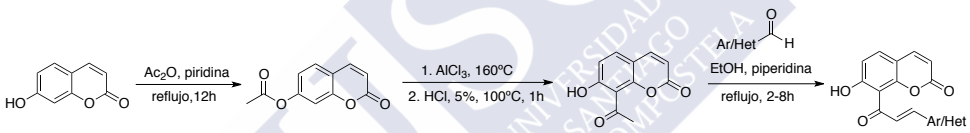


Figura 52. Síntesis de 8-cinamoilcumarinas

La síntesis de la 7-acetoxicumarina se realizó disolviendo la umbeliferona en anhídrido acético y tratando la mezcla de reacción con una cantidad catalítica de piridina a reflujo durante 12 horas. El precipitado obtenido se trató posteriormente con tricloruro de aluminio anhidro a 160 ºC durante 2 horas, y tras dejar enfriar la reacción, se agregó ácido clorhídrico al 5%, dejando la mezcla de reacción durante 1 hora a 100 ºC. El compuesto final, la 8-acetil-7-hidroxicumarina, que utilizaríamos como precursor, se purificó mediante cromatografía en columna con un rendimiento global de 85%.[249] Obtenida la cumarina acetilada en la posición 8, ésta se mezcló con el aril/heteroaril aldehído apropiado, se disolvieron en etanol y se trataron con piperidina a reflujo entre 2 y 8 horas. [254] Tras su purificación mediante cromatografía en columna y/o recristalización, se obtuvieron las 8-cinamoilcumarinas deseadas con unos rendimientos de entre 58 y 96%.

2.4. Optimización de la reacción de Nazarov: aplicaciones en la síntesis de productos naturales oxacíclicos.

Como se ha mencionado en la introducción, la reacción de ciclación de Nazarov supone una excelente herramienta sintética para la síntesis total de productos naturales oxacíclicos tales como la rocaglamida. Sin embargo, la formación de átomos de carbono cuaternarios adyacentes durante la ciclación de Nazarov está limitada a un número muy pequeño de ejemplos. La investigación para el desarrollo de una metodología sintética que nos permitiese generalizar este proceso ha sido objeto de nuestro estudio.

Para ello, se desarrolló y se puso a punto una metodología sintética que nos permitiese obtener primeramente los sustratos dienona tetrasustituídos, y a continuación, se optimizaron las condiciones de reacción que nos permitieron llevar a cabo la reacción de ciclación de Nazarov diastereoespecífica. Los resultados que se obtuvieron fueron muy sorprendentes y prometedores, ya que nos permitía expandir el ámbito de la reacción de Nazarov para la obtención de ciclopentenonas altamente sustutuidas.[255] En base a los resultados obtenidos, parece que la reacción de ciclación de Nazarov nos permite generar centros vecinales con todos los átomos de carbono cuaternarios con éxito y de forma diastereoespecífica empleando unos parámetros de reacción cuidadosamente definidos.

De forma resumida, la metodología sintética que se desarrolló consta de cuatro etapas fundamentales: 1) síntesis de ácidos carboxílicos α,β-insaturados completamente sustituídos; 2) síntesis de (((trimetilsilil)etoxi)metoxi)ésteres; 3) síntesis de los sustratos dienona; 4) síntesis de productos de la ciclación de Nazarov diastereoespecífica (Figura 53).

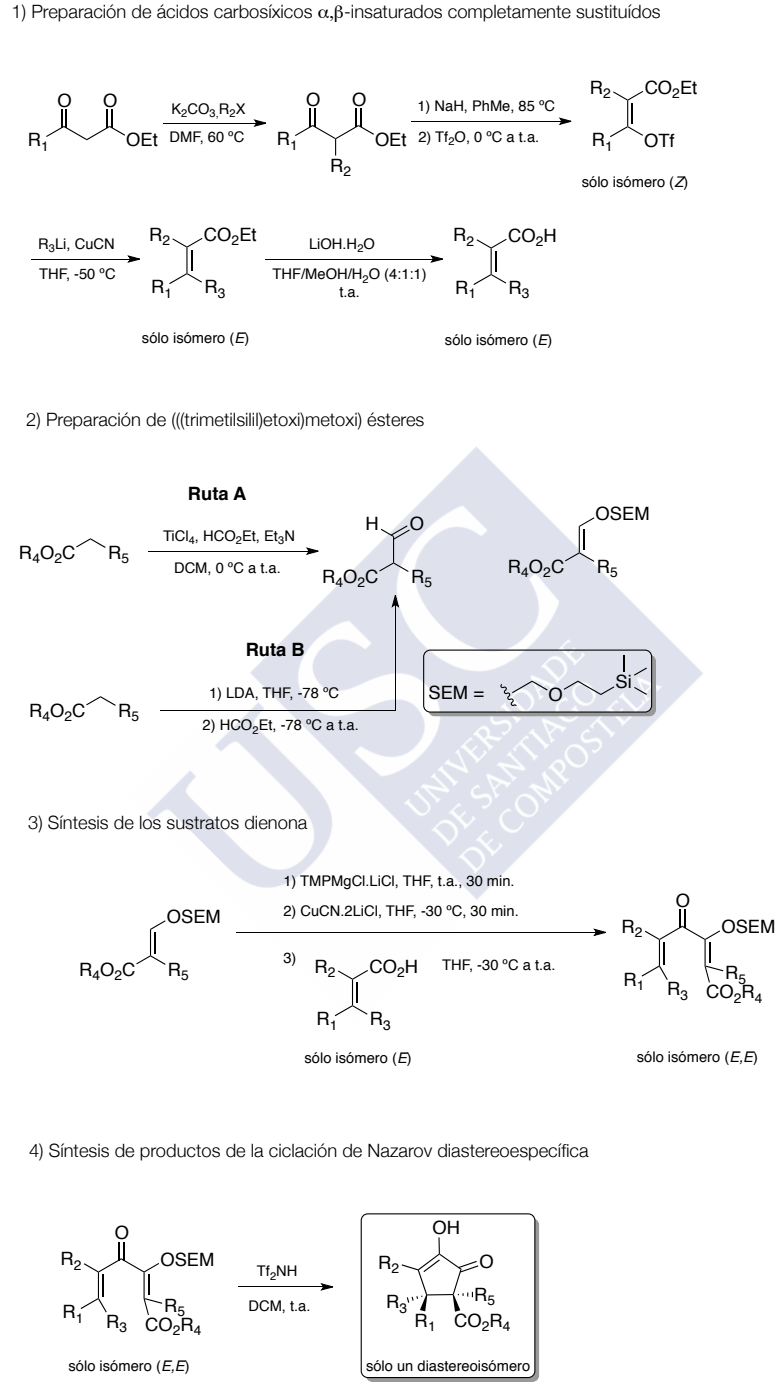


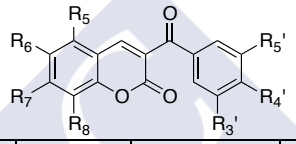
Figura 53. Metodología sintética para ciclación de Nazarov diastereoespecífica.

2.5. Series de compuestos sintetizados

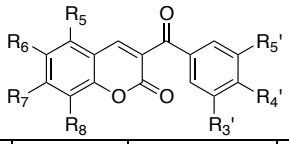
De las series de compuestos anteriormente descritas, se ha preparado una amplia quimioteca que se encuentra resumida en forma de tablas.

Las tablas se han dividido de acuerdo a los tipos de esqueletos sintetizados según las diferentes metodologías (secciones 2.2 y 2.3): 3-benzoilcumarinas (Tabla 1), 3-benzoil-4-hidroxicumarinas (Tabla 2), 3-cinamoilcumarinas (Tabla 3), 3-cinamoill-4-hidroxicumarinas (Tabla 4), 3-(3-aril-3-oxo-propenil)-4-clorocumarinas (Tabla 5) y 8-cinamoil cumarinas (Tabla 6).

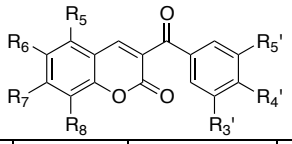
Tabla 1. 3-benzoilcumainas.



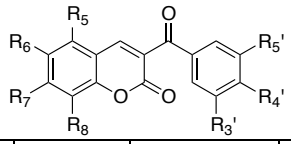
Compuesto	R_5	R_6	R_7	R_8	$R_{3'}$	R_4'	R_5'
1	H	H	H	H	H	H	H
2	H	OCH_3	H	H	H	H	H
3	H	Br	H	H	H	H	H
4	H	H	H	OCH_3	H	H	H
5	H	H	H	OCH_2CH_3	H	H	H
6	H	Br	H	OCH_3	H	H	H
7	H	CH_3	H	H	H	H	H
8	H	H	H	CH_3	H	H	H
9	H	NO_2	H	H	H	H	H
10	OCH_3	H	OCH_3	H	H	H	H
11	H	Cl	H	H	H	H	H



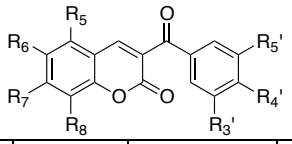
Compuesto	R_5	R_6	R_7	R_8	$R_{3'}$	R_4'	R_5'
12	H	H	OCH_3	H	H	H	H
13	H	H	H	H	H	OCH_3	H
14	H	OCH_3	H	H	H	OCH_3	H
15	H	Br	H	H	H	OCH_3	H
16	H	NO_2	H	H	H	OCH_3	H
17	H	H	H	CH_3	H	OCH_3	H
18	H	Br	H	OCH_3	H	OCH_3	H
19	OCH_3	H	OCH_3	H	H	OCH_3	H
20	H	CH_3	H	H	H	OCH_3	H
21	H	H	H	OCH_2CH_3	H	OCH_3	H
22	H	H	H	OCH_3	H	OCH_3	H
23	H	Cl	H	H	H	OCH_3	H
24	H	H	OCH_3	H	H	OCH_3	H
25	H	H	H	H	OCH_3	OCH_3	H
26	H	OCH_3	H	H	OCH_3	OCH_3	H
27	H	Br	H	H	OCH_3	OCH_3	H
28	H	H	H	OCH_3	OCH_3	OCH_3	H



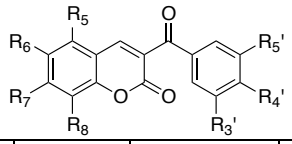
Compuesto	R_5	R_6	R_7	R_8	$R_{3'}$	R_4'	R_5'
29	H	Br	H	OCH_3	OCH_3	OCH_3	H
30	OCH_3	H	OCH_3	H	OCH_3	OCH_3	H
31	H	CH_3	H	H	OCH_3	OCH_3	H
32	H	H	H	CH_3	OCH_3	OCH_3	H
33	H	NO_2	H	H	OCH_3	OCH_3	H
34	H	H	H	OCH_2CH_3	OCH_3	OCH_3	H
35	H	Cl	H	H	OCH_3	OCH_3	H
36	H	H	OCH_3	H	OCH_3	OCH_3	H
37	H	H	H	H	H	NO_2	H
38	H	OCH_3	H	H	H	NO_2	H
39	H	Br	H	H	H	NO_2	H
40	H	H	H	OCH_3	H	NO_2	H
41	H	H	H	OCH_2CH_3	H	NO_2	H
42	H	Br	H	OCH_3	H	NO_2	H
43	H	CH_3	H	H	H	NO_2	H
44	H	H	H	CH_3	H	NO_2	H
45	H	NO_2	H	H	H	NO_2	H



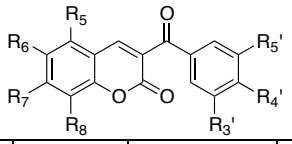
Compuesto	R_5	R_6	R_7	R_8	$R_{3'}$	R_4'	R_5'
46	OCH_3	H	OCH_3	H	H	NO_2	H
47	H	Cl	H	H	H	NO_2	H
48	H	H	OCH_3	H	H	NO_2	H
49	H	OCH_3	H	H	H	Cl	H
50	H	Br	H	H	H	Cl	H
51	H	H	H	OCH_3	H	Cl	H
52	H	Br	H	OCH_3	H	Cl	H
53	H	CH_3	H	H	H	Cl	H
54	H	NO_2	H	H	H	Cl	H
55	OCH_3	H	OCH_3	H	H	Cl	H
56	H	Cl	H	H	H	Cl	H
57	H	H	H	H	H	CH_3	H
58	H	OCH_3	H	H	H	CH_3	H
59	H	Br	H	H	H	CH_3	H
60	H	NO_2	H	H	H	CH_3	H
61	OCH_3	H	OCH_3	H	H	CH_3	H
62	H	H	OCH_3	H	H	CH_3	H



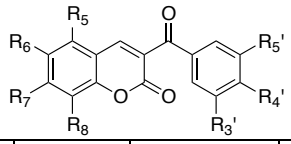
Compuesto	R_5	R_6	R_7	R_8	$R_{3'}$	R_4'	R_5'
63	H	H	H	H	OCH_3	OCH_3	OCH_3
64	H	OCH_3	H	H	OCH_3	OCH_3	OCH_3
65	H	Br	H	H	OCH_3	OCH_3	OCH_3
66	H	H	H	OCH_3	OCH_3	OCH_3	OCH_3
67	H	H	H	OCH_2CH_3	OCH_3	OCH_3	OCH_3
68	H	Br	H	OCH_3	OCH_3	OCH_3	OCH_3
69	H	CH_3	H	H	OCH_3	OCH_3	OCH_3
70	H	H	H	CH_3	OCH_3	OCH_3	OCH_3
71	H	NO_2	H	H	OCH_3	OCH_3	OCH_3
72	OCH_3	H	OCH_3	H	OCH_3	OCH_3	OCH_3
73	H	Cl	H	H	OCH_3	OCH_3	OCH_3
74	H	H	OCH_3	H	OCH_3	OCH_3	OCH_3
75	H	H	H	H	NO_2	H	H
76	H	OCH_3	H	H	NO_2	H	H
77	H	Br	H	H	NO_2	H	H
78	H	H	H	OCH_3	NO_2	H	H
79	H	H	H	OCH_2CH_3	NO_2	H	H



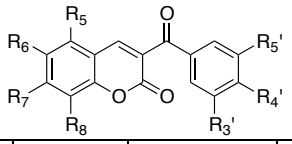
Compuesto	R_5	R_6	R_7	R_8	$R_{3'}$	R_4'	R_5'
80	H	Br	H	OCH_3	NO_2	H	H
81	H	CH_3	H	H	NO_2	H	H
82	H	H	H	CH_3	NO_2	H	H
83	H	NO_2	H	H	NO_2	H	H
84	OCH_3	H	OCH_3	H	NO_2	H	H
85	H	Cl	H	H	NO_2	H	H
86	H	H	OCH_3	H	NO_2	H	H
87	H	OH	H	H	H	H	H
88	H	Br	H	OH	H	H	H
89	H	H	H	OH	H	H	H
90	OH	H	OH	H	H	H	H
91	H	H	OH	H	H	H	H
92	H	H	H	H	H	OH	H
93	H	OH	H	H	H	OH	H
94	H	Br	H	H	H	OH	H
95	H	H	H	CH_3	H	OH	H
96	H	Br	H	OH	H	OH	H



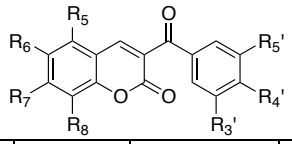
Compuesto	R_5	R_6	R_7	R_8	$R_{3'}$	R_4'	R_5'
97	OH	H	OH	H	H	OH	H
98	H	CH_3	H	H	H	OH	H
99	H	H	H	OH	H	OH	H
100	H	H	OH	H	H	OH	H
101	H	H	H	H	OH	OH	H
102	H	OH	H	H	OH	OH	H
103	H	Br	H	H	OH	OH	H
104	H	CH_3	H	H	OH	OH	H
105	H	H	H	OH	OH	OH	H
106	H	Br	H	OH	OH	OH	H
107	OH	H	OH	H	OH	OH	H
108	H	H	H	CH_3	OH	OH	H
109	H	H	OH	H	OH	OH	H
110	H	H	H	H	OH	OH	OH
111	H	OH	H	H	OH	OH	OH
112	H	Br	H	H	OH	OH	OH
113	H	CH_3	H	H	OH	OH	OH



Compuesto	R_5	R_6	R_7	R_8	$R_{3'}$	R_4'	R_5'
114	H	Cl	H	H	OH	OH	OH
115	H	Br	H	OH	OH	OH	OH
116	H	H	H	OH	OH	OH	OH
117	H	H	H	CH_3	OH	OH	OH
118	OH	H	OH	H	OH	OH	OH
119	H	H	OH	H	OH	OH	OH
120	H	OH	H	H	H	CH_3	H
121	H	H	OH	H	H	CH_3	H
122	OH	H	OH	H	H	CH_3	H
123	H	H	H	H	H	NH_2	H
124	H	Br	H	H	H	NH_2	H
125	H	OCH_3	H	H	H	NH_2	H
126	H	CH_3	H	H	H	NH_2	H
127	H	NH_2	H	H	H	NH_2	H
128	H	Cl	H	H	H	NH_2	H
129	H	H	H	CH_3	H	NH_2	H
130	H	H	H	OCH_2CH_3	H	NH_2	H

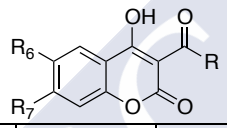


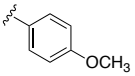
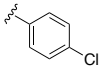
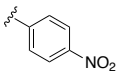
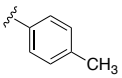
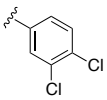
Compuesto	R_5	R_6	R_7	R_8	$R_{3'}$	R_4'	R_5'
131	H	H	H	OCH_3	H	NH_2	H
132	H	Br	H	OCH_3	H	NH_2	H
133	H	H	OCH_3	H	H	NH_2	H
134	OCH_3	H	OCH_3	H	H	NH_2	H
135	H	H	H	H	NH_2	H	H
136	H	Br	H	H	NH_2	H	H
137	H	OCH_3	H	H	NH_2	H	H
138	H	CH_3	H	H	NH_2	H	H
139	H	Cl	H	H	NH_2	H	H
140	H	H	H	CH_3	NH_2	H	H
141	H	H	H	OCH_2CH_3	NH_2	H	H
142	H	H	H	OCH_3	NH_2	H	H
143	H	Br	H	OCH_3	NH_2	H	H
144	H	H	OCH_3	H	NH_2	H	H
145	OCH_3	H	OCH_3	H	NH_2	H	H
146	H	NH_2	H	H	H	H	H
147	H	NH_2	H	H	H	OCH_3	H

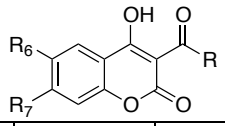


Compuesto	R_5	R_6	R_7	R_8	$R_{3'}$	R_4'	R_5'
148	H	NH_2	H	H	OCH_3	OCH_3	H

Tabla 2. 3-benzoil-4-hidroxicumarinas.

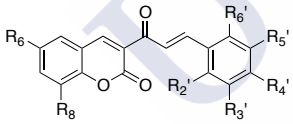


Compuesto	R_6	R_7	R
149	H	H	
150	H	H	
151	H	H	
152	H	H	
153	H	H	
154	H	H	
155	H	H	

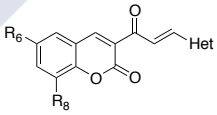


Compuesto	R_6	R_7	R
156	H	H	
157	CH_3		
158	CH_3		
159	CH_3	CH_3	

Tabla 3. 3-cinamoilcumarinas y análogos.

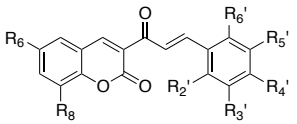


160-183

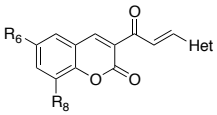


184-186

Compuesto	R_6	R_8	R_2'	R_3'	R_4'	R_5'	R_6'
160	H	H	H	H	H	H	H
161	H	H	H	H	OCH_3	H	H
162	H	H	H	H	Cl	H	H
163	H	H	H	H	CH_3	H	H
164	H	H	OCH_3	H	OCH_3	H	H
165	H	H	OCH_3	H	OCH_3	OCH_3	H

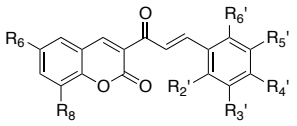


160-183

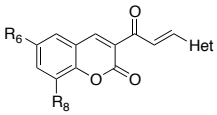


184-186

Compuesto	R_6	R_8	R_2'	R_3'	R_4'	R_5'	R_6'
166	H	H	OCH_3	H	OCH_3	H	OCH_3
167	H	H	OCH_3	H	NO_2	H	H
168	H	H	H	NO_2	H	H	H
169	H	H	H	OCH_3	OCH_3	H	H
170	H	H	OCH_3	OCH_3	H	H	H
171	H	H	OCH_3	H	H	OCH_3	H
172	Cl	H	H	H	OCH_3	H	H
173	Cl	H	H	H	CH_3	H	H
174	Cl	H	OCH_3	H	OCH_3	H	H
175	Cl	H	OCH_3	H	OCH_3	OCH_3	H
176	Cl	H	OCH_3	H	OCH_3	H	OCH_3
177	Cl	H	OCH_3	H	H	OCH_3	H
178	Cl	H	H	OCH_3	H	OCH_3	H
179	H	OCH_3	H	H	OCH_3	H	H
180	H	OCH_3	OCH_3	H	OCH_3	H	H
181	H	OCH_3	OCH_3	H	OCH_3	OCH_3	H



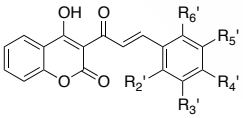
160-183



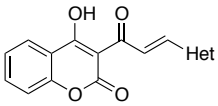
184-186

Compuesto	R_6	R_8	R_2'	R_3'	R_4'	R_5'	R_6'
182	H	OCH_3	OCH_3	H	H	OCH_3	H
183	H	OCH_3	H	OCH_3	H	OCH_3	H
184	H	H					
185	H	H					
186	Cl	H					

Tabla 4. 3-cinamoil-4-hidroxicumarinas y derivados.

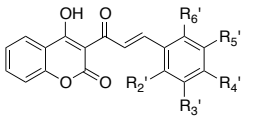
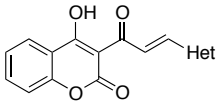


187-202



203-206

Compuesto	R_2'	R_3'	R_4'	R_5'	R_6'
187	H	H	H	H	H
188	H	H	OCH_3	H	H
189	H	H	Cl	H	H
190	H	H	CH_3	H	H

 187-202			 203-206		
Compuesto	R_2'	R_3'	R_4'	R_5'	R_6'
191	OCH_3	H	OCH_3	H	H
192	OCH_3	H	OCH_3	OCH_3	H
193	OCH_3	H	OCH_3	H	OCH_3
194	H	OCH_3	OCH_3	H	H
195	OCH_3	H	NO_2	H	H
196	OCH_3	H	H	OCH_3	H
197	OCH_3	OCH_3	H	H	H
198	Cl	H	H	NO_2	H
199	OCH_3	H	H	H	H
200	H	OCH_3	H	H	H
201	H	NO_2	H	H	H
202	H	OCH_3	H	OCH_3	H
203					
204					

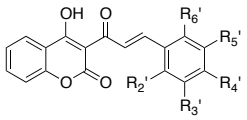
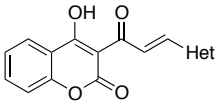
 187-202	 203-206				
Compuesto	R_2'	R_3'	R_4'	R_5'	R_6'
205					
206					

Tabla 5. 3-(3-aril-3-oxo-propenil)-4-clorocumarinas.

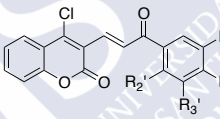
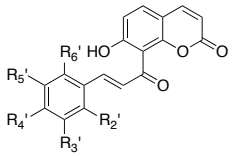
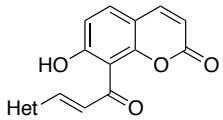
				
Compuesto	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$
207	H	H	H	H
208	H	H	CH_3	H
209	H	H	OCH_3	H
210	H	H	NO_2	H
211	H	OCH_3	H	H
212	H	NO_2	H	H
213	OCH_3	H	H	OCH_3

Tabla 6. 8-cinamoilcumarinas y derivados.

 214-223	 224

Compuesto	R_2'	R_3'	R_4'	R_5'	R_6'
214	H	H	OCH_3	H	H
215	OCH_3	H	OCH_3	H	H
216	OCH_3	H	OCH_3	OCH_3	H
217	OCH_3	H	OCH_3	H	OCH_3
218	H	OCH_3	OCH_3	H	H
219	OCH_3	H	NO_2	H	H
220	OCH_3	H	H	OCH_3	H
221	H	NO_2	H	H	H
222	OCH_3	OCH_3	H	H	H
223	H	OCH_3	H	OCH_3	H
224					

3. Estudios biológicos

Dado el elevado potencial de las moléculas sintetizadas sobre diferentes dianas farmacológicas, en esta Memoria se presenta de forma resumida los distintos tipos de ensayos biológicos llevados a cabo, así como la relación estructura-actividad (SAR) de dichas series de compuestos. La descripción completa de dichos ensayos, al igual que en el caso de la descripción sintética, se encontrará explícitamente detallada en los artículos publicados que se adjuntan en esta Memoria.

3.1. Estudio como inhibidores de MAO.

En base a los antecedentes de nuestro grupo de investigación con híbridos cumarina-resveratrol (3-arilcumarinas) como inhibidores MAO[256,239] se establecieron importantes conclusiones de la relación estructura-actividad de dichos compuestos, encontrándose que los compuestos descritos eran potentes inhibidores selectivos de la MAO-B[259,111] y que de todas las posiciones estudiadas, la introducción de grupos poco voluminosos (metilo, bromo o metoxilo) tanto en posición 6 y/o 8 como en las posiciones 3' y/o 4' del núcleo de la 3-arilcumarina eran muy favorables.[257]

Dada la estrecha relación estructural de los híbridos "endo" cumarina-chalcona, como se comentó en el apartado de antecedentes y objetivos, la primera diana farmacológica a estudiar frente a este tipo de compuestos fue la MAO. Según estos estudios, apoyados por estudios de *docking* (Figuras 54 y 55) se encontró que el hecho de introducir un grupo carbonílico entre el grupo arilo y el núcleo de cumarina hacía que la actividad inhibidora MAO frente a la isoforma B disminuyese. Sin embargo, para la serie estudiada, la afinidad por el receptor MAO-A aumentaba.[258] Por tanto observamos que un pequeño cambio como es la introducción de un grupo carbonilo, causaba un gran cambio en la afinidad y selectividad de las moléculas para este receptor. Un estudio continuado sobre este receptor y un amplio análisis bibliográfico nos permitió establecer qué tipo de derivados cumarínicos podrían ser más interesantes como inhibidores de MAO.[259,260]

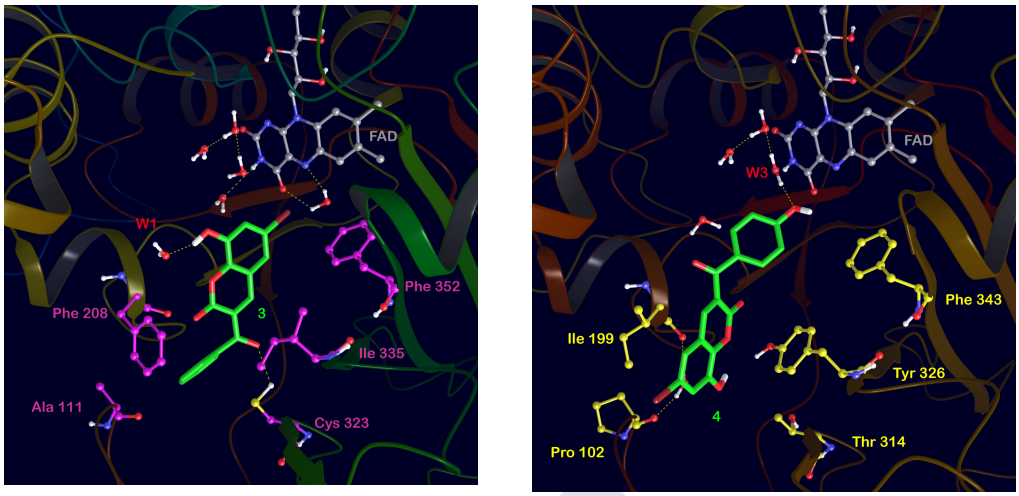


Figura 54. Interacción de los compuestos **88** (izquierda) y **96** (derecha) con el sitio activo de MAO-A.

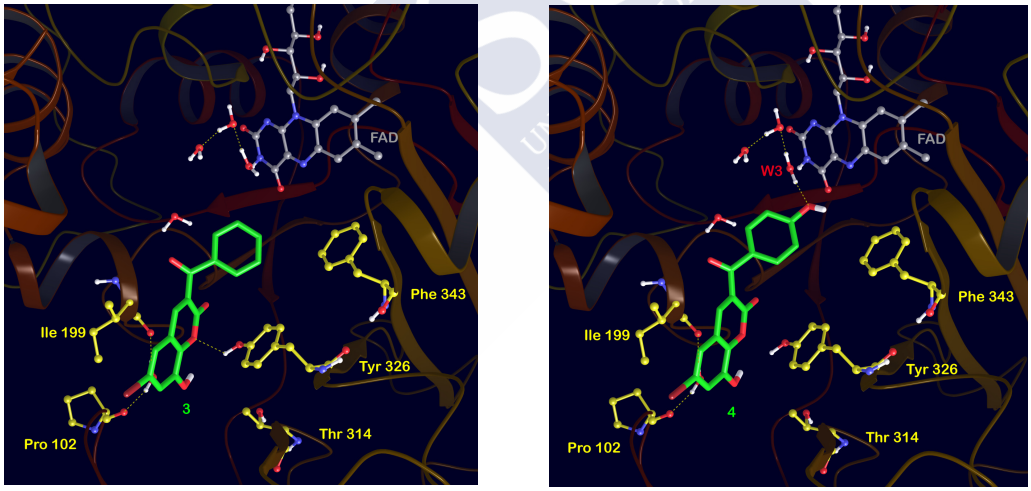


Figura 55. Interacción de los compuestos **88** (izquierda) y **96** (derecha) con el sitio activo de MAO-B.

3.2. Estudio de las propiedades antioxidantes.

El consumo de antioxidantes naturales, tales como los flavonoides, ayuda al sistema de defensa antioxidante endógeno. Tanto los antioxidantes procedentes de la dieta como los enzimáticos son componentes de sistemas interrelacionados que interactúan entre sí para controlar la producción de especies reactivas de oxígeno (ROS), asegurando así una defensa adecuada contra el estrés oxidativo.[261,262] En este sentido, se han llevado a cabo numerosos estudios sobre la capacidad antioxidante de compuestos fenólicos de diferentes tipos (flavonoides, cumarinas o taninas entre otros) y se han correlacionado sus características estructurales con sus propiedades antioxidantes.[263]

En general, el atrapamiento de radicales libres, el potencial redox y la actividad antioxidante de estos compuestos depende principalmente del número y posición de los grupos hidroxilo donadores de hidrógeno en el anillo aromático de los compuestos fenólicos, aunque existen otros factores que también afectan a estas propiedades.[83]

Teniendo en cuenta la importancia de los sustituyentes hidroxilo en las propiedades antioxidantes de los compuestos,[83, 264] inicialmente el estudio de las propiedades antioxidantes se ha centró en híbridos hidroxi-cumarina-chalcona tipo "endo" (compuestos **87,88, 92-95, 103, 105 y 106).** Se encontró que la serie de compuestos estudiados resultó tener unas propiedades antioxidantes excelentes, ya que los compuestos presentaron bajos potenciales de oxidación, elevados índices de capacidad de absorción del radical oxígeno (ORAC, por sus siglas en inglés), con valores mucho más elevados que los compuestos de referencia quercetina y catequina, y además, los compuestos presentaron baja o nula citotoxicidad en células BAEC y buenos efectos citoprotectores frente a peróxido de hidrógeno y peroxinitrito.[265] De esta serie de compuestos estudiada, el más prometedor fue el compuesto **93**, que presentó el mayor índice ORAC (14.1), con un valor que doblaba el de los patrones de referencia, buena capacidad para atrapar radicales, baja citotoxicidad y elevados valores de citoprotección.

Un estudio similar se llevó a cabo con los híbridos hidroxi-cumarina-chalcona que contenían un grupo catecol en su estructura (compuestos **101**, **102**, **104**, **106** y **107**) observándose también excelentes propiedades antioxidantes.[266] Esta serie de compuestos que contenían el grupo catecol, presentó una actividad antioxidante menor que la serie anteriormente mencionada a pesar de que los compuestos **106** y **107** presentan valores del índice ORAC del orden de la quercetina y catequina.

Otras series de derivados cumarínicos hidroxi-sustituidos como las 3-arilcumarinas preparadas en nuestro grupo también han sido estudiadas, obteniéndose también resultados prometedores que verifican el elevado potencial del núcleo cumarínico como esqueleto para agentes antioxidantes.[267,268]

3.3. Estudio de la actividad tripanocida.

La enfermedad de Chagas está causada por el parásito *Trypanosoma cruzi* (*T. cruzi*) y representa un problema de salud importante en la mayoría de los países de Latinoamérica y el sur de Estados Unidos. Durante el transcurso de la infección por *T. cruzi* y del desarrollo de la enfermedad, se pueden producir ROS como consecuencia de la destrucción de tejido causada por secreciones tóxicas del parásito, por reacciones citotóxicas mediadas por el sistema inmune y por daño secundario en el miocardio. Por tanto, la intervención con compuestos antioxidantes que puedan reducir las ROS, pueden presentar efectos beneficiosos para prevenir o detener el estrés oxidativo generado por el parásito tras la infección.[269,270]

Por tanto, dada la posible relación entre las propiedades antioxidantes y la actividad tripanocida, se realizaron estudios de la actividad tripanocida sobre la serie de compuestos previamente mencionados que contenían grupos catecol (compuestos **101**, **102**, **104**, **106** y **107**). Todos los compuestos, a excepción del compuesto **106**, presentaron una actividad tripanocida moderada. Sin embargo, se observó que aquellos compuestos que presentaban mayor actividad, eran aquellos que tenían valores de ORAC más bajos, de modo que estos resultados pueden ser tomados en cuenta para futuros estudios a cerca del mecanismo de acción de este tipo de compuestos frente a *T. cruzi*.

3.4. Estudio como ligandos de receptores de adenosina.

Los receptores de adenosina (ARs) son receptores acoplados a proteínas G (GPCRs) que exhiben un enorme número de actividades fisiológicas a través de la activación de cuatro subtipos diferentes de receptores: A_1, A_{2A}, A_{2B} y A_3.[271] La mayoría de los antagonistas de los receptores de adenosina son nuevos y prometedores candidatos a fármacos para un gran número de procesos patológicos, tales como inflamación, fallos renales y cardíacos o desórdenes neurológicos como las enfermedades de Parkinson y Alzheimer.[272,273] Entre los antagonistas no clásicos, los derivados de flavonas e isoflavonas presentan un papel destacado, como el caso de la genisteina, que ha sido descrita como un antagonista competitivo frente al receptor A_1

en células FRTL (tiroides),[274] o como la galanginina, que presenta actividad micromolar para el receptor A_3.[275]

Por tanto, dada la similitud estructural entre flavonas, cumarinas, y chalconas, hemos considerado evaluar la actividad de algunos tipos de híbridos cumarina-chalcona frente a los cuatro subtipos de receptores de adenosina humanos expresados en células ováricas de hámster chino (CHO cells). Los compuestos estudiados en este tipo de ensayos poseen el esqueleto de chalcona como núcleo estructural principal, y los diferentes derivados han sido preparados basándonos en la sustitución de uno o ambos anillos bencílicos (anillos A y/o B) de la chalcona por una cumarina y/o un grupo tiofeno con diferentes sustituyentes. Los compuestos estudiados y sobre los que hemos hecho el estudio de relación estructura-actividad han sido los compuestos **161**, **184**, **188**,**203**, **214** y **224**, llegando a la conclusión de que una selectividad hacia el receptor de adenosina *h*A_3 se conseguía fundamentalmente cuando el anillo B de la chalcona era sustituido por un tiofeno, y el anillo A era sustituido por el anillo de pirona incluido en el esqueleto de cumarina (compuesto **184**).[276]

Siguiendo la línea del trabajo anterior, decidimos seguir estudiando el potencial de los híbridos cumarina-chalcona como ligandos de adenosina. Realizamos un estudio sobre híbridos metoxi/hidroxi-3-benzoilcumarina (compuestos **19**, **25**, **26**, **30** y **97**, **101**, **102** y **107**, respectivamente). Un estudio preliminar de la relación estructura-actividad de los derivados estudiados mostró que, en general, los derivados metoxi sustituídos (**19**, **25**, **26**, **30**) presentaron buenas afinidades de unión y selectividad hacia el *h*A_3AR, mientras que los derivados hidroxi sustituídos (**101**, **102** y **107**) presentaron una actividad moderada pero selectividad hacia el *h*A_1AR. Se observó también que las posiciones 5 y 7 del anillo de cumarina resultaban cruciales par la potencia y selectividad de ambas series de compuestos.[277]

3.5. Estudio de la actividad antibacteriana.

Se conocen múltiples ejemplos de cumarinas[278] y chalconas[144] que presentan un amplio rango de actividades antibacterianas. Estudios realizados en nuestro grupo, muestran que tanto el núcleo cumarínico simple, como derivados de 3-arilcumarinas, presentan interesantes actividades frente a bacterias patógenas humanas.[279,280]

Con la intención de ampliar el tipo de estudios antibacterianos no sólo sobre patógenos humanos, y teniendo en cuenta la importancia de la acuicultura tanto en nuestra región como sobre la economía mundial, se realizaron ensayos antibacterianos

con patógenos marinos con el fin de encontrar candidatos para tratamientos en acuicultura. Así, además del estudio con bacterias humanas como *Escherichia coli*, *Pseudomona aeruginosa* o *Staphylococcus aureus*, los patógenos marinos a estudiar fueron aquellos pertenecientes al género *Tenacibaculum*, siendo *Tenacibaculum maritimum* el más estudiado. Esta bacteria es la causante de la tenacibaculosis, una enfermedad que produce desagradables lesiones sobre la superficie del cuerpo de los peces tales como úlceras, necrosis, malformaciones en la boca, o aletas y colas de aspecto putrefacto, entre otras,[281,282] por lo que aunque estos patógenos son inocuos para el ser humano, sus efectos sobre los organismos marinos producen daño y hacen que su aspecto no sea bueno para su comercialización.

Los compuestos empleados para estos estudios y sobre los que hemos hecho el estudio de relación estructura-actividad han sido los compuestos **1**, **3-5** y **123, 124**, **130, 131, 135, 136, 141** y **142**. Observamos que de la serie estudiada, ninguno de los compuestos fue activo frente a las bacterias patógenas humanas estudiadas. Sin embargo, los compuestos que poseían un grupo amino en su estructura (**123, 124**, **130, 131, 135, 136, 141** y **142**), mostraban de moderada a gran actividad frente a todas las cepas de *Tenacibaculum maritimum*, con órdenes iguales o superiores a los patrones empleados y que actualmente se emplean para el tratamiento de la tenacibaculosis, como la enrofloxacina, cuya actividad no es selectiva frente a *T. maritimum*, ya que también es altamente activa frente a las bacterias humanas ensayadas. La presencia de este grupo amino resultó ser clave para la actividad de los compuestos, así como la sustitución en la posición 8 del núcleo de cumarina, siendo los compuestos **130**, **131**, **141** y **142** los más activos.[283] Por tanto, la elevada actividad de estos compuestos y su selectividad solo frente a distintas cepas de la bacteria marina *T. maritimum* pueden evitar futuros problemas de resistencia en patógenos humanos, haciendo a estos compuestos buenos candidatos para el tratamiento de la tenacibaculosis.

Parte Experimental
Experimental Part

1. Artículos publicados o en fase de publicación 97

2. Compuestos preparados bajo estudio y pendientes de publicación 257

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La presentación de esta Memoria incluye los artículos publicados, enviados y en fase de preparación. Las rutas sintéticas utilizadas para los compuestos ya publicados, la caracterización de los mismos, los ensayos farmacológicos y electroquímicos realizados, así como los estudios estructurales y de SAR se presentan a continuación de forma detallada y discutida en cada uno de los respectivos manuscritos. Por tanto, dichos artículos serán incluidos en este apartado de parte experimental de esta Memoria.

Aquellos compuestos que a la hora de presentar esta Memoria todavía no han sido publicados pero que han sido preparados y se encuentran bajo estudio, se encuentran caracterizados en la sección 2 del apartado de Parte Experimental.

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1.1. Artículos publicados.

1. “Diastereospecific Nazarov Cyclization of Fully Substituted Dienones: Generation of Vicinal All-Carbon-Atom Quaternary Stereocenters” (Jolit, A.*; Vazquez-Rodriguez, S.; Yap, G.P.A.; Tius, M.A.) – *Angewandte Chemie International Edition* **2013**, *52*, 1-5.

2. Synthesis of 3-Arylcoumarins via Suzuki-cross-coupling Reactions of 3-Chlorocoumarin” (Matos, M.J.*; Vazquez-Rodriguez, S.; Borges, F.; Santana, L.; Uriarte, E.) – *Tetrahedron Letters* **2011**, *52*, 1225-1227.

3. “3-(4-Methoxybenzoyl)-6-nitrocoumarin” (Vazquez-Rodriguez, S.*; Uriarte, E.; Santana, L. *Acta Cryst.* **2013**, *E69*, o345.

4. “Focusing on New Monoamine Oxidase Inhibitors: Differently Substituted Coumarins As An Interesting Scaffold” (Matos, M.J.; Viña, D.; Vazquez-Rodriguez, S.; Uriarte, E.; Santana, L.*) – *Current Topics in Medicinal Chemistry* **2012**, *12*, 2210-2239.

5. “MAO Inhibitory Activity Modulation: 3-Phenylcoumarins versus 3-Benzoylcoumarins” (Matos, M.J.; Vazquez-Rodriguez, S.; Uriarte, E.; Santana, L.; Viña, D.*) – *Bioorganic & Medicinal Chemistry Letters* **2011**, *21*, 4224-4227.

6. “Hydroxycoumarins as Selective MAO-B Inhibitors” (Serra, S.*; Ferino, G.; Matos, M.J.; Vázquez-Rodríguez, S.; Delogu, G.; Viña, D.; Cadoni, E.; Santana, L.; Uriarte, E.) – *Bioorganic & Medicinal Chemistry Letters* **2012**, *22*, 258-261.

7. "Synthesis, electrochemical and biological studies on novel coumarin-chalcone hybrid compounds" (Pérez-Cruz, F.; Vazquez-Rodriguez, S.*; Matos, M.J.; Herrera-Morales, A.; Villamena, F.; Das, A.; Gopalakrishnan, B.; Olea-Azar, C.; Santana, L.; Uriarte, E.) – *Journal of Medicinal Chemistry*, **2013**, *56*, 6136-6145.

8. “Synthesis of coumarin-chalcone hybrids and evaluation of their antioxidant and trypanocidal properties“ (Vazquez-Rodriguez, S.*; Figueroa-Guíñez, R.; Matos, M.J.; Santana, L.; Uriarte, E.; Lapier, M.; Maya, J.D.; Olea-Azar, C.*) – *MedChemComm* **2013**, *4*, 993-1000.

9. “Synthesis and evaluation of antioxidant and trypanocidal properties of a selected series of coumarin derivatives” (Figueroa Guíñez, R.; Matos, M.J.*; Vazquez-Rodriguez, S.; Santana, L.; Uriarte, E.; Olea-Azar, C.*; Maya, J.D.) – *Future Medicinal Chemistry*, **2013**, *5*, 1911-1922.

10. “Remarkable antioxidant properties of a series of hydroxy-3-arylcoumarins” (Matos, M.J.*; Pérez-Cruz, F.; Vazquez-Rodriguez, S.; Uriarte, E.; Santana, L.; Borges, F.; Olea-Azar, C.) – *Bioorganic & Medicinal Chemistry* **2013**, *21*, 3900–3906.

11. “Chalcone-based derivatives as new scaffolds for hA_3 adenosine receptor antagonists” (Vazquez-Rodriguez, S.*; Matos, M.J.; Santana, L.; Uriarte, E.; Borges, F.; Kachler, S.; Klotz, K.-N.) – *Journal of Pharmacy & Pharmacology* **2013**, *65*, 697-703.

12. “Synthesis and Structure-Activity Relationships of Novel Amino/Nitro Substituted 3-Arylcoumarins as Antibacterial Agents” (Matos, M.J.*; Vazquez-Rodriguez, S.; Santana, L.; Uriarte, E.; Fuentes-Edfuf, C.; Santos, Y.; Muñoz-Crego, A.) - *Molecules* **2013**, *18*, 1394.

13. “Looking for New Targets: Simple Coumarins as Antibacterial Agents” (Matos, M.J.;* Vazquez-Rodriguez, S.; Santana, L.; Uriarte, E.; Fuentes-Edfuf, C.; Santos, Y.; Muñoz-Crego, A.) – *Medicinal Chemistry* **2012**, *8*, 1140-1145.

1.2. Artículos en fase de publicación.

14. “Synthesis and Antibacterial Evaluation of Coumarin-Chalcone Hybrids as New and Potent Agents for Tenacibaculosis” (Vazquez-Rodriguez, S.*; Díaz, A.; Matos, M.J.; Serra, S.; Uriarte, E.; Santana, L.; Muñoz-Crego, A.; Santos, Y.) –Enviado.

15. “3-Benzoylcoumarins as new selective scaffolds for modulation of the *h*A_1 and *h*A_3 binding affinity. Synthesis, Biological assays and docking studies.” (Vazquez-Rodriguez, S.*; Serra, S.; Uriarte, E.; Santana, L.; Kachler, S.; Klotz, K.N.; Vilar, S.) – Enviado.

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Synthetic Methods

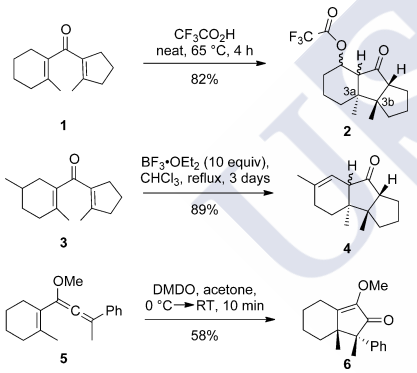
DOI: 10.1002/anie.201305218

Diastereospecific Nazarov Cyclization of Fully Substituted Dienones: Generation of Vicinal All-Carbon-Atom Quaternary Stereocenters**

*Anais Jolit, Saleta Vazquez-Rodriguez, Glenn P. A. Yap, and Marcus A. Tius**

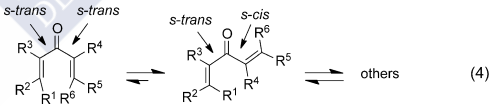
The joining of two carbon atoms through a σ bond so as to form vicinal all-carbon-atom quaternary centers remains a difficult challenge in organic synthesis.[1] An effective strategy to address the problem is to exploit an orbital-symmetry-controlled process.[2] We describe herein a diastereospecific Nazarov cyclization[3] that leads to cyclopentenones bearing adjacent all-carbon-atom quaternary centers.

We are aware of only three examples of Nazarov cyclizations that lead to two contiguous all-carbon-atom quaternary stereocenters during the ring-forming step (Scheme 1). The efforts of Harding and co-workers [Eqs. (1) and (2), Scheme 1] culminated in the total synthesis of DL-trichodiene.[4] The reaction conditions in both cases were harsh, but the yield of cyclic products **2** and **4**, which lack sensitive functionality, was high. In both reactions the C3a,C3b relative configuration (see compound **2**) was determined by the conrotation that is required by the selection rule governing the thermal 4π-electron process. The third example is the oxidative Nazarov cyclization of allenyl ether **5** that was reported by Frontier and co-workers in 2011 [Eq. (3) in Scheme 1].[5] The oxyallyl zwitterion that forms spontaneously from the allene oxide following the treatment of **5** with dimethyldioxirane (DMDO) underwent stereospecific cyclization to **6** in moderate yield. The zwitterion intermediate is strongly polarized, hence the mild reaction conditions.



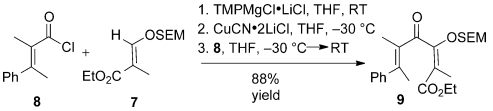
Scheme 1. Generation of vicinal all-carbon-atom quaternary stereocenters in the Nazarov cyclization.

Frontier and co-workers also described a diastereoselective tandem Nazarov–Wagner–Meerwein reaction that leads to cyclopentenones bearing vicinal all-carbon-atom stereocenters.[6] Finally, there are two reported examples of the Nazarov cyclization of 3,5-dibromo-2,6-dimethylhepta-2,5-dien-4-one; in each case the reaction led to vicinal *gem*-dimethyl groups in the cyclic product.[7,8] To the best of our knowledge, the formation of adjacent quaternary carbon atoms during the Nazarov cyclization is limited to this very small number of examples. These cyclizations represent a special challenge because the two "inside" substituents, R^1 and R^6 [Eq. (4)], destabilize the U-shaped *s-trans*/*s-trans* conformer of the dienone that is able to form a ring, thereby displacing the equilibrium to the *s-cis* conformers, which cannot cyclize. Herein we will demonstrate how this problem can be overcome through judicious experimental design.



(4)

It has been shown that polarized dienones undergo accelerated Nazarov cyclizations.[9,10] An especially effective way to activate the dienone toward cyclization is to incorporate a "push–pull" vinylogous carbonate.[11] A synthesis of activated dienones that are ideally suited for the present application is shown in Scheme 2. Metalation of the 2-(trimethylsilyl)ethoxymethyl (SEM) enol ether **7** according to a procedure described by Knochel and Bresser[12] and



Scheme 2. Preparation of the dienone substrate. TMP = 2,2,6,6-tetramethylpiperidinyl.

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[**] We thank the NIH-NIGMS (R01 GM57873) for partial support and the NSF for CRIF-MRI 1048367.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201305218.

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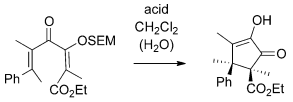
subsequent exposure to (*E*)-2-methyl-3-phenylbut-2-enoyl chloride (**8**) led to dienone **9** in 88% yield from the carboxylic acid as a single (*E*,*E*) geometrical isomer. The isomeric purity of **9** is critical because contamination by the geometrical isomer of either double bond would erode the stereoisomeric purity of the derived cyclic product. The substituted acryloyl chlorides used in this study were synthesized according to reported procedures from the appropriate acetoacetate esters.[13]

We optimized the reaction conditions for the Nazarov cyclization by using dienone **9** (Table 1). No special effort was made to exclude traces of water from the reaction mixture.

Table 1: Optimization of the reaction conditions.



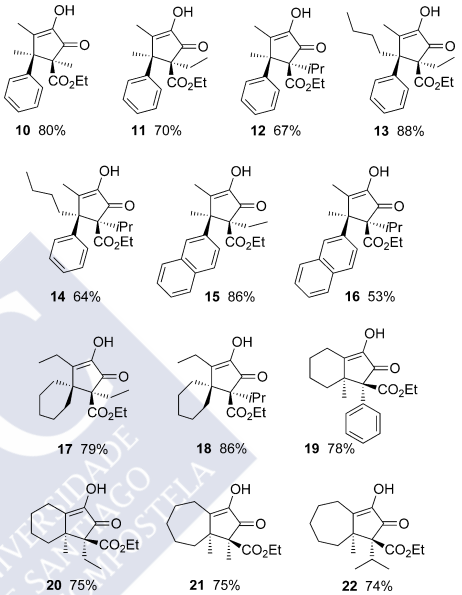
9 **10**

Entry	Acid (equiv)	*T* [°C]	*t* [min][a]	Yield [%][b]
1[c]	TFA (90)	0	1	63
2[c]	TFA (4)	0	10	–
3[c]	Tf_2NH (4)	0	2	81
4[c]	Tf_2NH (1)	0	2	–
5[d]	Tf_2NH (0.2)	0	2	–
6[e]	**Tf_2NH (0.2)**	**23**	**60**	**80**
7[f]	Tf_2NH (0.1)	23	180	78

[a] Total reaction time. [b] Yield of isolated **10**. [c] The acid was added neat. [d] Triflimide was added as a 0.27 M solution in CH_2Cl_2. [e] Inverse addition at 5 μL min^{-1} of a 1 M solution of **9** to a 1 M solution of triflimide. [f] Inverse addition at 1.7 μL min^{-1} of a 1 M solution of **9** to a 1 M solution of triflimide. Tf = trifluoromethanesulfonyl.

The first conditions that were examined, trifluoroacetic acid (TFA; 90 equiv) in dichloromethane at 0 °C, were successful and led to the α-hydroxycyclopentenone **10** as a single diastereomer in 63% yield after a reaction time of 1 min (Table 1, entry 1). A reduction in the amount of acid used to 4 equivalents resulted in a slower reaction that led to a mixture in which **10** was a minor product (Table 1, entry 2). One of the by-products appeared to be the acyclic α-diketone resulting from loss of the SEM group. We reasoned that a Brønsted acid with a less nucleophilic counterion would suppress this unwanted reaction. Support for this hypothesis was obtained by the exposure of **9** to triflimide (4 equiv), which led to the production of **10** in 81% yield as a single diastereomer (Table 1, entry 3). In an effort to reduce the amount of acid required, the reaction was conducted in the presence of 1 and 0.2 equivalents of triflimide (Table 1, entries 4 and 5), but in each case mixtures of products were observed. Because the reaction was only successful when excess acid was used, the mode of addition was changed. When a solution of **9** was added with a syringe pump to a concentrated solution of triflimide (0.2 equiv; Table 1, entry 6), **10** was produced in 80% yield by the time the transfer was complete. When even less triflimide was used (0.1 equiv), it was necessary to increase the addition time, and the yield of **10** was 78% (Table 1, entry 7). For all subsequent investigations we used the conditions described in entry 6 of Table 1. The requirement of a high acid concentration is consistent with the cyclization of a diprotonated intermediate of the type that West and co-workers postulated for certain Nazarov cyclizations.[14,15]

The reaction scope is indicated by the examples in Scheme 3 of compounds obtained under the optimized reaction conditions (Table 1, entry 6). All compounds were



Scheme 3. Products formed with vicinal quaternary carbon atoms in the Nazarov cyclization.

formed as single diastereomers, and their configuration was determined by NMR spectroscopy on the basis of NOE data. The configuration of **10** was confirmed by single-crystal X-ray crystallographic analysis.[16] The yield of the cyclic products varied from 53 to 88%. The cyclization is surprisingly tolerant of sterically demanding substrates. For example, even cyclopentenone **16**, which bears isopropyl and 2-naphthyl groups on adjacent carbon atoms, was formed in 53% yield. Significantly, there is no requirement for a β aryl group to activate the dienone, as demonstrated by examples **17**–**22**.

The success of these Nazarov reactions rests in a delicate balance, as the following examples demonstrate. Exposure of dienone **23**, which differs from **9** only in the presence of an isopropyl group in place of the phenyl group, to the optimized cyclization conditions led to an approximately 1.5:1 mixture of the anticipated product **24** and its diastereomer **25** in 81% yield [Eq. (5)]. The appearance of **25** is evidence of the occurrence of double-bond isomerization in **23** in competition with cyclization. It is unlikely that isomerization of the enol ether took place, because this isomerization would then have

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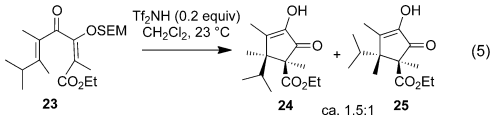
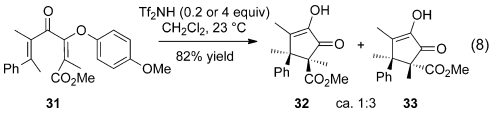


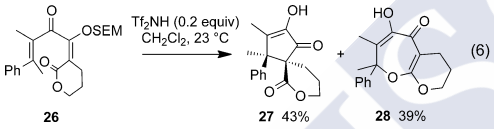
(5)



(8)

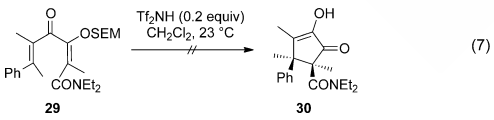
been observed for some of the compounds in Scheme 3 under the identical reaction conditions. Furthermore, double-bond isomerization was observed during the preparation of (*E*)-2,3,4-trimethylpent-2-enoyl chloride in the presence of an acid.[17] There is a higher barrier to the isomerization of **9** because it requires the disruption of conjugation to the phenyl group. The formation of **25** is remarkable, because it demonstrates that cyclization can even take place efficiently with an "inside" isopropyl group [see Eq. (4)].

The use of lactone **26** as a substrate revealed an unanticipated process [Eq. (6)]. Cyclization according to the predicted pathway led to nearly equal amounts of a single diastereomer of **27** and by-product **28**. The ketene acetal **28** may have been formed by means of an intramolecular Michael addition of the oxygen atom of the lactone carbonyl group. This process is facile in this example because of the enforced coplanarity of the lactone carbonyl group with the dienone. It is also possible that **28** was formed through an electrocyclization.[18]



(6)

Diethylamide **29** failed to undergo cyclization even upon exposure to triflimide (4 equiv) for 24 h and was recovered from the reaction mixture [Eq. (7)]. This result demonstrates the effect of double-bond polarization on the rate of cyclization. The amide group, which is less electron withdrawing than the ester, apparently does not enable the cyclization under the same conditions.



(7)

In dienone **31**, the SEM group has been replaced with a 4-methoxyphenyl group [Eq. (8)]. Cyclization proceeded in good yield but led to an approximately 1:3 mixture of diastereomers **32** and **33**. This result underscores the critical role that the SEM group plays in the successful cyclization. Favorably polarized **31** is expected to cyclize as fast as **9**, but the hydrolytic loss of the 4-methoxyphenyl group is probably slower than SEM cleavage, which does not require the participation of water. A slow termination step leading to a reversible Nazarov–*retro*-Nazarov[19] process might provide an opportunity for enol ether isomerization in **31** to compete with cyclization.

On the basis of these results, it appears that the Nazarov cyclization to produce vicinal all-carbon-atom quaternary centers is successful and diastereospecific within a carefully defined range of reaction parameters. The strongly polarized "push–pull" vinylogous carbonate is required to lower the barrier to cyclization. The SEM group also plays a critical role by collapsing rapidly through the loss of ethylene and formaldehyde to suppress processes that lead to erosion of the stereochemical integrity of the cyclic product. Inverse addition of the dienone to the acid leads to a rapid cyclization that suppresses undesired competing acid-catalyzed processes. The examples described in Scheme 3 suggest broad synthetic utility, and it is also likely that other "push–pull" alkenes can be used in place of the vinylogous carbonates that have been described herein. This study greatly expands the scope of the Nazarov reaction by making highly congested cyclopentenones readily available. An asymmetric version of this cyclization is under investigation.[20]

Experimental Section

A reaction vial equipped with a membrane cap was loaded with Tf_2NH (15 mg, 0.054 mmol, 0.2 equiv) in dry CH_2Cl_2 (0.05 mL, 1M). A small needle was inserted through the cap to relieve pressure. A solution of dienone **9** (112 mg, 0.268 mmol, 1 equiv) in dry CH_2Cl_2 (0.27 mL, 1M) was added dropwise with a syringe pump (5 μL min^{-1}) to the solution of Tf_2NH at room temperature. The reaction mixture was placed on a vortex stirrer during the addition. The reaction mixture was then diluted with CH_2Cl_2 (2 mL) and quenched with 5% aqueous $NaHCO_3$. The aqueous layer was extracted twice with CH_2Cl_2. The combined layers were washed with water and brine and then dried over Na_2SO_4. Evaporation of the solvent and column chromatography (silica gel, 2% Et_2O/CH_2Cl_2) afforded **10** (61 mg, 80%) as a white solid. M.p.: 141–145 °C; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.28–7.06$ (m, 5H), 5.31 (s, 1H, OH), 3.49–3.39 (m, 1H), 3.32–3.18 (m, 1H), 1.90 (s, 3H), 1.61 (s, 3H), 1.46 (s, 3H), 0.83 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 200.4$, 170.8, 148.2, 147.5, 141.5, 128.0 (2C), 127.8 (2C), 127.1, 61.5, 60.8, 52.7, 21.3, 18.4, 13.4, 10.9 ppm; IR (neat): $\tilde{\nu}$ =3366, 2982, 1708, 1661, 1445, 1404, 1363, 1249, 1193, 1083, 777, 733 cm^{-1}; HRMS (ESI^+): *m/z* calcd for $C_{17}H_{20}O_4$: 311.1260 $[M+Na]^+$; found: 311.1253.

Received: June 18, 2013
Revised: July 23, 2013
Published online: September 3, 2013

Keywords: diastereoselectivity · Nazarov cyclization · quaternary stereocenters · rearrangement · synthetic methods

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Tetrahedron Letters 52 (2011) 1225–1227

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of 3-arylcoumarins via Suzuki-cross-coupling reactions of 3-chlorocoumarin

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ARTICLE INFO

Article history:
Received 15 November 2010
Revised 5 January 2011
Accepted 12 January 2011
Available online 18 January 2011

Keywords:
3-Arylcoumarins
Suzuki reaction
3-Chlorocoumarin
N,N'-Bis(salicylidene)-ethylenediamino-palladium catalyst

ABSTRACT

A convenient, new, and effective protocol for a rapid synthesis of different substituted 3-arylcoumarins is reported. The developed synthetic route involves Pd-catalyzed cross-coupling reaction, using a catalytic complex Pd-salen. Under these conditions, a series of different substituted boronic acids have been successfully reacted with a coumarin halide to afford the coupling products in good yields.

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Coumarins are an important class of benzopyrones that are found in the vegetable kingdom, either in free or combined state.[1] They occupy an important place in the realm of natural products and synthetic organic chemistry. The diverse biological and pharmaceutical properties of natural and synthetic coumarins as antioxidants,[2] anti-HIV,[3] anticancer,[4] vasorelaxants[5] or enzymatic inhibitors[6] are well-known.[1] In the last few years our research group has been engaged in the synthesis of new biologically active coumarins. In particular, the 3-phenylcoumarin's scaffold has been the focal point of our most recent studies. In fact we have demonstrated that some 3-phenylcoumarins play an important role in the monoamino oxidase (MAO) enzymatic inhibition.[7–9] Accordingly, the interesting application potential of the 3-arylcoumarins in the Health Sciences promotes their preparation as an interesting topic in synthetic organic chemistry.

Two different strategies can be used to obtain the phenylcoumarins. One is the construction of the coumarin nucleus by condensation–cyclization-type reactions. Wittig,[10] Pechmann,[5,7,11,12] Perkin[8,9,13–17] or Knoevenagel[18,19] reactions are some of the synthetic routes frequently described to prepare 3-arylcoumarins, starting from phenols or aromatic carbonyl compounds. The classical Perkin condensation is perhaps the most direct and simple method known for the preparation of 3-arylcoumarins.[16] Although this method is often used, it has some drawbacks such as the application of strong acids and high temperatures and sometimes the request of multi-step reactions.

An alternative strategy consists in the direct 3-arylation of the coumarin scaffold by metal cross-coupling reactions. In recent years, palladium-catalyzed coupling reactions have been developing into a versatile and efficient method for the C–C bond formation. Suzuki[20,21] and Stille have described simple synthetic routes to obtain C–C liaisons throughout the coupling arylboronic or organotin compounds with organic halides catalyzed by a palladium-complex. In these reactions, Pd complexes are used as catalysts in which the ligands are P and/or N lone pairs donors or active Pd complexes are generated in situ.[22] In the first case, some of these ligands are expensive, toxic, and sensitive to air and a major challenge lies in the separation of product from the expensive catalyst, much to the dismay of large-scale producers.[22] Ionic liquids,[23] free of organic solvent systems or phase-transfer catalysts[24] have been used to provide a green alternative chemistry to the conventional organic methodologies, but they are not devoid of toxicity. In turn, phosphine ligands[25] had played an important role in C–C bond formation, in homogeneous reaction medium. Several efforts have been made in order to discover air and moisture-stable palladium ligands.[25] However, these ligands are air- and moisture-sensitive and the P–C bond degradation frequently occurs at elevated temperature.[26] The degradation of the catalyst strongly affects conversion and selectivity of the derivatives.[27] So, different methods using phosphine-free ligands have been explored for application in this kind of reactions.[28]

Because of its versatility, palladium catalyzed coupling reactions were widely used to synthesize different arylcoumarins. The arylation in 4-position of the coumarin nucleus is usually achieved by arylation of 4-hydroxycoumarins with arylboronic

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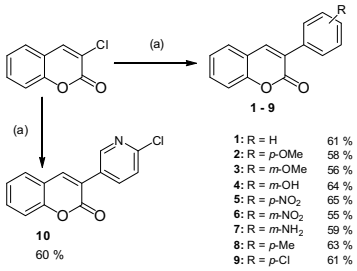
0040-4039/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2011.01.048



Scheme 1. Synthesis of 3-arylcoumarin derivatives. Reagents and conditions: (a) 3-chlorocoumarin (1.0 equiv), phenylboronic acid/2-chloro-5-pyridineboronic acid (1.25 equiv), sodium carbonate (2.0 equiv), palladium complex (0.5 mol %), DMF/H_2O (1:1), 110 °C, 120–180 min.

acids via C–OH bond activation.[29] Accordingly, the 4-hydroxy group is converted in *pseudo*halides, such as triflates or tosylates which are used instead of halides in a coupling reaction with arylboronic acids (via a Suzuki type reaction). In other cases, the aryl substituents have been successfully incorporated in 4-position of the hydroxycoumarin using organostannanes[30] or organozinc compounds[31] under Stille or Negishi conditions, respectively.

However, the formation of the aryl C–C bond using coumarin as starting material is till now an uncommon subject.[32,33] To our knowledge, few papers have been published so far concerning the 3-arylation of coumarin nucleus by direct coupling under Suzuki conditions: in all these cases, the halide was 3-bromocoumarin. And the catalysts were the classic palladium acetate ($Pd(OAc)_2$), tetrakis(triphenylphosphine)palladium (0) $Pd(PPh3)_4$[34,35], and [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride.[36,37] Salen ligand has been found to be a highly active catalyst for the Suzuki reaction performed with aryl iodides, bromides, and chlorides, giving excellent yields, in a short reaction time.[38,39] Salen ligand is a bright yellow solid, soluble in polar organic solvents, and effective in C–C bond forming reactions with high selectivity. This symmetrical palladium(II) complex was a product of the reaction of the Salen ligand with palladium acetate (for 20 h at room temperature) in a good yield. This complex promotes a catalytic activity in a low concentration (0.5 mol %).[40] Noteworthy to mention that Salen is a chelating ligand generally used in coordination chemistry and homogeneous catalysis.[41]

Although significant advances have been performed in the metal-catalyzed synthesis, the palladium-catalyzed cross-coupling reaction using 3-chlorocoumarin to afford 3-arylcoumarins has never been described. This constraint encourages us to explore this synthetic strategy. Accordingly, preliminary experiments were done to examine the effect of various ligands for Pd-based coupling.[42,43] The most promising one was *N,N′*-bis(salicylidene)-ethylenediamino-palladium (salen-Pd). In fact, $Pd(OAc)_2$-salen couple in presence of a Na_2CO_3 in DMF/H_2O (1:1) was found to be an efficient catalytic system to obtain a variety of 3-arylcoumarin derivatives (Scheme 1).[40] As coumarins are sensitive substrates to alkaline conditions, and may result in the cleavage of the lactone ring, the solvent, and base for the coupling reaction were carefully studied. To optimize the reaction the influence of the palladium source in the catalytic activity was also performed. $Pd(OAc)_2$ was found to be the best option.[40] After reaction optimization, the influence of the Pd-salen complex in the Suzuki reaction involving cross-coupling of a coumarin halide and different arylboronic acids was investigated. The Suzuki reaction developed herein was found to be effective when using arylboronic acids with a variety of functional groups. Furthermore the reaction works with iodo-, bromo- or chloro-coumarin substrates. Different reaction times are required for each coumarin halide type. Although it is well-known that in Suzuki reaction the chloride derivatives are less reactive than the bromide or the iodide ones, the use of the 3-chlorocoumarin as starting material in the reaction is related with its commercial availability. The synthesis of 3-iodocoumarin and 3-bromocoumarin involves the introduction of other synthetic steps in the overall reaction.

Comparing the synthetic route herein proposed with the classic one, previously reported by us,[7,8] one can verify that both are easy and not too expensive methodologies. The main advantage of this new synthetic approach is that it can afford 3-arylcoumarins with different substitution patterns. By means of the traditional methodology, hydroxyl and nitro derivatives could not be obtained and/or purified. The reaction was not clean and the obtained crude product was a complex mixture with several sub-products and the purification process was worthless. The reaction versatility is the main advantage of this synthetic strategy comparing to the classic Perkin reaction. Compounds **4**–**7**[44–46] and **10**[47] are exclusively prepared by the Suzuki methodology while compounds **1**–**3**, **8**, and **9** can be prepared by both methods (Table 1).[48–50] The proposed synthetic strategy operates from micro- to macro-scale and avoids the formation of undesired sub-products. The palladium complex can be easily and almost totally recuperated at the end of the process. The catalyst is recovered from the reaction mixture when the product is purified by flash chromatography and it is ready to be reused. This subject has great interest because of the toxicity and the cost of palladium complexes.

In conclusion, a novel and versatile route for the synthesis of 3-arylcoumarins is proposed. The palladium cross-coupling reaction can be applied to obtain a series of different functionalized derivatives. In addition, we have shown that *N,N′*-bis(salicylidene)-ethylenediamino-palladium proved to be a good catalyst for the Suzuki reaction of heterocyclic activated chlorides with arylboronic acids, under aerobic conditions. This methodology is an efficient synthetic route for obtaining different 3-arylcoumarins.

Table 1
Synthesis of 3-arylcoumarins (**1**–**10**) by Perkin reaction and/or via Suzuki-cross-coupling reaction

Compounds	Perkin reaction[a]		Suzuki reaction[b]	
	Time (min)	Yield (%)	Time (min)	Yield (%)
1	1440	60	120	61
2	1440	65	120	58
3	1440	68	120	56
4	*	*	180	64
5	*	*	160	65
6	*	*	160	55
7	*	*	160	59
8	1440	70	120	63
9	2160	63	140	61
10	*	*	140	60

* Compounds **4**–**7** and **10** are exclusively prepared by the Suzuki methodology.
[a] Conditions: phenylacetic acids, DCC, DMSO, 110 °C, 24–36 h.
[b] Reactions with 3-chlorocoumarin. Compound **1** was synthesized using 3-bromocoumarin—reaction time: 100 min; yield: 70%.

Acknowledgments

The authors thank the Spanish Ministry (PS0900501) and Xunta da Galicia (INCITE09E2R203035ES and PGIDIT09CSA030203PR) for the financial support. M.J.M. thanks Fundação de Ciência e Tecnologia for the SFRH/BD/61262/2009 scholarship.

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46. *General method to prepare 3-arylcoumarins*: To a 20 mL two neck round-bottomed flask was added a solution of 3-chlorocoumarin (0.83 mmol), aryl boronic acid (1.04 mmol), Na_2CO_3 (1.66 mmol), and Pd-salen complex (0.5 mol %) in DMF/H_2O (1:1). The reaction mixture was heated at 110 °C for 120–180 min. The reaction was monitored by chromatography. After the completion of the reaction, the mixture was extracted with ethyl acetate (3 × 20 mL). The organic extracts were dried over anhydrous sodium sulphate, filtrated, and the solvent was evaporated under vacuum. The obtained crude product was purified by column chromatography (hexane/ethyl acetate 9:1) to give the coumarins (**1**–**9**). *3-(3′-Nitrophenyl)coumarin* (**6**): yellow solid, yield 55%; mp 252–3 °C. ^{1}H NMR ($CDCl_3$): δ = 7.17–7.22 (m, 2H, H-7, H-8), 7.39–7.48 (m, 3H, H-5, H-6, H-5′), 7.52–7.54 (m, 1H, H-6′), 7.60–7.71 (m, 2H, H-4, H-4′), 8.44 (s, 1H, H-2′). ^{13}C NMR ($CDCl_3$): δ = 110.0, 114.2, 116.7, 117.6, 119.3, 122.8, 125.5, 128.5, 131.1, 132.6, 132.7, 141.4, 149.1, 152.8, 158.6. MS: *m/z* (%) = 268 (26), 267 (M$^+$, 100), 239 (24), 221 (38), 193 (17) 165 (52), 164 (16), 163 (19), 139 (12), 82 (11). Anal. calcd. for $C_{15}H_9NO_4$ (267.24): C, 67.42; H, 3.39. Found: C, 67.38; H, 3.36. *3-(3′-aminophenyl)coumarin* (**7**): white solid, yield 59%; mp 154–5 °C. ^{1}H NMR ($CDCl_3$): δ = 4.12 (s, 2H, –NH_2), 7.27–7.38 (m, 4H, H-2′, H-4′, H-6′, H-8), 7.47 (dd, 2H, H-6, H-7, *J* = 7.74, *J* = 1.31), 7.53–7.58 (m, 2H, H-5, H-5′), 7.88 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$): δ = 116.8, 118.8, 122.4, 125.0, 125.2, 127.2, 127.8, 128.7, 131.9, 133.3, 140.1, 152.6, 155.2, 157.3, 158.8. MS: *m/z* (%) = 239 (10), 238 (19), 237 (M$^+$, 100), 182 (36), 181 (12), 152 (50), 124 (18), 89 (45), 63 (28), 62 (18). Anal. calcd. for $C_{15}H_{11}NO_2$ (237.25): C, 75.94; H, 4.67. Found: C, 76.00; H, 4.72.
47. *3-(4′-Chloropyridin-3′-yl)coumarin* (**10**): white solid, yield 60%; mp 234–5 °C. ^{1}H NMR ($CDCl_3$): δ = 7.32–7.37 (m, 2H, H-5′, H-8), 7.46 (d, 2H, H-6, H-7, *J* = 7.51), 7.52–7.58 (m, 2H, H-5, H-6′), 7.88 (s, 1H, H-4), 8.58 (s, 1H, H-2′). ^{13}C NMR ($CDCl_3$): δ = 116.8, 118.8, 122.3, 124.7, 125.0, 125.2, 127.2, 131.8, 137.0, 140.0, 147.7, 149.6, 152.7, 157.3. MS: *m/z* (%) = 260 (5), 259 (32), 258 (15), 257 (M$^+$, 100), 223 (18), 182 (34), 152 (44) 123 (13), 89 (40), 85 (23), 71 (30), 63 (18), 57 (35). Anal. calcd. for $C_{14}H_8ClNO_2$ (257.57): C, 65.26; H, 3.13. Found: C, 65.24; H, 3.10.
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Acta Crystallographica Section E
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ISSN 1600-5368
Editors: **W. T. A. Harrison, H. Stoeckli-Evans,**
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3-(4-Methoxybenzoyl)-6-nitrocoumarin

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Acta Cryst. (2013). E**69**, o345

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3-(4-Methoxybenzoyl)-6-nitrocoumarin

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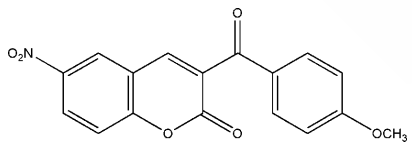
Received 21 January 2013; accepted 29 January 2013

Key indicators: single-crystal X-ray study; T = 100 K; mean σ(C–C) = 0.003 Å; R factor = 0.043; wR factor = 0.126; data-to-parameter ratio = 13.1.

In the title coumarin derivative (also known as 2*H*-chromen-2-one or 2*H*-1-benzopyran-2-one), $C_{17}H_{11}NO_6$, the coumarin ring system is nearly planar, with a dihedral angle of 3.35 (9)° between the pyrone and the benzene rings. The dihedral angle between the planes formed by the coumarin ring system and the benzene substituent is 54.60 (7)°, clearly showing the non-coplanarity of the whole aromatic system. The crystal studied was a non-merohedral twin; the minor component refined to approximately 0.44.

Related literature

For the synthesis of the title compound, see: Raju *et al.* (2010). For examples of the biological activity of coumarin derivatives, see: Borges *et al.* (2009), Matos *et al.* (2011*a*,*b*,*c*), Viña *et al.* (2012*a*,*b*); Vazquez-Rodriguez *et al.* (2013).



Experimental

Crystal data

$C_{17}H_{11}NO_6$
M_r = 325.27
Monoclinic, $P2_1/n$
a = 8.875 (3) Å
b = 17.266 (5) Å
c = 9.174 (3) Å
β = 95.401 (15)°
V = 1399.6 (7) Å^3
Z = 4
Mo $K\alpha$ radiation
μ = 0.12 mm^{-1}
T = 100 K
0.67 × 0.14 × 0.03 mm

Data collection

Bruker APEXII CCD diffractometer
Absorption correction: multi-scan (*SADABS*; Bruker, 2007)
T_{min} = 0.604, T_{max} = 0.745
30736 measured reflections
2864 independent reflections
2200 reflections with $I > 2\sigma(I)$
R_{int} = 0.054

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.043
$wR(F^2)$ = 0.126
S = 0.91
2864 reflections
219 parameters
H-atom parameters constrained
$\Delta\rho_{max}$ = 0.39 e Å^{-3}
$\Delta\rho_{min}$ = −0.26 e Å^{-3}

Data collection: *APEX2* (Bruker, 2007); cell refinement: *SAINT* (Bruker, 2007); data reduction: *SAINT*; program(s) used to solve structure: *SIR97* (Altomare *et al.*, 1999); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *ORTEP-3 for Windows* (Farrugia, 2012); software used to prepare material for publication: *WinGX* (Farrugia, 2012).

This work was partially supported by the funds of the Xunta da Galicia (09CSA030203PR) and the Ministerio de Sanidad y Consumo (PS09/00501). SVR thanks the FPU program for her PhD grant AP2008–04263.

Supplementary data and figures for this paper are available from the IUCr electronic archives (Reference: GO2080).

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supplementary materials

Acta Cryst. (2013). E**69**, o345 [doi:10.1107/S1600536813002948]

3-(4-Methoxybenzoyl)-6-nitrocoumarin

Saleta Vazquez-Rodriguez, Eugenio Uriarte and Lourdes Santana

Comment

Coumarin derivative compounds present a great interest in the medicinal chemistry field due to the displayed biological properties that they present (Borges *et al.* 2009, Matos *et al.* 2011*a*, Matos *et al.* 2011*b*, Matos *et al.* 2011*c*, Vazquez-Rodriguez *et al.* 2013, Viña *et al.* 2012*a* and Viña *et al.* 2012*b*). The title structure is a 3-substituted coumarin derivative containing a 4-methoxybenzoyl ring at the mentioned position and a nitro group at position 6 of the coumarin scaffold. Therefore, the X-ray analysis of this compound (figure 1) aims to contribute to the elucidation of structural requirements needed to understand the partial planarity of the compound (coumarin nucleus) and the torsion of the 3-benzoyl moiety regarding to this nucleus. From the single-crystal diffraction measurements one can conclude that both the pyrone and benzene rings in the coumarin motif are essentially planar, presenting dihedral angle of 3.35 (9)°. The planarity of the coumarin moiety is also evident by the torsion angle value between their carbons C3—C2—C7—C8 (-175.89 (18)°).

In addition, the torsion angles of the carbonyl group *versus* the coumarin moiety and the phenyl ring are C10—C9—C15—O16 (43.2 (2)°) and O16—C15—C17—C18 (-152.9 (2)°) respectively. These values are typical of the torsion permitted by the rotation present at position 3. Presence of the carbonyl group at position 3 provokes a non coplanarity of the benzoyl moiety regarding to the coumarin scaffold. This fact is evident taking into account the dihedral angles formed by the planes of the coumarin, the carbonyl and the phenyl groups. Dihedral angle between the coumarin moiety and the carbonyl group is 38.66 (9)°; between the carbonyl and the phenyl group is 25.76 (10)° and between the coumarin scaffold and the phenyl group is 54.60 (7)°.

Experimental

3-(4-Methoxybenzoyl)-6-nitrocoumarin was prepared according to the following protocol: to a solution of 2-hydroxy-5-nitrobenzaldehyde (1 mmol) and ethyl 4-methoxybenzoylacetate (1 mmol) in ethanol (4 ml), a catalytic amount of piperidine (5%) was added dropwise and the reaction was stirred at refluxed for 4 h. The precipitated was filtered and the solid obtained was recrystallized in dichlomethane/methanol in a 73% yield. Mp 257–259 °C.

Refinement

H atoms were treated as riding atoms with C—H(aromatic), 0.95Å with U_{iso} = 1.2U_{eq}(C), C—H(methyl) = 0.98Å, with U_{iso} = 1.5U_{eq}(C). The positions of methyl hydrogens were checked on a final difference map. The structure was refined as a two-component non-merohedral twin with a BASF parameter of 0.4374.

Computing details

Data collection: *APEX2* (Bruker, 2007); cell refinement: *SAINT* (Bruker, 2007); data reduction: *SAINT* (Bruker, 2007); program(s) used to solve structure: *SIR97* (Altomare *et al.*, 1999); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *ORTEP-3 for Windows* (Farrugia, 2012); software used to prepare material for publication: *WinGX* (Farrugia, 2012).

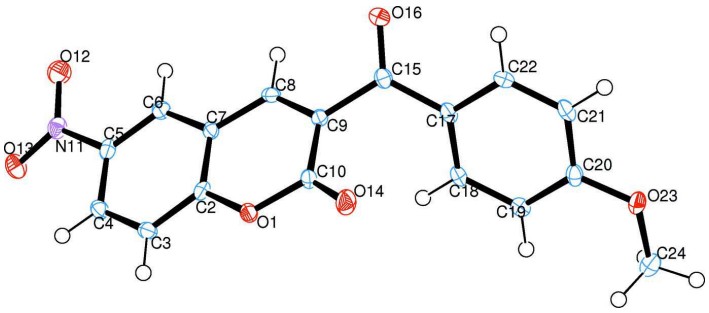

Figure 1

Molecular structure of the title compound with the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level.

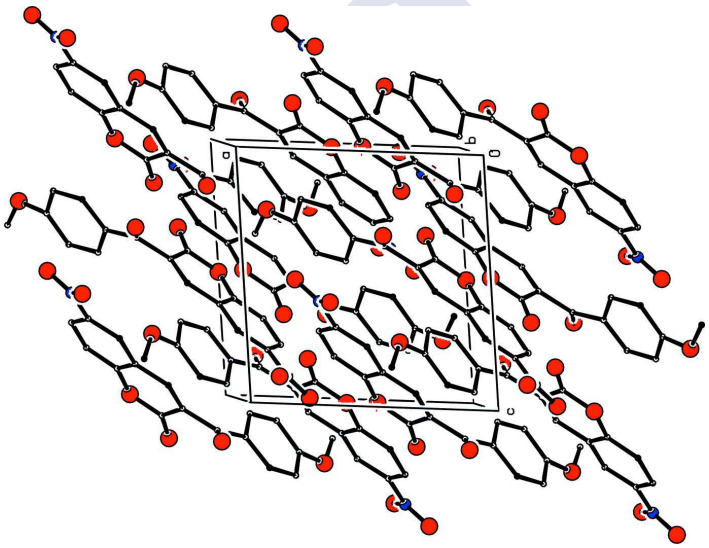

Figure 2

Packing diagram of the title structure viewed along the *b* axis.

3-(4-Methoxybenzoyl)-6-nitrocoumarin

Crystal data

$C_{17}H_{11}NO_6$
$M_r = 325.27$
Monoclinic, $P2_1/n$
$a = 8.875$ (3) Å
$b = 17.266$ (5) Å
$c = 9.174$ (3) Å
$\beta = 95.401$ (15)°
$V = 1399.6$ (7) Å^3
$Z = 4$
$F(000) = 672$
$D_x = 1.544$ Mg m^{-3}
Mo $K\alpha$ radiation, $\lambda = 0.7107$ Å

Cell parameters from 1848 reflections
θ = 2.4–26.2°
μ = 0.12 mm^{-1}

T = 100 K
Prism, colourless
0.67 × 0.14 × 0.03 mm

Data collection

Bruker APEXII CCD diffractometer
Radiation source: fine-focus sealed tube
Graphite monochromator
ω and phi scans
Absorption correction: multi-scan (*SADABS*; Bruker, 2007)
T_{min} = 0.604, T_{max} = 0.745

30736 measured reflections
2864 independent reflections
2200 reflections with $I > 2\sigma(I)$
R_{int} = 0.054
θ_{max} = 26.5°, θ_{min} = 2.4°
h = −11→11
k = 0→21
l = 0→11

Refinement

Refinement on F^2
Least-squares matrix: full
$R[F^2 > 2\sigma(F^2)]$ = 0.043
$wR(F^2)$ = 0.126
S = 0.91
2864 reflections
219 parameters
0 restraints
Primary atom site location: structure-invariant direct methods

Secondary atom site location: difference Fourier map
Hydrogen site location: inferred from neighbouring sites
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0871P)^2 + 0.3903P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} < 0.001$
$\Delta\rho_{max}$ = 0.39 e Å^{-3}
$\Delta\rho_{min}$ = −0.26 e Å^{-3}

Special details

Experimental. ^{1}H NMR (250 MHz, DMSO-d_6) δ p.p.m. 8.29 (d, J = 3.2 Hz, 1H, H-4), 7.75 (dd, J = 9.8, 3.1 Hz, 1H, H-7), 7.69–7.56 (m, 3H, H-5, *o*-H-2, *o*-H-6), 7.04 (d, J = 8.3 Hz, 2H, *m*-H-3, *m*-H5), 6.08 (d, J = 9.6 Hz, 1H, H-8), 3.83 (s, 3H, –OMe); ^{13}C NMR (63 MHz, DMSO-d_6) δ p.p.m. 192.91, 177.98, 166.65, 162.34, 138.75, 131.40, 130.83, 130.47, 129.32, 128.05, 127.28, 121.24, 120.67, 113.84, 55.61; MS EI *m/z* (%): 326 ([*M*+1]+, 25), 325 ([*M*]+, 93), 190 (34), 135 (100), 92 (27), 77 (37); Elem. Anal. Calcd. for $C_{17}H_{11}NO_6$: C, C, 62.77; H, 3.41; Found: C, 62.72; H, 3.32.

Geometry. All s.u.'s (except the s.u. in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell s.u.'s are taken into account individually in the estimation of s.u.'s in distances, angles and torsion angles; correlations between s.u.'s in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell s.u.'s is used for estimating s.u.'s involving l.s. planes.

Refinement. Refinement of F^2 against ALL reflections. The weighted R-factor wR and goodness of fit S are based on F^2, conventional R-factors R are based on F, with F set to zero for negative F^2. The threshold expression of $F^2 > 2\sigma(F^2)$ is used only for calculating R-factors(gt) *etc*. and is not relevant to the choice of reflections for refinement. R-factors based on F^2 are statistically about twice as large as those based on F, and R- factors based on ALL data will be even larger.

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2)

	x	*y*	*z*	U_{iso}*/U_{eq}
C2	0.0307 (2)	0.13803 (12)	0.5978 (2)	0.0137 (4)
C3−	0.0864 (2)	0.16362 (12)	0.6744 (2)	0.0153 (5)
H3−	0.1256	0.2145	0.6595	0.018*
C4−	0.1455 (2)	0.11473 (12)	0.7723 (2)	0.0164 (5)
H4−	0.2265	0.1310	0.8257	0.020*
C5−	0.0842 (2)	0.04053 (13)	0.7918 (2)	0.0156 (5)
C6	0.0352 (2)	0.01480 (13)	0.7193 (2)	0.0147 (5)
H6	0.0751	−0.0358	0.7364	0.018*
C7	0.0969 (2)	0.06452 (12)	0.6200 (2)	0.0131 (4)

C8	0.2244 (2)	0.04597 (12)	0.5416 (2)	0.0135 (4)
H8	0.2701−	0.0036	0.5544	0.016*
C9	0.2809 (2)	0.09697 (11)	0.4506 (2)	0.0131 (4)
C10	0.2034 (2)	0.17126 (12)	0.4186 (2)	0.0149 (5)
C15	0.4152 (2)	0.07478 (12)	0.3707 (2)	0.0146 (5)
C17	0.5336 (2)	0.13255 (12)	0.3473 (2)	0.0147 (5)
C18	0.5631 (2)	0.19667 (12)	0.4378 (2)	0.0144 (5)
H18	0.5055	0.2041	0.5189	0.017*
C19	0.6746 (2)	0.24980 (13)	0.4121 (2)	0.0154 (5)
H19	0.6941	0.2928	0.4756	0.018*
C20	0.7579 (2)	0.23946 (12)	0.2922 (2)	0.0154 (5)
C21	0.7319 (2)	0.17463 (12)	0.2022 (2)	0.0171 (5)
H21	0.7901	0.1671	0.1216	0.021*
C22	0.6222 (2)	0.12166 (12)	0.2302 (2)	0.0146 (5)
H22	0.6064	0.0773	0.1694	0.018*
C24	0.9071 (3)	0.35368 (12)	0.3471 (2)	0.0205 (5)
H24A	0.9528	0.3331	0.4406	0.031*
H24B	0.8180	0.3847	0.3641	0.031*
H24C	0.9809	0.3862	0.3027	0.031*
N11−	0.1513 (2)−	0.01184 (11)	0.89252 (19)	0.0186 (4)
O1	0.08130 (16)	0.18773 (8)	0.49739 (15)	0.0152 (3)
O12−	0.11434 (19)−	0.08064 (9)	0.89156 (18)	0.0273 (4)
O13−	0.24056 (19)	0.01484 (9)	0.97419 (17)	0.0248 (4)
O14	0.23187 (18)	0.21826 (9)	0.32929 (17)	0.0224 (4)
O16	0.42438 (17)	0.00770 (9)	0.32955 (17)	0.0196 (4)
O23	0.86233 (17)	0.29040 (8)	0.24994 (16)	0.0185 (4)

Atomic displacement parameters (Å²)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
C2	0.0131 (10)	0.0141 (10)	0.0138 (10)	−0.0044 (9)	0.0010 (8)	0.0002 (8)
C3	0.0147 (11)	0.0139 (11)	0.0172 (11)	0.0022 (9)	0.0008 (9)	−0.0025 (8)
C4	0.0141 (11)	0.0192 (11)	0.0163 (11)−	0.0001 (9)	0.0030 (9)−	0.0046 (9)
C5	0.0140 (10)	0.0202 (11)	0.0127 (10)	−0.0056 (9)	0.0020 (8)−	0.0014 (9)
C6	0.0145 (11)	0.0150 (11)	0.0143 (11)−	0.0009 (9)	0.0004 (9)−	0.0011 (8)
C7	0.0102 (10)	0.0157 (10)	0.0132 (10)	−0.0015 (9)	0.0003 (8)	−0.0033 (8)
C8	0.0139 (10)	0.0110 (10)	0.0154 (10)	0.0009 (9)	−0.0006 (8)	−0.0031 (8)
C9	0.0120 (10)	0.0137 (11)	0.0140 (10)	−0.0018 (9)	0.0023 (8)	−0.0032 (8)
C10	0.0112 (11)	0.0165 (11)	0.0172 (11)−	0.0016 (9)	0.0034 (9)−	0.0018 (9)
C15	0.0134 (11)	0.0176 (12)	0.0130 (10)	0.0009 (9)	0.0017 (8)	0.0011 (8)
C17	0.0132 (11)	0.0170 (12)	0.0143 (10)	0.0037 (9)	0.0038 (8)	0.0031 (8)
C18	0.0120 (11)	0.0180 (12)	0.0136 (10)	0.0028 (9)	0.0037 (8)	0.0011 (8)
C19	0.0155 (11)	0.0161 (10)	0.0145 (11)	0.0022 (9)	0.0014 (8)	−0.0008 (9)
C20	0.0112 (10)	0.0180 (12)	0.0171 (11)	0.0019 (9)	0.0020 (8)	0.0054 (9)
C21	0.0154 (11)	0.0212 (12)	0.0155 (11)	0.0034 (9)	0.0063 (8)	0.0008 (9)
C22	0.0163 (11)	0.0129 (11)	0.0149 (10)	0.0034 (9)	0.0020 (8)−	0.0015 (8)
C24	0.0198 (12)	0.0183 (11)	0.0232 (12)−	0.0040 (10)	0.0013 (9)	0.0031 (9)
N11	0.0188 (10)	0.0222 (11)	0.0151 (10)−	0.0038 (8)	0.0033 (8)−	0.0001 (8)
O1	0.0138 (8)	0.0141 (7)	0.0183 (8)	0.0015 (6)	0.0047 (6)	0.0019 (6)
O12	0.0319 (10)	0.0213 (9)	0.0304 (10)−	0.0009 (7)	0.0119 (7)	0.0048 (7)

O13	0.0268 (9)	0.0303 (9)	0.0195 (8)−	0.0015 (8)	0.0134 (7)−	0.0017 (7)
O14	0.0203 (9)	0.0209 (8)	0.0271 (9)	0.0003 (7)	0.0076 (7)	0.0086 (7)
O16	0.0204 (8)	0.0160 (8)	0.0234 (9)	0.0007 (7)	0.0070 (7)−	0.0037 (6)
O23	0.0174 (8)	0.0188 (8)	0.0200 (8)−	0.0043 (7)	0.0060 (6)	0.0018 (6)

Geometric parameters (Å, º)

C2—O1	1.365 (2)	C15—C17	1.479 (3)
C2—C3	1.381 (3)	C17—C18	1.394 (3)
C2—C7	1.405 (3)	C17—C22	1.403 (3)
C3—C4	1.372 (3)	C18—C19	1.386 (3)
C3—H3	0.9500	C18—H18	0.9500
C4—C5	1.397 (3)	C19—C20	1.393 (3)
C4—H4	0.9500	C19—H19	0.9500
C5—C6	1.377 (3)	C20—O23	1.361 (3)
C5—N11	1.459 (3)	C20—C21	1.397 (3)
C6—C7	1.400 (3)	C21—C22	1.377 (3)
C6—H6	0.9500	C21—H21	0.9500
C7—C8	1.433 (3)	C22—H22	0.9500
C8—C9	1.343 (3)	C24—O23	1.442 (3)
C8—H8	0.9500	C24—H24A	0.9800
C9—C10	1.472 (3)	C24—H24B	0.9800
C9—C15	1.506 (3)	C24—H24C	0.9800
C10—O14	1.197 (3)	N11—O13	1.230 (2)
C10—O1	1.387 (3)	N11—O12	1.233 (2)
C15—O16	1.223 (3)		
O1—C2—C3	116.95 (18)	C18—C17—C22	118.4 (2)
O1—C2—C7	120.40 (19)	C18—C17—C15	123.04 (19)
C3—C2—C7	122.65 (19)	C22—C17—C15	118.55 (19)
C4—C3—C2	119.25 (19)	C19—C18—C17	121.4 (2)
C4—C3—H3	120.4	C19—C18—H18	119.3
C2—C3—H3	120.4	C17—C18—H18	119.3
C3—C4—C5	118.7 (2)	C18—C19—C20	119.3 (2)
C3—C4—H4	120.7	C18—C19—H19	120.3
C5—C4—H4	120.7	C20—C19—H19	120.3
C6—C5—C4	122.8 (2)	O23—C20—C19	124.6 (2)
C6—C5—N11	118.98 (19)	O23—C20—C21	115.35 (19)
C4—C5—N11	118.18 (19)	C19—C20—C21	120.0 (2)
C5—C6—C7	118.8 (2)	C22—C21—C20	120.1 (2)
C5—C6—H6	120.6	C22—C21—H21	119.9
C7—C6—H6	120.6	C20—C21—H21	119.9
C6—C7—C2	117.68 (19)	C21—C22—C17	120.7 (2)
C6—C7—C8	124.40 (19)	C21—C22—H22	119.6
C2—C7—C8	117.91 (19)	C17—C22—H22	119.6
C9—C8—C7	121.61 (19)	O23—C24—H24A	109.5
C9—C8—H8	119.2	O23—C24—H24B	109.5
C7—C8—H8	119.2	H24A—C24—H24B	109.5
C8—C9—C10	120.03 (19)	O23—C24—H24C	109.5
C8—C9—C15	119.64 (18)	H24A—C24—H24C	109.5

C10—C9—C15	120.09 (18)	H24B—C24—H24C	109.5
O14—C10—O1	116.33 (19)	O13—N11—O12	123.53 (18)
O14—C10—C9	127.0 (2)	O13—N11—C5	118.57 (18)
O1—C10—C9	116.64 (18)	O12—N11—C5	117.90 (18)
O16—C15—C17	121.65 (19)	C2—O1—C10	123.03 (16)
O16—C15—C9	118.01 (19)	C20—O23—C24	117.96 (17)
C17—C15—C9	120.31 (18)		

O1—C2—C3—C4	177.38 (18)	O16—C15—C17—C18−	152.9 (2)
C7—C2—C3—C4−	2.6 (3)	C9—C15—C17—C18	25.3 (3)
C2—C3—C4—C5	0.4 (3)	O16—C15—C17—C22	25.9 (3)
C3—C4—C5—C6	1.4 (3)	C9—C15—C17—C22−155.83 (19)	
C3—C4—C5—N11−	177.98 (19)	C22—C17—C18—C19	1.4 (3)
C4—C5—C6—C7−	1.0 (3)	C15—C17—C18—C19−	179.72 (19)
N11—C5—C6—C7	178.44 (18)	C17—C18—C19—C20	0.8 (3)
C5—C6—C7—C2−	1.2 (3)	C18—C19—C20—O23	175.13 (19)
C5—C6—C7—C8	177.66 (19)	C18—C19—C20—C21−2.1 (3)	
O1—C2—C7—C6−	176.97 (17)	O23—C20—C21—C22−	176.26 (18)
C3—C2—C7—C6	3.1 (3)	C19—C20—C21—C22	1.2 (3)
O1—C2—C7—C8	4.1 (3)	C20—C21—C22—C17	1.0 (3)
C3—C2—C7—C8−	175.89 (19)	C18—C17—C22—C21	−2.3 (3)
C6—C7—C8—C9−	178.2 (2)	C15—C17—C22—C21	178.75 (19)
C2—C7—C8—C9	0.6 (3)	C6—C5—N11—O13	168.4 (2)
C7—C8—C9—C10−	5.7 (3)	C4—C5—N11—O13−12.2 (3)	
C7—C8—C9—C15	179.94 (18)	C6—C5—N11—O12	−11.2 (3)
C8—C9—C10—O14	−171.9 (2)	C4—C5—N11—O12	168.27 (19)
C15—C9—C10—O14	2.5 (3)	C3—C2—O1—C10	176.38 (18)
C8—C9—C10—O1	6.1 (3)	C7—C2—O1—C10−	3.6 (3)
C15—C9—C10—O1−	179.58 (17)	O14—C10—O1—C2	176.75 (18)
C8—C9—C15—O16	35.9 (3)	C9—C10—O1—C2−	1.4 (3)
C10—C9—C15—O16−	138.5 (2)	C19—C20—O23—C24	9.7 (3)
C8—C9—C15—C17	−142.4 (2)	C21—C20—O23—C24−	172.89 (18)
C10—C9—C15—C17	43.2 (3)		

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2210 *Current Topics in Medicinal Chemistry*, 2012, *12*, 2210-2239

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Focusing on New Monoamine Oxidase Inhibitors: Differently Substituted Coumarins As An Interesting Scaffold

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Abstract: It is commonly accepted that monoamine oxidase (MAO) may play a critical role in the regulation of the central nervous system activity and contribute to the pathogenesis of human neurodegenerative and depressive disorders. This has encouraged an active research in the development of new drugs, MAO inhibitors, since they may represent an important advance in the treatment of complex diseases such as Alzheimer and Parkinson, which are becoming prevalent pathologies due to the increase of aging population of developed countries societies. Different research groups are intensively working in this area with the aim of finding new MAO selective inhibitors. Differently substituted coumarins have been synthesized and evaluated as MAO-A and MAO-B inhibitors. The purpose of this review is to summarize the findings reported in this area, particularly focuses on the coumarin scaffold.

Keywords: Coumarins, Alzheimer disease, parkinson disease, monoamine oxidase, MAO inhibitors.

1. INTRODUCTION

Monoamine oxidase (MAO) is a FAD-depending enzyme found in the outer mitochondrial membrane of neuronal, glial and other mammalian cells [1]. This enzyme is responsible for catalyzing the oxidative deamination of neurotransmitters and dietary amines, regulating intracellular levels of biogenic amines in the brain and the peripheral tissues [2, 3]. It is well-known that MAO plays a critical role in the regulation of central nervous system activity and contributes to the pathogenesis of human neurodegenerative and depressive disorders [4]. Fifty years ago the first generation of MAO inhibitors (MAOIs) was developed and applied in therapy as anti-depressive compounds. However, for many years MAOIs were considered useless in therapy due to the serious side effects induced by these drugs [5]. Later, two enzymatic isoforms, named MAO-A and MAO-B, have been identified on the basis of their amino acid sequences [6], three-dimensional structure [7], tissue distribution [8], inhibitor selectivity [9] and substrate preferences [10]. The MAO-A isoform has higher affinity for serotonin and noradrenaline, whereas the MAO-B isoform, preferentially deaminates β-phenylethylamines and benzylamine [11, 12]. On the other hand, dopamine and tyramine are common substrates for both isoforms. As a result of these physiological properties, MAOIs became again the center of scientific and pharmacological interest, providing new drugs for the therapy of Parkinson´s diseases (PD), Alzheimer´s disease (AD) and various types of depression [13]. Additionally, the description of the crystal structure of the two MAO isoforms by C. Binda *et al.* has provided relevant information about the selective interactions and the pharmacophoric requirements needed for the design of potent and selective inhibitors [14-18].

MAO-A plays an important role in neuropsychiatric and behavioural disorders. The importance of this isoform is suggested by the aggressive phenotype seen in male mice deficient in MAO-A and in males in a Dutch kindred bearing a spontaneous mutation (resulting in a premature stop) in the *mao*-A gene [19]. Similarly, maltreated children, whose genotype confers low levels of MAO-A expression, more often develop conduct disorder, antisocial personality and adult violent crime than children with a high-activity MAO-A genotype do [20]. Relatively modest changes in MAO-A activity/function can have important neuropsychiatric consequences as demonstrated by the fact that [^{11}C]-harmine-labeled is elevated by only 34% throughout the brain of untreated depressed patients compared to controls, yet it appears to be the major contributor to the monoamine metabolism in these same patients [21]. Depression not only may promote cognitive impairment, but also may be a risk factor for AD [22]. Not surprisingly, MAO-A which is often a target for the treatment of depression, is also a potential risk factor for late-onset AD [23-26]. In contrast to irreversible MAOI-A as clorgyline, reversible inhibitors are better tolerated and have been particularly efficacious in treating depression [27] and cognitive disorders [28] in the elderly. In addition, inhibition of MAO-A activity protect against striatal damage produced by the mitochondrial poison malonate and appears to rely on attenuation of dopamine-derived reactive oxygen species (ROS) [29], while apoptosis following serum starvation is reduced in MAO-A deficient cortical brain cells [30].

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This manuscript is dedicated to Prof Francisco Orallo in memoriam, who had introduced and incentivated us in the study of the MAOIs.

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On the other hand, it has been demonstrated that selective and irreversible MAOIs-B, such as selegiline and rasagiline, are important molecules in the therapy of PD as well as in patients with mild AD-type dementia [31-33]. Both MAO-B positive astrocytes [34] and ROS [35] have been found in the vicinity of *β*-amyloid (A*β*) plaques. Selegiline and rasagiline can also exert neuroprotection at lower concentrations than those required for inhibition of the enzyme [36]. It has been associated with activation of Bcl-2 family members, interaction with the mitochondrial pore complex and modulation of amyloid precursor protein cleavage [37].

The importance of the discovery of drugs associated with neurodegenerative diseases (ND) has emerged with the significant increase of life expectancy.

Coumarins are a wide family of compounds present in remarkable amounts in the nature [38, 39]. Representative compounds occur for instance in the vegetable kingdom, either in free or combined state [40, 41]. The versatile and efficient synthetic accessibility and the substitution capability make them privileged scaffolds not only in organic chemistry but also in the medicinal chemistry field [42]. In the literature, coumarin derivatives have been described as anticancer [43, 44], antioxidant or anti-inflammatory [45, 46], vasorelaxant [47], antimicrobial [48, 49], antiviral [50, 51], and enzymatic inhibitors [52-54].

In 1981, it was found for the first time an interesting MAOI activity of the coumarin moiety, when an organophosphate group was attached to the umbelliferone ring [55]. But it was only in the 1990´s when the coumarin nucleus emerged as a promising scaffold for MAOIs [56]. In particular, some coumarins isolated from *Psoralea corylifolia* L., *Peucedanum japonicum* L. or *Monascus anka* K. have been described as MAOIs [57-59]. Although natural coumarins generally show low MAO inhibitory potency, properly modified natural coumarins have been characterized as potent and selective MAOIs. For example, the desmethyl congener of geiparvarin, a natural 7-substitued coumarin from the leaves of *Geijera parviflora* L., exhibites potent and selective MAO-B inhibition Fig. (**1**) [60].

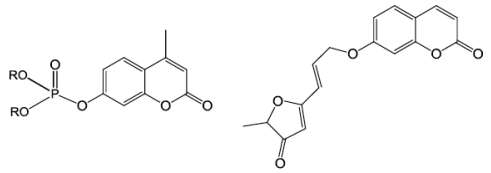


Fig. (1). Umbelliferone and geiparvarin derivatives.

Several research groups have been exploring the importance of the number and position of different substituents on the coumarin nucleus, taking into account volume, lipophilicity, steric impediment, electron withdrawing and electron donating characteristics. Different substituents were attached to the coumarin skeleton with the aim of improving both activity and selectivity Fig. (**2**). Although positions 3 and 7 of the coumarin ring were the most studied, all the substitution possibilities were taken into account and a big number of coumarin derivatives have been synthesized and evaluated as MAOIs, being most of them active and selective against MAO-B isoenzyme [61-81].

It is important to mention that the IC_{50} values given in the following tables resulted from different experimental settings.

2. COUMARIN DERIVATIVES AND MAO ACTIVITY

The first structure-activity relationship studies (SAR) about coumarin derivatives suggested that selectivity was mainly determined by the nature of the linkage between the coumarin and the substituent group at position 7. A series of 7-hydroxycoumarin derivatives **I** bearing at position 7 ether, ester or carbamate functions of varying size and lipophilicity have been investigated for their *in vitro* MAO-A and -B inhibitory activities. Most of these compounds resulted to be preferentially MAOIs-B. SAR studies also showed that lipophilicity is an important property to modulate the MAO-B inhibition potency of the above mentioned coumarins [61-68]. A 7-benzyloxy group proved to be the favouring substituent for the binding of coumarins to MAO-B, whereas a π-electron-poor phenyl ring at position 7 seems to improve both MAO-A and MAO-B inhibitiory activities, leading to more active but slightly less selective inhibitors [62]. A. Carotti *et al.* described a large family of compounds related to these structures. On the other hand, it has been proved that an alkylsulfonyloxy group at position 7 provided MAOIs-A like esuprone. This effect is even more pronounced in the phenyl sulfonates in which the substitution with electron withdrawing groups lead to more potent and highly selective MAOIs-A. Also, B. Rendenbach-Mueller *et al.* synthesized and studied the potential of ether derivatives and sulfonic acid esters Fig. (**3**), checking that introduction of a sulfonic ester linkage, instead of the ether bridge, dramatically changed the activity profile of the new compounds [63].

Substitutions at position 3 and/or 4 of the coumarin nucleus also contribute to modulate MAO-B inhibitory activity and A/B selectivity of the previously described derivatives. It can be inferred that the MAO-B binding site should accept lipophilic substituents of limited size at positions 3 and/or 4 of the coumarin nucleus, whereas hydrophilic or larger hydrophobic groups are not well tolerated [62]. However, derivatives of 7-benzyloxycoumarins bearing properly selected polar substituents at position 4 led to new MAOIs-B with an improved pharmacokinetic profile and a higher druggability [64].

B. Rendenbach-Müller *et al.* also explored the importance of the presence of a heteroarylalkoxy group at position 7 of the coumarin scaffold to modulate the activity against MAO isoforms [56]. In this study, small alkyl, phenyl or halogen groups were introduced at positions 3 and 4 of the skeleton. The same authors evaluated and described heteroarylalkoxycoumarins as potent and selective MAOIs-B [63, 65, 68]. Only small substituents, as methyl groups, were introduced at positions 3 and 4 of this coumarin nucleus.

Based on previously described SAR studies, introduction of cycloaliphatic or benzene rings fused at 3,4-positions was performed on 7-hydroxycoumarin derivatives. A. Carotti *et al.* and B. Rendenbach-Mueller *et al.* described series of these derivatives with interesting MAO-B properties [62, 65,

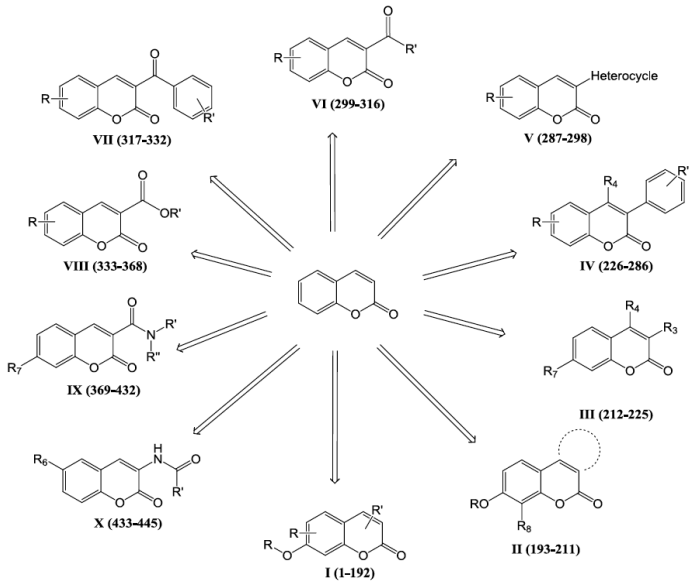


Fig. (2). Chemical structures of known coumarins as MAOIs.

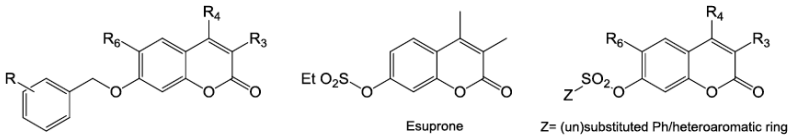


Fig. (3). 7-Alkyloxy and sulfonyloxy coumarin derivatives.

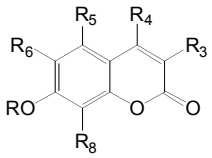
66]. L. Santana *et al.* have reported that annelation with 5- and 6-membered rings on the coumarin nucleus, in addition to 7-acetonyloxy-substitutents, showed higher activity against both MAO isoforms than the 7-hydroxy precursors [66]. These derivatives presented different MAO-A/MAO-B selectivity profiles, as shown in the (Table **2**).

In order to complete the study of 7-substituted coumarin derivatives, A. Carotti *et al.* synthesized and evaluated the *in vitro* biological MAO activity of novel series of coumarins bearing different substituents at position 7. Such substituents consisted of a phenyl ring linked to a coumarin core by bridges of different size, length and lipophilic and electronic nature. In general, isosteric variations of the oxymethylene linker lead to inhibitors with lower MAO-B affinity and B/A selectivity [62, 67].

The reported results for 7-substituted coumarins containing a benzene ring fused at 3,4 positions encouraged M. J. Matos *et al.* to analyze the importance of the aryl substituent on the pyrone ring, synthesizing a series of 3-arylcoumarin derivatives **IV**. The substitution at C7 was removed and small substituents were introduced either at C6 [69-74] or/and C8 [75, 76] positions. The high MAO-B selectivity found for this new 3-arylcoumarin scaffold encouraged the authors to synthesize and study the MAO inhibitory activity of new analogues. A variety of groups with different size, electronic and lipophilic properties were introduced in both aromatic rings, in order to clarify the influence of the substitution pattern in the MAO inhibitory activity and selectivity of the 3-arylcoumarin skeleton [69-76]. Most active compounds present *para* or *meta* substitutions in the 3-phenyl ring. The best compound of the described series is the 6-methyl-3-(4'-methylphenyl)coumarin (compound **245**), with an IC_{50} of 0.31 nM. This compound proved to be not only 64 times more active *in vitro* than selegiline (the reference compound) but also several times more selective than it (compound **245** is > 97 fold more selective than selegiline) [72].

Additionally, a hydroxyl group at position 4 of the coumarin skeleton was introduced. This modification allowed to consider the similarity of the core structure of the 4-hydroxycoumarin comparing with the 2-hydroxyisoflavone. Taking into account the obtained results, it could be estab-

Table 1. Chemical Structures and MAO Inhibition Data of 7-hydroxycoumarin Derivatives I (Compounds 1-192)



Cpds	R	R_3	R_4	R_5	R_6	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
1	H	H	CH_3	H	H	OCH_3	80.30	> 100	66
2	H	H	CH_3	H	H	CH_3	> 100	71.32	66
3	H	H	CH_3	H	H	H	> 100	80.41	66
4	H	NH_2	H	H	H	OCH_3	> 100	> 100	66
5		CH_3	CH_3	H	H	H	11.0	0.004	61
6		CH_3	CH_3	H	H	H	9.20	0.94	61
7		CH_3	CH_3	H	H	H	5.90	0.11	61
8		CH_3	CH_3	H	H	H	7.70	0.014	61
9		CH_3	CH_3	H	H	H	4.10	0.004	61
10		CH_3	CH_3	H	H	H	20 (15%)	0.028	61
11		CH_3	CH_3	H	H	H	10 (0%)	10 (27%)	61
12		CH_3	CH_3	H	H	H	34.0	0.89	61
13		CH_3	CH_3	H	H	H	0.70	0.84	61
14		CH_3	CH_3	H	H	H	1.13	0.14	61
15		H	H	H	H	H	> 100	0.044	66
16		H	CH_3	H	H	H	0.041	0.006	66
17		H	CH_3	H	H	OCH_3	2.66	0.86	66
18		H	CH_3	H	H	CH_3	0.007	0.011	66
19		H	CH_3	H	H	H	43.0	> 100	66
20		H	CH_3	H	H	OCH_3	15.45	> 100	66
21		H	CH_3	H	H	CH_3	0.40	0.004	66
22	CH_3	CH_3	CH_3	H	H	H	0.071	0.0005	63

(Table 1) contd….

Cpds	R	R_3	R_4	R_5	R_6	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
23		CH_3	CH_3	H	H	H	31.60	3.24	67
24		Cl	CH_3	H	H	H	> 10	0.004	56
25		H	H	H	H	H	6.76	0.055	62
26		CH_3	H	H	H	H	5.50	0.007	67
27		H	CH_3	H	H	H	1.95	0.018	62
28		CH_3	CH_3	H	H	H	0.69	0.004	62
29		H	OH	H	H	H	15 (26%)	1.58	62
30		H	CF_3	H	H	H	> 5	1.38	62
31			CH_3	H	H	H	3 (4%)	3 (8%)	62
32		H		H	H	H	> 4	> 4	62
33		CH_3	CH_3	H	H	CH_3	0.56	3.31	62
34		CH_3	CH_3	H		H	> 0.4	> 0.4	62
35		CH_3	CH_3	H	OH	H	112.20	3.09	62
36		CH_3	CH_3	H	H	H	3.55	0.32	62
37		CH_3	CH_3	H	H	H	> 0.54	0.001	56
38		CH_3	CH_3	H	H	H	0.39	0.001	56
39		CH_3	CH_3	H	H	H	0.56	0.001	56
40		CH_3	CH_3	H	H	H	> 10	0.004	56
41		H	H	H	H	H	> 10	0.002	56
42		H	CH_3	H	H	H	> 10	0.005	56
43		CH_3	H	H	H	H	> 10	0.003	56
44		CH_3	CH_3	H	H	H	> 10	0.007	56
45		CH_3	CH_3	H	Cl	H	> 10	0.031	56
46		CH_3	CH_3	H	H	CH_3	> 100	> 100	63
47		CH_3	CH_3	H	H	H	> 10	10.0	63
48		CH_3	CH_3	H	H	H	> 10	0.032	56
49		CH_3	CH_3	H	H	H	0.30	0.001	56
50		CH_3	CH_3	H	H	H	> 10	0.002	56

(Table 1) contd….

Cpds	R	R_3	R_4	R_5	R_6	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
51		CH_3	CH_3	H	H	H	1.51	0.004	62
52		CH_3	CH_3	H	H	H	2.10	0.0006	56
53		CH_3	CH_3	H	H	H	5.89	0.011	62
54		CH_3	CH_3	H	H	H	0.58	0.003	62
55		CH_3	CH_3	H	H	H	0.12	0.003	62
56		CH_3	CH_3	H	H	H	0.12	0.001	62
57		CH_3	CH_3	H	H	H	0.68	0.003	62
58		CH_3	CH_3	H	H	H	0.11	0.007	62
59		CH_3	CH_3	H	H	H	0.69	0.006	62
60		CH_3	H	H	H	H	12.59	0.005	67
61		CH_3	CH_3	H	H	H	1.12	0.003	62
62		CH_3	CH_3	H	H	H	0.12	0.003	62
63		H	CH_3	H	H	H	0.13	0.013	62
64		CH_3	CH_3	H	H	H	0.076	0.003	62
65		CH_3	CH_3	H	H	H	0.83	0.009	62
66		CH_3	CH_3	H	H	H	0.18	0.006	62
67		CH_3	CH_3	H	H	H	1.0	0.035	62
68		CH_3	CH_3	H	H	H	7.08	0.25	62
69		CH_3	CH_3	H	H	H	0.42	0.010	62
70		CH_3	CH_3	H	H	H	0.42	0.023	62
71		CH_3	CH_3	H	H	H	0.22	0.011	62
72		CH_3	CH_3	H	H	H	0.10	0.004	62
73		CH_3	CH_3	H	H	H	1.0	0.006	62
74		CH_3	CH_3	H	CH_2CH_3	H	> 10	0.010	56
75		CH_3	CH_3	H	H	H	10.0	0.071	67

(Table 1) contd….

Cpds	R	R_3	R_4	R_5	R_6	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
76		CH_3	CH_3	H	H	H	9.77	0.018	67
77		H	H	H	H	H	1.50	0.004	56
78		CH_3	CH_3	H	H	H	1.20	0.004	56
79		CH_3	CH_3	H	H	H	10.23	0.027	67
80		CH_3	CH_3	H	H	H	31.62	0.11	67
81		H	H	H	H	H	100.0	0.058	67
82		CH_3	CH_3	H	H	H	1.80	0.009	63
83		CH_3	CH_3	H	Cl	H	> 10	0.0007	65
84		CH_3	CH_3	H	H	H	> 10	0.011	65
85		CH_3	CH_3	H	H	H	10.0	0.008	65
86		CH_3	CH_3	H	H	H	> 10	0.002	65
87		CH_3	CH_3	H	H	H	110.0	> 100	63
88		CH_3	CH_3	H	H	H	0.008	5.0	63
89		CH_3	CH_3	H	H	H	0.016	2.20	63
90		CH_3	CH_3	H	H	H	0.034	> 10	63
91		H	H	H	H	H	0.45	54.95	67
92		CH_3	CH_3	H	H	H	0.024	1.10	63
93		CH_3	CH_3	H	H	H	0.047	15 (17%)	62
94		CH_3	CH_3	H	H	H	0.071	16.98	62
95		CH_3	CH_3	H	H	H	0.005	0.004	63
96		CH_3	CH_3	H	H	H	0.008	> 100	63
97		CH_3	CH_3	H	H	H	0.006	> 100	63
98		CH_3	CH_3	H	H	H	0.013	2 (20%)	62
99		CH_3	CH_3	H	H	H	2.0	100.0	67
100		CH_3	CH_3	H	H	H	2.82	0.87	67
101		CH_3	CH_3	H	H	H	> 10	0.017	65
102		CH_3	CH_3	H	H	H	0.35	0.0005	65
103		CH_3	CH_3	H	H	H	0.23	0.0005	65
104		CH_3	CH_3	H	H	H	> 10	0.001	65

(Table 1) contd….

Cpds	R	R_3	R_4	R_5	R_6	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
105		CH_3	CH_3	H	H	H	10.0	0.003	65
106		CH_3	CH_3	H	H	H	3.20	0.006	65
107		CH_3	CH_3	H	H	H	10.0	0.001	68
108		CH_3	CH_3	H	H	H	> 10	0.003	65
109		Cl	CH_3	H	Cl	H	> 10	0.002	65
110		CH_3	CH_3	H	H	H	10.0	0.001	65
111		Cl	CH_3	H	Cl	H	> 10	0.002	65
112		CH_3	CH_3	H	H	H	1.50	0.002	65
113		CH_3	CH_3	H	H	H	10.0	0.002	65
114		CH_3	CH_3	H	H	H	10.0	0.005	65
115		CH_3	CH_3	H	H	H	6.0	0.0007	65
116		CH_3	CH_3	H	H	H	2.90	0.001	65
117		CH_3	CH_3	H	H	H	10.0	0.002	65
118		CH_3	CH_3	H	H	H	> 10	0.001	65
119		CH_3	CH_3	H	H	H	> 10	0.001	65
120		CH_3	CH_3	H	H	H	7.0	0.001	65
121		CH_3	CH_3	H	H	H	> 10	0.001	65
122		CH_3	CH_3	H	H	H	> 10	0.001	65
123		CH_3	CH_3	H	H	H	> 10	0.014	65
124		CH_3	CH_3	H	H	H	20.0	0.0006	65
125		CH_3	CH_3	H	H	H	5.0	0.004	65
126		CH_3	CH_3	H	H	H	10.0	0.005	65
127		CH_3	CH_3	H	H	H	> 10	0.002	65

(Table 1) contd….

Cpds	R	R_3	R_4	R_5	R_6	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
128		Cl	CH_3	H	H	H	> 10	0.002	65
129		CH_3	CH_3	H	CH_2CH_3	H	> 10	0.009	65
130		CH_3	CH_3	H	H	H	> 10	0.010	65
131		CH_3	CH_3	H	H	H	5.60	0.015	65
132		CH_3	CH_3	H	H	H	10.0	0.001	65
133		CH_3	CH_3	H	H	H	> 10	0.008	65
134		CH_3	CH_3	H	H	H	4.0	0.004	65
135		CH_3	CH_3	H	H	H	> 10	0.001	65
136		CH_3	CH_3	H	H	H	7.0	0.001	65
137		CH_3	CH_3	H	H	H	> 10	0.013	65
138		CH_3	CH_3	H	H	H	> 10	0.001	68
139		CH_3	CH_3	H	Cl	H	> 10	0.003	68
140		CH_3	CH_3	H	H	H	3.60	0.003	65
141		CH_3	CH_3	H	CH_2CH_3	H	> 10	0.007	65
142		CH_3	H	H	H	H	> 10	0.015	65
143		Cl	CH_3	H	H	H	> 10	0.006	65
144		CH_3	CH_3	H	Br	H	> 10	0.001	65
145		CH_3	CH_3	H	H	H	> 10	0.002	68
146		H		H	H	H	1.44	0.040	64
147		H		H	H	H	2.50	0.090	64
148		H		H	H	H	> 100	> 10	64

(Table 1) contd….

Cpds	R	R_3	R_4	R_5	R_6	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
149		H		H	H	H	8.0	0.20	64
150		H		H	H	H	26.0	0.030	64
151		H		H	H	H	70.0	0.050	64
152		H		H	H	H	4.27	1.0	64
153		H		H	H	H	2.80	0.015	64
154		H		H	H	H	0.50	0.024	64
155		H		H	H	H	> 100	> 10	64
156		H		H	H	H	> 100	5.0	64
157		H		H	H	H	23.10	0.40	64
158		H		H	H	H	> 100	0.040	64
159		H		H	H	H	> 100	> 10	64
160		H		H	H	H	21.0	1.30	64
161		H		H	H	H	26.50	2.0	64
162		H		H	H	H	6.26	0.10	64
163		H		H	H	H	2.0	0.10	64
164		H		H	H	H	15.40	0.028	64
165		H		H	H	H	5.94	0.013	64
166		H		H	H	H	13.50	0.018	64

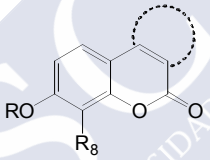
(Table 1) contd….

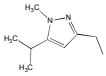
Cpds	R	R_3	R_4	R_5	R_6	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
167		H		H	H	H	5.40	0.020	64
168		H		H	H	H	22.60	0.10	64
169		H		H	H	H	91.0	3.80	64
170		H		H	H	H	> 100	3.60	64
171		H		H	H	H	100.0	1.80	64
172		H		H	H	H	50.80	1.12	64
173		H		H	H	H	> 100	0.51	64
174		H		H	H	H	> 100	8.0	64
175		H		H	H	H	4.40	0.021	64
176		H		H	H	H	2.0	0.015	64
177		H		H	H	H	0.20	0.23	64
178		H		H	H	H	0.47	0.016	64
179		H		H	H	H	100.0	0.68	64
180		H		H	H	H	10 (35%)	0.21	64
181		H		H	H	H	99.80	16.10	64
182		H		H	H	H	10 (5%)	9.60	64
183		H		H	H	H	> 100	2.40	64
184		H		H	H	H	22.70	1.80	64
185		H		H	H	H	35.0	0.38	64
186		H		H	H	H	> 100	0.85	64

(Table 1) contd….

Cpds	R	R_3	R_4	R_5	R_6	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
187		H		H	H	H	31.80	0.25	64
188		H		H	H	H	74.0	0.30	64
189		H		H	H	H	25.90	0.46	64
190	H	H	H	H		H	23.44	2.04	62
191	H	CH_3	CH_3	H		H	112.20	3.09	62
192	H	CH_3	CH_3		H	H	30 (35%)	30 (18%)	62

Table 2. Chemical Structures and MAO Inhibition Data of Substituted Coumarins with 3,4-fused Cycloaliphatic or Benzene Rings II (Compounds 193-211)

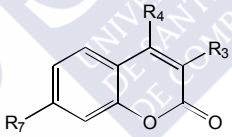


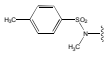
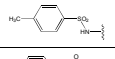
Cpds	R	R_3	R_4	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
193				H	1.58	0.003	62
194				H	1.58	0.003	62
195				H	1 (7%)	0.050	62
196				H	> 10	0.007	65
197				H	> 10	0.10	65
198	H			H	48.20	> 100	66
199	H			OCH_3	63.53	> 100	66
200	H			CH_3	> 100	> 100	66
201	H			OCH_3	> 100	50.20	66
202	H			CH_3	60.11	> 100	66
203				OCH_3	0.12	12.98	66

(Table 2) contd….

Cpds	R	R_3	R_4	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
204				CH_3	0.24	0.17	66
205				OCH_3	0.37	12.12	66
206				CH_3	0.004	17.30	66
207				OCH_3	11.93	9.36	66
208				CH_3	2.56	28.13	66
209				CH_3	0.13	1.18	66
210				CH_3	0.70	0.24	66
211				CH_3	2.38	0.001	66

Table 3. Chemical Structures and MAO Inhibition Data of Coumarins Bearing a Carbo, Thio or Aza Spacer at Position 7 III (212-225)

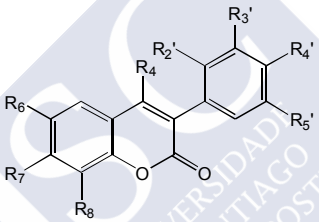


Cpds	R_7	R_3	R_4	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
212		CH_3	CH_3	0.41	0.028	62
213		CH_3	CH_3	10.0	0.040	67
214		CH_3	CH_3	18.62	2.75	67
215		CH_3	CH_3	4.57	31.62	67
216		CH_3	CH_3	67.61	40 (29%)	62
217		CH_3	CH_3	1.38	0.19	62
218		CH_3	CH_3	1.58	0.16	62
219		CH_3	CH_3	7.08	0.46	67

(Table 3) contd….

Cpds	R_7	R_3	R_4	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref.
220		H	H	0.39	0.085	62
221		H	H	41.69	2.14	62
222		H	H	3.80	1.29	67
223		H	H	6.17	0.58	67
224		H	H	21.88	25.70	67
225		H	H	9.55	2.40	67

Table 4. Chemical Structures and MAO Inhibition Data of Differently Substituted 3-arylcoumarins IV (Compounds 226-286)



Cpds	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	R_4	R_6	R_7	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
226	H	H	H	H	H	H	H	H	> 100	11.81	74
227	OH	H	H	H	H	Cl	H	H	> 100	0.44	69
228	H	OH	H	H	H	Cl	H	H	> 100	0.21	69
229	H	H	OH	H	H	Cl	H	H	> 100	0.13	69
230	OCH_3	H	H	H	H	Cl	H	H	> 100	> 100	69
231	H	OCH_3	H	H	H	Cl	H	H	> 100	0.001	69
232	H	H	OCH_3	H	H	H	H	H	> 100	0.004	69
233	OH	H	H	H	H	Br	H	H	> 100	0.48	69
234	H	OH	H	H	H	Br	H	H	> 100	0.29	69
235	H	H	OH	H	H	Br	H	H	> 100	0.64	69
236	OCH_3	H	H	H	H	Br	H	H	> 100	> 100	69
237	H	OCH_3	H	H	H	Br	H	H	> 100	0.002	69
238	H	H	OCH_3	H	H	Br	H	H	> 100	0.006	69
239	H	H	H	H	H	Br	H	OCH_3	> 100	0.083	73
240	H	H	H	H	H	Br	H	OH	> 100	30.91	73
241	H	H	OCH_3	H	H	Br	H	OCH_3	> 100	1.35	73

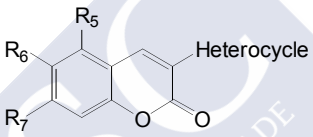
(Table 4) contd….

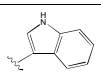
Cpds	R_2,	R_3,	R_4,	R_5,	R_4	R_6	R_7	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
242	H	H	OH	H	H	Br	H	OH	20.74	16.87	73
243	H	H	H	H	H	CH_2Br	H	H	> 100	0.043	69
244	H	H	H	H	H	CH_3	H	H	> 100	0.28	72
245	H	H	CH_3	H	H	CH_3	H	H	> 100	$0.31x\ 10^{-3}$	72
246	H	CH_3	H	H	H	CH_3	H	H	> 100	0.015	72
247	H	OCH_3	H	H	H	CH_3	H	H	> 100	$0.80x\ 10^{-3}$	72
248	H	OH	H	H	H	CH_3	H	H	35.04	0.65	72
249	H	H	OCH_3	H	H	CH_3	H	H	> 100	0.013	72
250	H	H	OH	H	H	CH_3	H	H	> 100	0.16	72
251	H	OCH_3	OCH_3	H	H	CH_3	H	H	25.14	0.003	72
252	H	OCH_3	H	OCH_3	H	CH_3	H	H	> 100	0.009	72
253	H	OCH_3	OCH_3	OCH_3	H	CH_3	H	H	> 100	0.16	72
254	H	Br	OCH_3	H	H	CH_3	H	H	> 100	$0.74x\ 10^{-3}$	72
255	H	OCH_3	Br	H	H	CH_3	H	H	> 100	0.003	72
256	H		H	H	H	CH_3	H	H	> 100	0.18	72
257	OCH_3	H	H	H	H	CH_3	H	H	> 100	> 100	72
258	OH	H	H	H	H	CH_3	H	H	21.58	0.12	72
259	Br	H	H	H	H	CH_3	H	H	> 100	4.34	75
260	Br	OCH_3	H	OCH_3	H	CH_3	H	H	> 100	54.03	72
261	Br	OCH_3	OCH_3	OCH_3	H	CH_3	H	H	> 100	> 100	72
262	H	H	H	H	H	CH_3	H	Br	> 100	0.011	75
263	H	H	OCH_3	H	H	CH_3	H	Br	> 100	0.003	75
264	H	OCH_3	H	OCH_3	H	CH_3	H	Br	> 100	0.007	75
265	H	OCH_3	OCH_3	OCH_3	H	CH_3	H	Br	31.20	4.89	75
266	H	CH_3	H	H	H	OCH_3	H	H	> 100	0.018	72
267	H	H	CH_3	H	H	OCH_3	H	H	> 100	0.002	72
268	H	H	CH_3	H	H	OH	H	H	24.90	0.067	72
269	H	H	CH_3	H	H		H	H	> 100	5.52	72
270	H	H	CH_3	H	H		H	H	> 100	14.47	72
271	H	H	H	H	H	H	H	CH_3	6.26	0.078	76
272	H	H	OCH_3	H	H	H	H	CH_3	21.60	0.003	76
273	H	H	CH_3	H	H	H	H	CH_3	> 100	0.005	76
274	H	H	Br	H	H	H	H	CH_3	> 100	0.020	76
275	H	H	H	H	H	H	H	OCH_3	> 100	0.15	76
276	H	H	Br	H	H	H	H	OCH_3	> 100	0.003	76
277	H	H	H	H	H	H	H	OCH_2CH_3	> 100	1.06	76

(Table 4) contd….

Cpds	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	R_4	R_6	R_7	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
278	H	H	OCH_3	H	H	H	H	OCH_2CH_3	> 100	0.21	76
279	H	H	CH_3	H	H	H	H	OCH_2CH_3	> 100	0.15	76
280	H	H	H	H	OH	H	H	H	> 100	> 100	77
281	H	H	OCH_3	H	OH	H	H	H	> 100	69.59	77
282	H	H	OCH_3	H	OH	CH_3	H	H	> 100	32.04	77
283	H	H	OCH_3	H	OH	Cl	H	H	> 100	> 100	77
284	H	Cl	OCH_3	H	OH	H	H	H	> 100	9.26	77
285	H	Cl	OCH_3	H	OH	CH_3	H	H	> 100	42.68	77
286	H	Cl	OCH_3	H	OH	Cl	H	H	> 100	2.79	77

Table 5. Chemical Structures and MAO Inhibition Data of Differently Substituted 3-heteroarylcoumarins V (Compounds 287-298)

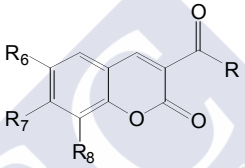


Cpds	R_3	R_5	R_6	R_7	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
287		H	H	H	> 100	6.37	79
288		H	OCH_3	H	> 100	0.63	79
289		H	H	OCH_3	> 100	0.26	79
290		OCH_3	H	OCH_3	> 100	> 100	79
291		H	H	H	> 100	13.30	79
292		H	OCH_3	H	> 100	0.23	79
293		H	H	OCH_3	4.16	0.056	79
294		OCH_3	H	OCH_3	> 100	> 100	79
295		H	H	H	> 100	1.92	79

(Table 5) contd….

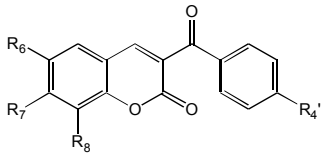
Cpds	R_3	R_5	R_6	R_7	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
296		H	OCH_3	H	> 100	> 100	79
297		H	H	OCH_3	9.67	0.046	79
298		OCH_3	H	OCH_3	> 100	> 100	79

Table 6. Chemical Structures and MAO Inhibition Data of 3-carbonylcoumarin Derivatives VI (Compounds 299-316)



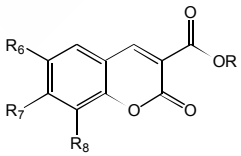
Cpds	R	R_6	R_7	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
299	CH_3	H	H	H	> 100	14.45	78
300	CH_3	H	H	OH	39.36	42.13	78
301	CH_3	H	OH	H	> 100	> 100	78
302	CH_3	OH	H	H	100 (45%)	64.17	78
303	CH_3	H	OCH_3	H	100 (45%)	15.40	78
304	CH_3	H	$N(CH_2CH_3)_2$	H	> 100	18.07	78
305	CH_3	CH_3	H	H	> 100	100 (45%)	78
306	CH_3	Cl	H	H	> 100	17.72	78
307	CH_3	Br	H	H	51.63	100 (45%)	78
308	CH_3			H	100 (45%)	2.01	78
309	CH_3	Cl	H	Br	55.16	0.14	78
310	CH_3	Br	H	Br	> 100	0.20	78
311	CH_3	I	H	I	> 100	>100	78
312	Cl	H	H	H	0.060	0.12	80
313	Cl	CH_3	H	H	0.029	0.10	80
314	Cl	Br	H	H	0.020	0.11	80
315	Cl	Cl	H	H	0.030	0.010	80
316	Cl	NO_2	H	H	0.029	0.10	80

Table 7. Chemical Structures and MAO Inhibition Data of 3-benzoylcoumarin Derivatives VII (Compounds 317-332)



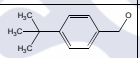
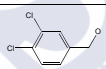
Cpds	$R_{4'}$	R_6	R_7	R_8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
317	H	H	H	H	> 100	> 100	78
318	H	Br	H	H	> 100	> 100	78
319	H	Br	H	OCH_3	> 100	> 100	73
320	H	Br	H	OH	46.81	73.92	73
321	OH	Br	H	OH	19.17	> 100	73
322	OCH_3	Br	H	OCH_3	> 100	> 100	73
323	H	H	H	OH	40.47	27.11	78
324	H	OH	H	H	51.66	17.97	78
325	H	H	OH	H	> 100	> 100	78
326	H	H	OCH_3	H	> 100	100 (45%)	78
327	H	H	$N(CH_2CH_3)_2$	H	> 100	23.84	78
328	H	CH_3	H	H	> 100	100 (45%)	78
329	H	Cl	H	H	> 100	> 100	78
330	H	Cl	H	Br	> 100	100 (45%)	78
331	H	Br	H	Br	> 100	100 (45%)	78
332	H	I	H	I	> 100	> 100	78

Table 8. Chemical Structures and MAO Inhibition Data of 3-carboxycoumarin Derivatives VIII (Compounds 333-368)



Cpds	R	R^6	R^7	R^8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
333	CH_2CH_3	H	H	H	0.23	5.01	80
334	CH_2CH_3	H	H	OH	40.55	14.81	78
335	CH_2CH_3	H	OH	H	> 100	> 100	78
336	CH_2CH_3	OH	H	H	49.07	10.06	78
337	CH_2CH_3	H	OCH_3	H	100 (45%)	23.46	78

(Table 8) contd….

Cpds	R	R^6	R^7	R^8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
338	CH_2CH_3	OCH_3	H	H	> 100	21.63	78
339	CH_2CH_3	H	$N(CH_2CH_3)_2$	H	> 100	45.52	78
340	CH_2CH_3	Cl	H	Br	> 100	17.51	78
341	CH_2CH_3	Br	H	Br	> 100	100 (45%)	78
342	CH_2CH_3	I	H	I	> 100	> 100	78
343	CH_2CH_3	H		H	46.35	0.021	78
344	CH_2CH_3	CH_3	H	H	0.19	1.0	80
345	CH_2CH_3	Br	H	H	0.21	1.0	80
346	CH_2CH_3	Cl	H	H	0.50	1.0	80
347	CH_2CH_3	NO_2	H	H	0.26	1.0	80
348	CH_2CH_3	H		H	> 100	0.007	78
349	CH_2CH_3	H		H	> 100	0.061	78
350	CH_2CH_3	H		H	> 100	3.75	78
351	CH_2CH_3	H		H	> 100	0.009	78
352	H	H	H	H	15.14	0.017	80
353	H	CH_3	H	H	12.02	0.019	80
354	H	Br	H	H	1.0	0.033	80
355	H	Cl	H	H	0.50	0.023	80
356	H	NO_2	H	H	0.068	0.019	80
357	H	H	H	OH	59.61	100 (45%)	78
358	H	OH	H	H	40.20	100 (45%)	78
359	H	H	OCH_3	H	> 100	100 (45%)	78
360	H	OCH_3	H	H	> 100	> 100	78
361	H	H	$N(CH_2CH_3)_2$	H	> 100	> 100	78
362	H	Cl	H	Br	> 100	> 100	78
363	H	Br	H	Br	> 100	> 100	78
364	H	I	H	I	> 100	17.78	78
365	H	H		H	> 100	8.62	78
366	H	H		H	> 100	1.80	78

(Table 8) contd….

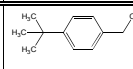
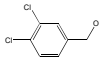
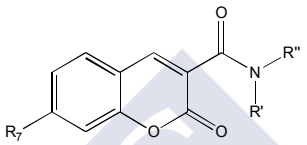
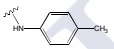
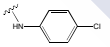
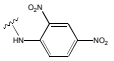
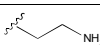
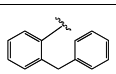
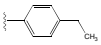
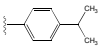
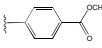
Cpds	R	R^6	R^7	R^8	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
367	H	H		H	> 100	> 100	78
368	H	H		H	> 100	0.41	78

Table 9. Chemical Structures and MAO Inhibition Data of 3-carboxyhydrazide and 3-carboxamide Coumarin Derivatives IX (Compounds 369-432)

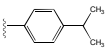


Cpds	R'	R''	R7	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
369	NH_2	H	H	> 100	0.003	78
370		H	H	41.40	4.52	78
371		H	H	39.56	> 100	78
372		H	H	58.43	> 100	78
373		H	H	48.02	1.19	78
374		H	H	60.49	56.12	78
375		H	H	> 100	0.50	81
376		H	H	> 100	25.36	81
377		H	H	> 100	3.27	81
378		H	H	> 100	> 100	81
379		H	H	> 100	7.68	81
380		H	H	13.12	0.76	81
381		H	H	> 100	0.91	81

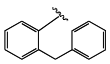
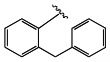
(Table 9) contd….

Cpds	R'	R''	R7	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
382		H	H	14.50	0.60	81
383		H	H	> 100	0.64	81
384		H	H	> 100	0.25	81
385		H	H	> 100	0.25	81
386		H	H	19.45	0.71	81
387		H	H	15.67	0.89	81
388		H	H	15.14	0.65	81
389		H	H	15.98	> 100	81
390		H	H	> 100	0.82	81
391		H	H	14.76	2.84	81
392		H	H	13.06	0.067	81
393		H	H	9.97	1.14	81
394		H	H	9.67	> 100	81
395		H	H	9.33	0.001	81
396		H	H	> 100	0.80	81
397		H	H	> 100	19.07	81
398		H	H	15.95	22.45	81
399		H	H	12.32	13.74	81
400		H	H	20.63	18.27	81
401		H	H	9.79	1.29	81

(Table 9) contd….

Cpds	R'	R''	R7	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
402		H	H	12.76	9.23	81
403		H	H	18.77	0.38	81
404		H	H	9.45	0.86	81
405		H	H	17.56	0.70	81
406		H	H	61.46	4.18	81
407		H	H	> 100	0.46	81
408		H	H	16.04	0.86	81
409		H	H	11.43	0.58	81
410		H	H	> 100	1.96	81
411		H	H	> 100	> 100	81
412		CH_3	H	> 100	8.89	81
413		CH_3	H	> 100	> 100	81
414			H	> 100	> 100	81
415		H		> 100	64.45	81
416		H		63.52	> 100	81
417		H		58.64	9.59	81
418		H		60.93	3.69	81
419		H		61.38	16.54	81

(Table 9) contd….

Cpds	R'	R''	R7	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
420		H		51.89	63.52	81
421		H		49.75	61.49	81
422		H		> 100	4.39	81
423		H		52.89	3.36	81
424		H		> 100	0.99	81
425		H		41.40	4.52	81
426		H		58.49	3.45	81
427		H		55.83	> 100	81
428		H		59.56	41.64	81
429		H		49.20	2.87	81
430		H		52.62	21.56	81
431		H		> 100	> 100	81
432		CH_3		> 100	2.37	81

lished a potential therapeutic application of these flavonoids/coumarins analogues as a new interesting scaffold with interest against age-related neurodegenerative diseases such as PD or AD [77].

G. Delogu *et al.* synthesized new coumarin derivatives **V** in which the 3-aryl ring is changed for a heteroaryl group and explored the importance of the number and position of methoxy groups on (1H)-benzopyran structure [79]. In general, these derivatives were found to be selective for the MAO-B isoform, with 7-methoxy-3-heteroarylcoumarins exhibiting inhibition potencies in the low nanoMolar range [79].

Substitution of the coumarin nucleus at position 3 has been widely carried out with phenyl and methyl groups, but less data have been reported with polar groups like ketone alkylic (Table **6**), ketone arylic (Table **7**) and carboxylic acid or ethyl esther (Table **8**). D. Secci *et al.* have dedicated particular attention to the study of the influence of these groups on MAO inhibitory activity and on A/B selectivity. For these derivatives, the steric hindrance of the substituent at position 6 is particulary important, in which only small substituents are tolerated [78, 80]. 3-Carbonyl chloride coumarins showed a high potency against both isoforms [80].

By analyzing the data reported in the previous (Table **6**), it can be seen that 3-methylketone coumarin derivatives showed better inhibitory activity and selectivity against MAO-B than their corresponding arylketones **VII** (Table **7**). Likewise, an important lost of the MAO-B inhibitory activity was verified when the 3-phenyl skeleton was substituted for the 3-benzoyl one [73]. Therefore, the simple introduction of a carbonyl bridge between the coumarin and the 3-phenyl ring changes dramatically the activity profiles.

Compounds bearing a CO_2Et moiety at C3 position, improved the inhibitory activity and selectivity towards MAO-B. However, the hydrolysis of ethyl esters to the corresponding carboxylic acids decreased the selectivity and the inhibitory activity towards MAO-B [78, 80]. Results of MAO activity are showed in (Table **8**).

D. Secci *et al.* also described a series of 3-carboxy hydrazide coumarin derivatives [78] as well as a series of 3-carboxamide coumarin derivatives (Table **9**) [81]. It can be seen that the majority of the compounds show a selective inhibitory activity toward the MAO-B isoenzyme. Substitution at position 7 on the coumarin ring by a benzyloxy group leads to a decrease in the activity against both MAO isoforms. Inhibitory activity increased when the nitrogen is substituted by a phenyl group.

Taking into account the activity of the above mentionated amido coumaris, M. J. Matos *et al.* synthetized analogs **X** and studied the possibility of linking the amidic group to the coumarin by the nitrogen atom [74]. These compounds could be considered inverse amidic derivatives with respect to the last described molecules **IX**. In spite of these considerations, the new compounds resulted to be poor MAOIs. However, some of them showed AChE inhibitory activity, being considered therefore as dual MAO/AChE inhibitors [74]. Compounds with such dual inhibitory activities are expected to have potential for the treatment of AD because they might decrease β-amyloid deposition [82].

3. SYNTHETIC METHODOLOGIES

Due to the interest of coumarins in industry as additives in food, agrochemicals, cosmetics and pharmaceuticals, synthesis of the coumarin template is a topic of current interest in organic chemistry. Different methodologies can be used to obtain the described compounds, depending on the starting materials and on the desired final substituents. Classically, the most important synthetic methodologies to obtain simple coumarins are the Pechmann, Perkin, Knoevenagel and Wittig reactions.

The Pechmann reaction allows to obtain the coumarin scaffold by condensation of phenols with β-ketoesters, in the presence of acid catalysts (usually sulphuric acid) (Scheme **1**). Although this is the most classical synthetic route to obtain the coumarin nucleus with alkyl, aryl, halogen, halogenomethyl or hydroxy substituents, some slight modifications to the traditional Pechmann reaction were performed, depending on the stability and reactivity of the used phenols and β-dicarbonyls [62, 64, 66, 83-85]. Several efforts are still being devoted to improve its performance by the use of specific catalysts and/or microwave radiation in ionic liquids media or solvent-free conditions [86-89]. The use of perchloric acid as the condensing agent allows, for example, the synthesis of differently substituted 7-hydroxycoumarin derivatives [90].

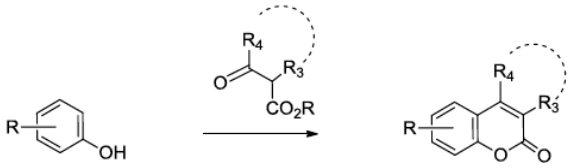
The Perkin reaction is an aldolic condensation of an *ortho*-hydroxybenzaldehyde and the conveniently substituted acid anhydrides, in the presence of an alkali salt of the acid (Scheme **2**). This methodology is perhaps the most direct and simple method known for the preparation of 3-arylcou marins. However, the overall yields of the final products in these reactions are invariably low and the variety of substrates is, in most cases, limited [47]. Thus, a lightly modified version of this methodology is commonly used for the synthesis of the 3-aryl or 3-heteroaryl substituted coumarins [69-73, 75, 76, 91]. In this reaction, the coumarin nucleus is obtained starting from the *ortho*-hydroxybenzaldehyde and the conveniently substituted arylacetic acid, in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC), the dehydrating agent, under DMSO reflux.

The Knoevenagel reaction is a condensation reaction that affords the coumarin derivatives starting from *ortho*-hydroxybenzaldehydes and active methylene compounds, in presence of ammonia or amines (Scheme **2**). The reaction is usually catalysed by weak bases or by suitable combinations of amines (usually piperidine) and carboxylic or Lewis acids under homogeneous conditions [78, 81]. The use of Meldrum´s acid in presence ionic liquids, results a simple and alternative method to obtain 3-carboxycoumarins [92]. A large list of coumarins has been synthesised using the Knoevenagel condensation. Benzoyl coumarins [73], as well as amino [93], amides [94], and 3-alkylamino substituted coumarins are some examples of the potential of this methodology [56, 68].

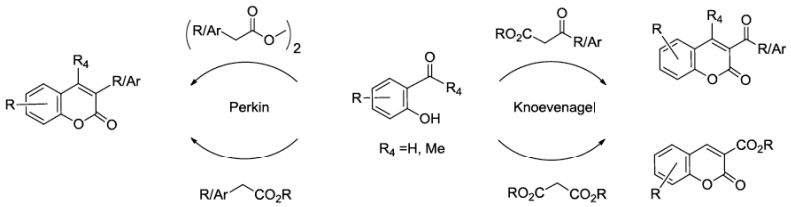
In order to afford multigram scale synthesis and easy purification, F. Chimenti *et al.* described the preparation of the 3-carboxycoumarin scaffold by the heterocyclization reaction of 2-hydroxybenzaldoximes and carbon suboxide (Scheme **3**) [80]. The basic hydrolysis of the carboxamide intermediate and the subsequent functionalization of the carboxylic acid intermediate, affords carboxycoumarin derivatives with high MAO inhibitory activity.

Wittig reaction is also an efficient and easy method for the synthesis of coumarins (Scheme **4**). This method consists in the reaction of *ortho*-hydroxycarbonyl aromatic compounds with phosphonium ylides and the subsequent intramolecular cyclization [95]. This type of reaction is a useful method to obtain prenylated coumarins [96, 97]. Also, aryl-, alkoxy-, or carboxycoumarin derivatives can be obtained via Wittig reaction, under classical conditions or improved protocols [70, 98, 99]. A variety of nitro/amine coumarins was prepared using microwave irradiation in solvent free conditions [96] and large combinatorial libraries of 3-halogenocoumarins were obtained by *one-pot* three component bromination/Wittig/cyclization process [100].

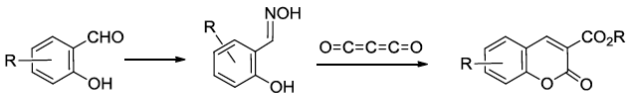
The palladium catalysed chemistry is an alternative methodology for the preparation of 4-substituted coumarin derivatives. 4-Aryl/alkylcoumarins can be obtained from the corresponding phenols by palladium-catalysis reaction with the appropriated propiolates or acrylates [101-103].



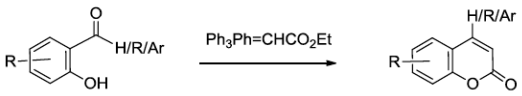
Scheme 1. Pechmann reaction.



Scheme 2. Perkin and Knoevenagel reactions.



Scheme 3. Heterocyclization reaction of 2-hydroxybenzaldoximes and carbon suboxide.



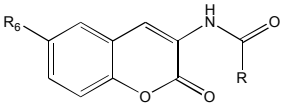
Scheme 4. Wittig reaction.

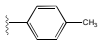
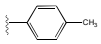
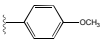
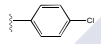
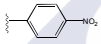
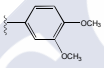
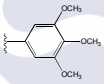
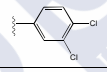
Special mention must be made regarding the arylcoumarin derivatives. Additionally to the above mentioned cyclo-condensation reactions, 3- and or 4-arylcoumarins can be obtained, starting from halogen or hydroxycoumarins by direct cross-coupling palladium arylation. The most common halogencoumarin used as starting material is the 3-bromo derivative. Therefore, libraries of 3-arylcoumarins were prepared starting from the 3-bromocoumarin by Pd-catalyzed coupling with boronic esters, acetylenes, or vinyl compounds [104]. However, very recently our research group has used for the first time the 3-chlorocoumarin as starting material and has prepared different 3-arylcoumarins by a Pd-catalyzed cross-coupling reaction using a catalytic complex Pd-salen [105].

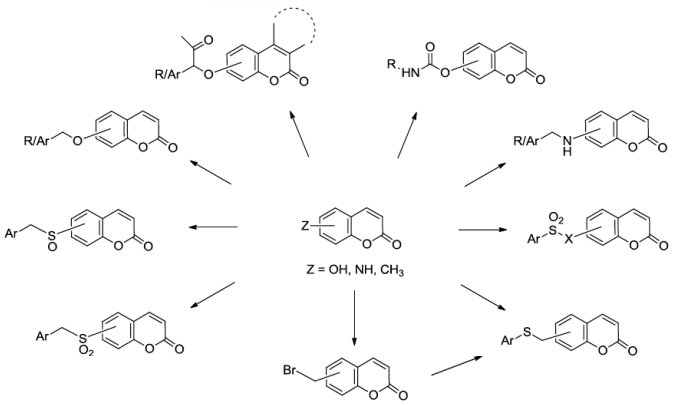
Other starting materials for the arylation of the coumarin ring are the 4-hydroxycoumarin derivatives. Due to the special reactivity of the 4-hydroxycoumarin ring, the coupling arylation can take place in either 3 or 4 positions, depending on the reaction conditions. The general palladium protocol or the Suzuki-Miyaura couplings are an efficient method for coupling 4-hydroxycoumarins. Through the intermediated triflate and tosylate derivatives, 4-arylcoumarins can be prepared in high yields [98, 106, 107]. However, the coupling arylation can take place also at position 3. Reaction of 4-hydroxycoumarins with iodobenzene diacetate gives the 3-phenyliodonium zwitterion intermediates which couple with substituted arylboronic acids in the palladium-catalyzed Suzuki-type reactions [77]. The excellent yields make this method a valuable tool for generating diversified 4-hydroxy-3-arylcoumarin derivatives. According to these methodologies, when the starting compounds are 4-hydroxy-3-bromocoumarins, it is possible to design an efficient protocol for the synthesis of 3- and/or 4-arylcoumarin derivatives [108].

The SAR studies performed with coumarin derivatives suggested that activity and selectivity were mainly determined by the characteristics of the substituents in the coumarin moiety [61, 64]. For this reason, during the last years new synthetic strategies have been described using as starting materials the previously synthesized coumarin ring. Most of the coumarin derivatives were prepared by nucleophilic substitution of simple hydroxy, amine or halogencoumarins under reflux of acetone, xylene, ethanol, or other solvents in the presence of potassium carbonate (Scheme **5**). Therefore, coumarins with benzyloxy [64, 84] and some alkylsulfonyloxy [67] groups were extensively synthesized as active and

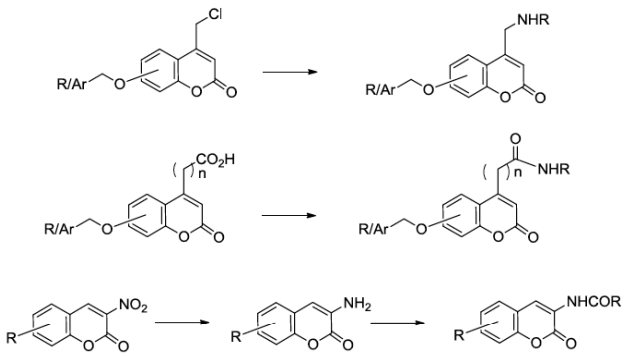
Table 10. Chemical Structures and MAO Inhibition Data of 3-carboxamidocoumarin Derivatives X (Compounds 433-445)



Cpds	R_6	R	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Ref
433	H		> 100	0.76	74
434	CH_3		45.97	0.17	74
435	H		> 100	7.96	74
436	H		> 100	30.50	74
437	H		> 100	1.95	74
438	H		> 100	> 100	74
439	H		> 100	58.38	74
440	H		> 100	72.58	74
441	H		> 100	5.95	74
442	H		> 100	49.96	74
443	H	CH_3	38.93	> 100	74
444	H	CH_2Br	> 100	> 100	74
445	H	CH_2Cl	> 100	> 100	74



Scheme 5. Synthetic strategies using as starting materials the previously synthesized coumarin ring.



Scheme 6. Functionalization of coumarins to afford the corresponding amino, carboxamido or amidocoumarin derivatives.

selective MAO inhibitors. The etherification of hydroxycoumarins and aminocoumarins were commonly performed by Williamson-type reactions [74]. So, different substituted bromides were furnished target coumarins in the presence of potassium carbonate and ethanol, under reflux [64, 66, 67, 84]. The same etherification reaction occurs adding suitable bromides and potassium carbonate in dry acetone, using *N,N'*-dicyclohexyl-18-crown-6-ether as chelating agent [81]. This type of reaction was also possible starting from the formation of the bromomethylcoumarin and the subsequent nucleophilic displacement of bromide by hydroxy or amine substituted molecules (for example phenol or aniline) [62]. In general, these etherifications were the most common substitutions under the coumarin nucleus to afford the desired MAO activity. The synthesis of alkylsulfonyloxy derivatives was carried out starting from the corresponding hydroxycoumarins with a conveniently substituted sulfonyl chloride in pyridine [67]. The thioether derivatives were prepared by refluxing bromides and thiols in MeCN in the presence of potassium carbonate. Sulfoxide and sulfone derivatives were obtained by oxidation of thioethers with sodium periodate or potassium permanganate, respectively [61]. Some functionalizations can also be performed by microwave irradiation [84].

Halogeno, carboxy or nitrocoumarins can also be used as starting synthons for the subsequent functionalization affording an array of the corresponding amino, carboxamido or amidocoumarins, compounds with inhibitory MAO activity (Scheme **6**).

Finally it must be mentioned that 3-aryl and 3-benzoylcoumarins can be further substituted in different positions of both aromatic rings using the same aforementioned methodologies [69-73, 75, 76]. Bromination of the derivatives was performed with *N*-bromosuccinimide (NBS), in carbon tetrachloride (CCl_4) under reflux, using 2,2'-azo-bis-iso-butyronitrile (AIBN) as catalyst [69, 70]. The methoxy/ethoxy derivatives can be hydrolyzed either with hydriodic acid, in the presence of acetic acid and acetic anhydride or BBr_3 in DCM [69, 72, 73, 75]. The Williamson reaction of the hydroxyl substituted arylcoumarins, as described for simple coumarins, was performed with differently substituted chlorides or bromide to give the corresponding ethers [72].

3. CONCLUSION

Since the pioneering work of H. A. Kadir *et al.* [55], and the discovery of the potential of the coumarin scaffold against both MAO isoforms, new generations of differently substituted molecules have been synthesized and evaluated *in vitro* in pharmacological assays. Some of the described derivatives are very active and selective compounds, with IC_{50} in the low nanomolar range. These findings pave the way for new promise for efficient and safe therapeutic solutions of an increasing and important problem of the developed countries: the neurodegenerative diseases. Furthermore, based on these important studies, there are several ongoing projects for the rational structure-based drug design of new molecules.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

Declared none.

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Received: March 20, 2012 **Revised: June 15, 2012** **Accepted: June 18, 2012**

Bioorganic & Medicinal Chemistry Letters 21 (2011) 4224–4227

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

MAO inhibitory activity modulation: 3-Phenylcoumarins versus 3-benzoylcoumarins

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ARTICLE INFO

Article history:
Received 5 April 2011
Revised 18 May 2011
Accepted 20 May 2011
Available online 30 May 2011

Keywords:
Perkin reaction
Knoevenagel reaction
3-Phenylcoumarins
3-Benzoylcoumarins
MAOI activity
Comparative study

ABSTRACT

With the aim of finding the structural features for the human MAO inhibitory activity and selectivity, in the present communication we report the synthesis, pharmacological evaluation and a comparative study of a new series of 3-phenylcoumarins (compounds **1**–**4**) and 3-benzoylcoumarins (compounds **5**–**8**). A bromo atom and a methoxy/hydroxy substituent were introduced in these scaffolds, at six and eight positions of the coumarin moiety, respectively. The synthesized compounds **1–8** were evaluated as MAO-A and B inhibitors using R-(−)-deprenyl and iproniazide as reference compounds. The presence or absence of a carbonyl group between the coumarin and the phenyl substituent in 3 position remarks, respectively, the MAO-A or MAO-B inhibitory activity. Some of the new compounds showed MAO-B inhibitory activities in the low nanomolar range. Compound **2** (IC_{50} = 1.35 nM) showed higher inhibitory activity than the R-(−)-deprenyl (IC_{50} = 19.60 nM) and higher MAO-B selectivity, with more than 74,074-fold inhibition level, respecting to the MAO-A isoform.

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Coumarins, chalcone and their natural and/or synthetic derivatives are biologically interesting compounds because of their structural diversity. Due to this variability, these heterocyclic compounds occupy an important role not only in the Organic Chemistry but also in the Medicinal Chemistry realm.[1–6] They are described as anticancer, anti-inflammatory, antimicrobial, and antioxidant agents.[7–16] A number of studies pay special attention to coumarin derivatives as monoamine oxidase (MAO)[17–23] inhibitors. Recently, chalcone structure has also been identified as a valid scaffold for monoamine oxidase inhibitors (MAOI).[24] Therefore, recent findings reveal that MAO-A and MAO-B affinity and selectivity can be efficiently modulated by appropriate substitutions in different positions of the coumarin and chalcone moiety (Fig. 1).[19,25–27]

MAO is a FAD-containing enzyme (flavoenzyme) bound to the outer mitochondrial membrane in neuronal, glial and many other cells.[28,29] Two isoforms namely as MAO-A and MAO-B have been identified based on their amino acid sequences, three-dimensional structure, substrate preference and inhibitor selectivity.[30,31] These isoenzymes are responsible for the oxidative deamination of neurotransmitters and dietary amines. Therefore, they are responsible for the regulation of intracellular levels of biogenic amines in the brain and the peripheral tissues.[32,33] MAO-B preferentially deaminates phenylethylamine and benzylamine, while MAO-A has a higher affinity for noradrenaline and serotonin.[34,35] Despite of these differences, dopamine and tyramine are common substrates for both isoforms. These properties determine the pharmacological interest of MAOIs. Selective and irreversible MAO-B inhibitors, such as selegiline (R-(−)-deprenyl) and rasagiline are useful compounds for the treatment of Parkinson[36,37] and Alzheimer's diseases.[38,39] Selective MAO-A inhibitors, such as clorgyline (irreversible) and moclobemide (reversible), are useful for the treatment of neurological disorders, such as depression and anxiety.[40,41] All these findings have led us to an intensive search for novel, selective and efficient MAO inhibitors.

With the aim of finding novel and selective MAO-B inhibitors, we have previously synthesized 3-arylcoumarin derivatives in which both the coumarin nucleus and a 3-phenyl ring were differently substituted. The experimental data show that those compounds are potent and selective MAO-B inhibitors.[20–23] In particular, the 6,8-disubstituted coumarins proved to be very interesting derivatives.[22] Based on the previous 3-phenylcoumarins experimental results, in this paper we describe a new project with a comparative study between 3-phenylcoumarins (compounds **1**–**4**) and 3-benzoylcoumarins (compounds **5**–**8**), which are

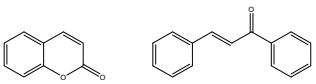

Figure 1. Chemical structures of coumarin and *trans*-chalcone.

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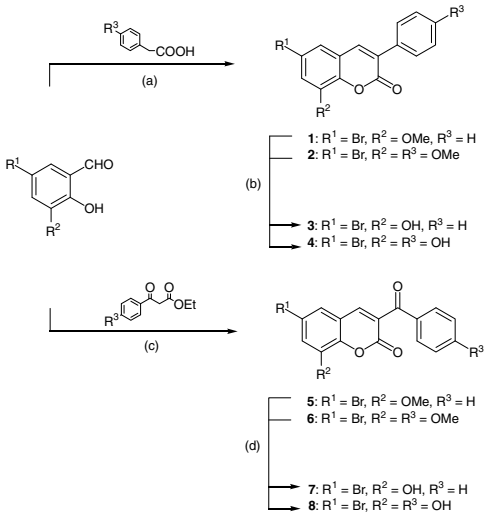
doi:10.1016/j.bmcl.2011.05.074



Scheme 1. Reagents and conditions: (a) DCC, DMSO, 110 °C, 24 h; (b) HI, AcOH, Ac_2O, 0 °C to reflux, 4 h; (c) ethanol, piperidine, reflux, 2–5 h; (d) BBr_3, DCM, 80 °C, 48 h.

interesting semi-rigid chalcones with the α,β-unsaturated double bond included in the coumarin skeleton (Scheme 1).

The 6-bromo-8-methoxycoumarins[42] were efficiently synthesized by Perkin[43–45] (**1** and **2**) and Knoevenagel[46,47] (**5** and **6**) reactions. The hydroxy derivatives (**3**, **4**, **7** and **8**)[42] were obtained by two different hydrolysis reactions,[48–50] according to the synthetic protocol outlined in Scheme 1. Treatment of the corresponding salicylaldehyde and the conveniently substituted phenylacetic acid with *N,N'*-dicyclohexylcarbodiimide (DCC) as dehydrating agent, in dimethyl sulfoxide (DMSO) at 110 °C during 24 h, afforded the 3-phenylcoumarins **1** and **2**. The consequent hydrolysis of **1** and **2** in acetic acid and acetic anhydride, with hydriodic acid 57%, for 4 h, yielded the hydroxy derivatives **3** and **4**.[42] The synthesis of the 3-benzoylcoumarins **5** and **6** was performed via condensation of the conveniently substituted salicylaldehyde with the corresponding β-ketoester, in ethanol at reflux temperature for 5 or 2 h respectively, using piperidine as basic catalyst. The resulting methoxy derivatives were treated with boron tribromide at 80 °C for 48 h, to give the corresponding hydroxy derivatives **7** and **8**. Starting from the same salicylaldehyde, we can afford two series, differing just in the presence (compounds **5**–**8**) or absence (compounds **1**–**4**) of a carbonyl group between the phenyl substituent at the 3 position and the coumarin scaffold.

The inhibitory MAO activity of compounds **1–8** was evaluated *in vitro* by the measurement of the enzymatic activity of human recombinant MAO isoforms expressed in BTI insect cells infected with baculovirus.[51] Subsequently, the IC_{50} values and MAO-B selectivity indexes [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] for inhibitory effects of both new types of compounds and reference inhibitors were calculated (Table 1).[51–53]

In the present communication, the effect of the presence or the absence of a carbonyl group between the coumarin and the 3-phenyl ring is studied. Substituents and their positions in the 3-phenylcoumarin nucleus have been selected based on previous results which showed very high MAOI activity for some derivatives. It was shown that introduction of both bromo and methoxy substituents at 6 and 8 positions respectively (compounds **1** and **2**) enhances the MAO-B inhibitory properties (potency and selectivity) of the described 3-phenylcoumarins, comparing to some of the other previously described compounds.[20–22] In addition, the introduction of a substituent in the *para* position of the 3-phenyl ring might help to improve the activity. Consequently, when this substituent is a methoxy group (compound **2**), the MAO-B inhibitory activity and selectivity improve in a great extent respecting to the other derivatives. However, replacement of the methoxy groups for hydroxyl groups at the indicated positions decreases the activity (compounds **3** and **4**) validating the previous information we already had[21] and it can even decrease selectivity. On the other hand, when we analyze the second series where a 3-benzoyl group has replaced the 3-phenyl substituent, the introduced carbonyl group decreases the MAOI activity against B isoform. The 6-bromo-8-hydroxy derivatives **7** and **8** are more active than the corresponding 6-bromo-8-methoxy ones (compounds **5** and **6**), being the structure activity relationship just the opposite of the 3-phenylcoumarins. Compounds **5** and **6** do not present any MAO inhibitory activity at the higher tested concentration (100 μM). However, compounds **7** and **8** increase the affinity for the MAO-A receptor, losing the MAO-B selectivity of the first series. A small change in the structure causes a big change in the affinity of the molecules for the receptor. These preliminary results allow us to understand slightly better interactions between molecule and receptor and the molecular fragments that are essential to maintain and improve the MAO activity and selectivity.

As conclusion, it was verified an important lost of the MAO-B inhibitory activity and selectivity when the 3-phenyl skeleton is substituted for the 3-benzoyl one. However, in some of the 3-benzoyl derivatives it was shown not only inactivity against MAO-B

Table 1
MAO-A and MAO-B inhibitory activity results for the synthesized compounds **1–8** and reference inhibitors

Compounds	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Selectivity Index
1	*	$83.48x10^{-3} \pm 5.60x10^{-3}$	>1,197[b]
2	*	$1.35x10^{-3} \pm 0.09x10^{-3}$	>74,074[b]
3	*	30.91 ± 2.09	>3.2[b]
4	20.74 ± 1.40	16.87 ± 1.14	1.2
5	*	*	–
6	*	*	–
7	46.81 ± 3.18[a]	73.92 ± 4.99	0.63
8	19.17 ± 1.29	**	0.19[c]
R-(−)-Deprenyl	67.25 ± 1.02[a]	$19.60x10^{-3} \pm 0.86x10^{-3}$	3,431
Iproniazide	6.56 ± 0.76	7.54 ± 0.36	0.87

* Inactive at 100 μM (highest concentration tested). At higher concentrations compound precipitate.
** 100 μM inhibits enzymatic activity around (by approximately) 45–50%. At higher concentrations compound precipitate.
[a] $P < 0.05$ versus the corresponding IC_{50} values obtained against MAO-B, as determined by ANOVA/Dunnett's.
[b] Values obtained under the assumption that the corresponding IC_{50} against MAO-A is the highest concentration tested (100 μM).
[c] Value obtained under the assumption that the corresponding IC_{50} against MAO-B is the highest tested concentration (100 μM).

isoenzyme, but showed MAO-A inhibitory activity and selectivity. Therefore, selectivity seems to depend on the nature of the coumarins' substituent. In the present study it was shown that 6-bromo-8-methoxy-3-phenylcoumarins are an interesting scaffold for MAO-B inhibitory studies, whereas the 6-bromo-8-hydroxy-3-benzoylcoumarins are an interesting moiety for MAO-A inhibitory ones. Compound **2**, with a *p*-methoxy substituent in the 3-phenyl ring was the most potent and selective molecule of the first series against MAO-B isoenzyme, with an IC_{50} in the low nanomolar range (IC_{50} = 1.35 nM). This compound is fifteen times more active and several times more selective than the R-(−)-deprenyl (IC_{50} = 19.6 nM, reference MAO-B inhibitor). The MAO selectivity is an important factor to discriminate the different potential therapeutic applications of these molecules. These findings encourage us to continue the efforts towards the optimization of the pharmacological profile of these structural types as important scaffolds in the neurodegenerative diseases realm.

Acknowledgments

Thanks to the Spanish Ministry (PS09/00501) and to Xunta de Galicia (PGIDIT09CSA030203PR and 10PXIB203303PR). M.J.M. thanks FCT for a PhD grant (SFRH/BD/61262/2009). S.V.R. thanks to Ministerio de Educación y Ciencia for a PhD grant (AP2008-04263).

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42. *General procedure for the preparation of 3-phenylcoumarins (**1** and **2**):* a solution of the conveniently substituted salicylaldehyde (0.56 mmol) and the correspondent phenylacetic acid (0.70 mmol) in DMSO and DCC (0.87 mmol) was heated in an oil-bath at 110 °C for 24 h. Triturate ice (20 mL) and acetic acid (3.0 mL) were added to the reaction mixture. After keeping it at room temperature for 2 h, the mixture was extracted with ether (3 x 25 mL). The organic layer was extracted with sodium bicarbonate solution (50 mL, 5%) and then with water (20 mL). The solvent was evaporated under vacuum and the dry residue was purified by FC (hexane/ethyl acetate 9:1) to give the desired compound.
*6-Bromo-8-methoxy-3-phenylcoumarin (**1**)*: it was obtained with a yield of 50%. Mp 153–154 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3.97 (s, 3H, –OCH_3), 7.16 (d, 1H, H-7, *J* = 2.0), 7.26 (d, 1H, H-5, *J* = 2.0), 7.43–7.48 (m, 3H, H-3', H-4', H-5'), 7.68–7.73 (m, 3H, H-2', H-6', H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 57.2, 117.0, 117.3, 121.9, 122.1, 129.2, 129.8, 130.3, 134.8, 139.1, 142.8, 148.3, 160.0. MS *m*/*z* (%): 332 (99), 331 (30), 330 (M^+, 100), 304 (40), 302 (40), 261 (25), 259 (26), 194 (16), 165 (12), 153 (14), 152 (88), 151 (23), 102 (19), 76 (34). Anal. Calcd for $C_{16}H_{11}BrO_3$: C, 58.03; H, 3.35. Found: C, 58.01; H, 3.30.
*6-Bromo-8-methoxy-3-(4'-methoxyphenyl)coumarin (**2**)*: it was obtained with a yield of 53%. Mp 184–185 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3.85 (s, 3H, –OCH_3), 3.96 (s, 3H, –OCH_3), 6.93–6.96 (m, 2H, H-3', H-5'), 7.12 (d, 1H, H-7, *J* = 1.8), 7.23 (d, 1H, H-5, *J* = 2.0), 7.63–7.67 (m, 2H, H-2', H-6'), 7.69 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 55.7, 56.8, 114.2, 116.2, 116.8, 121.5, 126.8, 129.4, 130.2, 130.8, 137.3, 142.2, 147.8, 159.8, 160.6. MS *m*/*z* (%): 363 (19), 362 (M^+, 100), 361 (19), 360 (59), 334 (24), 332 (23), 319 (33), 317 (34), 291 (11), 289 (11), 182 (18), 167 (17), 139 (21), 91 (11). Anal. Calcd for $C_{17}H_{13}BrO_4$: C, 56.53; H 3.63. Found: C, 56.55; H, 3.68.
*General procedure for the preparation of hydroxylated 3-phenylcoumarins (**3** and **4**).* a solution of the corresponding methoxy-3-phenylcoumarin (0.50 mmol) in acetic acid (5.0 mL) and acetic anhydride (5.0 mL), at 0 °C, was prepared. Hydriodic acid 57% (10.0 mL) was added dropwise. The mixture was stirred under reflux temperature for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by recrystallization with CH_3CN.
*6-Bromo-8-hydroxy-3-phenylcoumarin (**3**)*: it was obtained with a yield of 61%. Mp 160–161 °C. ^{1}H NMR (DMSO-d_6) δ (ppm), J (Hz): 7.19 (d, 1H, H-7, *J* = 2.0), 7.43–7.49 (m, 4H, H-5, H-3', H-4', H-5'), 7.70 (dd, 2H, H-2', H-6', *J* = 7.6, *J* = 1.6), 8.13 (s, 1H, H-4), 10.79 (s, 1H, -OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 115.5, 119.9, 120.4, 121.8, 127.9, 128.2, 128.5, 128.8, 134.4, 139.7, 141.1, 145.6, 159.1. MS *m*/*z* (%): 319 (16), 318 (99), 317 (17), 316 (M^+, 100), 291 (12), 290 (78), 289 (13), 288 (81), 153 (22), 152 (51), 151 (12), 76 (25). Anal. Calcd for $C_{15}H_9BrO_3$: C, 56.81; H, 2.86. Found: C, 56.78; H, 2.82.
*6-Bromo-8-hydroxy-3-(4'-hydroxyphenyl)coumarin (**4**)*: It was obtained with a yield of 53%. Mp 249–250 °C. ^{1}H NMR (DMSO-d_6) δ (ppm), J (Hz): 6.83 (d, 2H, H-3', H-5', *J* = 8.8), 7.14 (t, 1H, H-7, *J* = 1.5), 7.36 (d, 1H, H-5, *J* = 2.0), 7.56 (d, 2H, H-2', H-6', *J* = 8.5), 8.00 (s, 1H, H-4). ^{13}C NMR (DMSO-d_6) δ (ppm): 115.6, 116.0, 119.9, 120.6, 122.5, 125.4, 128.2, 130.4, 138.0, 141.2, 146.0, 158.6, 159.8. MS *m*/*z* (%): 335 (35), 334 (99), 333 (45), 332 (M^+, 100), 307 (31), 306 (99), 305 (35), 304 (99), 225 (26), 197 (29), 169 (35), 168 (46), 153 (24), 141 (21), 140 (16), 139 (51), 118 (17), 115 (28), 98 (13), 89 (14), 84 (18), 84 (46), 75 (11), 63 (13). Anal. Calcd for $C_{15}H_9BrO_4$: C, 54.08; H, 2.72. Found: C, 54.00; H 2.69.
*General procedure for the preparation of 3-benzoylcoumarins (**5** and **6**).* To a solution of the appropriate β-ketoester and the corresponding salicylaldehyde in ethanol was added piperidine in catalytic amount. The reaction mixture was refluxed for 2–5 h and after completion, the reaction was cooled and the precipitated was filtered and washed with cold ethanol and ether to afford the desired compound. Compounds were further recrystallized in methanol/CH_2Cl_2.
*6-Bromo-8-methoxy-3-benzoylcoumarin (**5**)*: it was obtained with a yield of 49%. Mp 207–208 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3.82 (s, 3H, –OCH_3), 7.09 (s, 1H, H-7), 7.14 (s, 1H, H-5), 7.24–7.51 (m, 3H, H-3', H-4', H-5'), 7.69 (d, 2H, H-2', H-6', *J* = 7.3), 7.78 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 57.2, 116.6, 118.6, 120.5, 123.0, 128.0, 129.2, 130.0, 134.5, 136.3, 143.3, 144.6, 147.8, 157.8, 191.9. MS *m*/*z* (%): 360 (18), 359 (M^+, 58), 358 (18), 357 (58), 105 (100), 77 (70). Anal. Calcd for $C_{17}H_{11}BrO_4$: C, 56.85; H, 3.09; Found: C, 56.79; H, 3.06.

6-Bromo-8-methoxy-3-(4́-methoxybenzoyl)coumarin (**6**): It was obtained with a yield of 90%. Mp 224–225 °C. ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3.71 (s, 3H, $-OCH_3$), 3.82 (s, 3H, $-OCH_3$), 6.77 (d, 2H, H-3', H-5', *J* = 8.9), 7.14–7.16 (m, 2H, H-5, H-7), 7.69 (d, 2H, H-2', H-6', *J* = 8.9), 7.72 (s, 1H, H-4). ^{13}C NMR ($CDCl_3$) δ (ppm): 55.5, 56.1, 92.8, 95.2, 103.8, 113.7, 121.2, 129.7, 132.1, 141.5, 157.9, 158.3, 159.2, 163.8, 165.8, 190.6. MS *m*/*z* (%): 389 (87), 388 (17), 387 (M^+, 90), 135 (100), 77 (56). Anal. Calcd for $C_{18}H_{13}BrO_5$: C, 55.55; H, 3.37. Found: C, 55.53; H, 3.34.

*General procedure for the preparation of hydroxylated 3-benzoylcoumarins (**7** and **8**).* To the corresponding methoxy-3-benzoylcoumarin in DCM (1 mmol), BBr_3 in DCM (20 mmol) was added in a Schlenk tube. Tube was sealed, and the reaction mixture was heated at 80 °C for 48 h. The resulting crude was treated with MeOH and rotated to dryness. The obtained precipitated was recrystallized in MeOH to afford the desired hydroxy derivative.

*6-Bromo-8-hydroxy-3-benzoylcoumarin (**7**):* it was obtained with a yield of 98%. Mp 240–242 °C. ^{1}H NMR (DMSO-d_6) δ (ppm), J (Hz): 7.29 (d, *J* = 2.2 Hz, 1H, H-7), 7.61–7.39 (m, 3H, H-3', H-4', H-5'), 7.76–7.67 (m, 1H, H-5), 7.93 (d, 2H, H-2', H6', *J* = 8.0), 8.30 (s, 1H, H-4), 10.92 (s, 1H, -OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 39.3, 39.5, 39.7, 39.9, 40.1, 40.4, 40.6, 116.2, 120.9, 121.8, 122.0, 127.8, 129.2, 130.0, 134.5, 136.3, 142.7, 144.8, 146.3, 157.9, 191.9. MS *m*/*z* (%): 345 (46), 343 (M^+, 45), 105 (100), 77 (58). Anal. Calcd for $C_{16}H_9BrO_4$: C, 55.68; H, 2.63. Found: C, 55.63; H, 2.59.

*6-Bromo-8-hydroxy-3-(4́-hydroxybenzoyl)coumarin (**8**):* it was obtained with a yield of 40%. Mp 293–295 °C. ^{1}H NMR (DMSO-d_6) δ (ppm), J (Hz): 6.86 (d, 2H, H-3', H-5', *J* = 8.7), 7.27 (d, 1H, H-7, *J* = 2.2), 7.46 (d, 1H, H-5 *J* = 2.2), 7.82 (d, 2H, H-2', H-6' *J* = 8.7), 8.18 (s, 1H, H-4), 10.62 (s, 1H, -OH), 10.89 (s, 1H, -OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 115.9, 116.2, 121.0, 121.5, 121.6, 127.6, 128.5, 133.0, 142.7, 143.5, 146.3, 157.9, 163.6, 190.0. MS *m*/*z* (%): 362 (29), 360 (M^+, 30), 121 (100), 93 (16), 65 (15). Anal. Calcd for $C_{16}H_9BrO_5$: C, 53.21; H, 2.51. Found: C, 52.97; H, 4.41.

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51. Determination of human monoamine oxidase (hMAO) isoform activity. The effects of the tested compounds on hMAO isoform enzymatic activity were evaluated by a fluorimetric method. Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing the tested drugs in several concentrations and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity [165 pmol of *p*-tyramine/min (hMAO-A: 1.1 mg protein; specific activity: 150 nmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min/mg protein; hMAO-B: 7.5 mg protein; specific activity: 22 nmol of *p*-tyramine transformed/min/mg protein)] were placed in the dark fluorimeter chamber and incubated for 15 min at 37 °C. The reaction was started by adding (final concentrations) 200 μM AmplexÒ Red reagent, 1 U/mL horseradish peroxidase and 1 mM *p*-tyramine. The production of H_2O_2 and, consequently, of resorufin was quantified at 37 °C in a multidetection microplate fluorescence reader (FLX800TM, Bio-TekÒ Instruments, Inc., Winooski, VT, USA) based on the fluorescence generated (excitation, 545 nm, emission, 590 nm) over a 15 min period, in which the fluorescence increased linearly. Control experiments were carried out simultaneously by replacing the tested drugs with appropriate dilutions of the vehicles. In addition, the possible capacity of the above tested drugs for modifying the fluorescence generated in the reaction mixture due to non-enzymatic inhibition (e.g., for directly reacting with AmplexÒ Red reagent) was determined by adding these drugs to solutions containing only the AmplexÒ Red reagent in a sodium phosphate buffer. The specific fluorescence emission (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from vials containing all components except the hMAO isoforms, which were replaced by a sodium phosphate buffer solution.
52. All IC_{50} values shown in the table are expressed as means ± SEM from five experiments.
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Bioorganic & Medicinal Chemistry Letters 22 (2012) 258–261

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Hydroxycoumarins as selective MAO-B inhibitors

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ARTICLE INFO

Article history:
Received 12 October 2011
Revised 4 November 2011
Accepted 6 November 2011
Available online 11 November 2011

Keywords:
4-Hydroxycoumarin
MAO inhibitory activity
Suzuki reaction
Docking experiments

ABSTRACT

A series of 3-aryl-4-hydroxycoumarin derivatives was synthesized with the aim to find out the structural features for the MAO inhibitory activity and selectivity. Methoxy and/or chloro substituents were introduced in the 3-phenyl ring, whereas the position 6 in the coumarin moiety was not substituted or substituted with a methyl group or a chloro atom due to their different electronic, steric and/or lipophilic properties. Most of the synthesized compounds presented MAO-B inhibitory activity. The presence of methoxy and chloro groups, respectively in the *para* and *meta* positions of the 3-phenyl ring, have an important influence on the inhibitory activity. Moreover, the presence of a chloro atom in the six position of the moiety (compound **7**) improved the inhibitor activity as well as its selectivity against MAO-B compared with iproniazide, used as reference compound. Docking experiments were carried out to understand which are the most energetically preferred orientations adopted by compounds **5**, **6** and **7** inside the MAO-B binding pocket.

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Neurodegenerative diseases (ND) constitute the third most important health problem in developed countries. Alzheimer's disease (AD) is the most prevalent of the ND followed by Parkinson's disease (PD). AD is a neurodegenerative and progressive disorder associated, in the most of the cases, with senile dementia. It seems to have a multiple etiology although the main cause it is thought to be the accumulation of β-amyloid plaques in brain that can provoke a degeneration or atrophy of the cholinergic neurons. PD is also a chronic and progressive neurodegenerative disorder, characterized by a predominant motor symptomatology, usually accompanied by non motor symptoms such as depression and anxiety. It seems to be caused by a decrease in the dopamine levels in neuronal synapses. In the treatment of this disease, dopaminergic agonists are generally employed. However, other therapeutic alternatives can be employed, such as the use of monoamine oxidase B inhibitors (MAOI-B), or the use of antioxidant compounds in order to prevent the oxidative cells damage.

MAO is an important FAD-containing enzyme (flavoenzyme) present in the outer mitochondrial membrane of neuronal, glial and many other cells.[1,2] It exists in two isoforms namely as MAO-A and MAO-B that have been identified based on their amino acid sequences, three-dimensional structure, substrate preference and inhibitor selectivity.[3,4] MAO-A preferentially deaminates serotonin and noradrenaline, whereas MAO-B has a higher affinity to phenylethylamine and benzylamine.[5,6] Therefore, these isoenzymes play a vital role in the monoamines degradation and, as consequence, in the inactivation of neurotransmitters. Their regulation determines the interest of the MAOI compounds as drugs used in the treatment of neurodegenerative and neurological disorders. In particularly, MAO-A inhibitors are effective in the treatment of depression,[7,8] while MAO-B inhibitors are useful in the treatment of PD.[9,10] In the last years, it has been proved that in patients who suffer ND the activity of brain MAO-B is increased. This fact provokes an increase of free radicals that are responsible for the oxidative stress, neuronal cell death and also for the development of the β-amyloid plaques.[11] In this sense, it is an accepted theory that the beneficial effects of the MAO-B inhibitors in the prevention of the neuronal damage is mostly derived to the decrease of hydrogen peroxide generated by the inhibition of this enzyme.

Coumarins are an important group of organic compounds from natural and/or synthetic origin, that show, due to their structural diversity, numerous biological activity.[12–14] There are coumarins described as anticancer, anti-inflammatory, antimicrobial, cardioprotective, vasorelaxant, and antioxidant agents.[15–20] Recently studies pay special attention to the MAO inhibitory properties of the 3-arylcoumarin derivatives.[21–26]

Flavonoids are naturally occurring polyphenolic compounds that are found in diets and medicinal herbs. The physiological benefits of dietary flavonoids (flavones and isoflavones) have been attributed to their antioxidant and free radical-scavenging abilities.[27] Additionally, flavonoids have been revealed to exhibit

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doi:10.1016/j.bmcl.2011.11.020

anti-amiloidogenic properties such as inhibitory effects on β-secretase enzimes[28,29] and also MAO-B inhibitory activity.[30]

In previous works, we reported the synthesis and biological evaluation of a series of 3-arylcoumarins as potent and selective MAO-B inhibitors.[23–25] These interesting results prompted us to synthesize and evaluate new 3-arylcoumarin derivatives, in which we introduce a hydroxyl group in the 4 position of coumarin skeleton (Fig. 1).

This modification allows us considering the similarity of the core structure of the 4-hydroxycoumarin (B) comparing with the 2-hydroxyisoflavone (C). Taking into account these data we could establish a potential therapeutic application of these analogues flavonoids/coumarins as a new interesting scaffold with interest against age-related neurodegeneration such as PD or AD.

The synthesis of 3-aryl-4-hydroxycoumarin derivatives (**1–7**)[31–33] is outlined in Scheme 1.

The key step for the synthesis of the 3-arylcoumarin skeleton was achieved by a palladium-catalyzed coupling Suzuki reaction between phenyliodonium zwitterions and aryl conveniently substituted phenyl boronic acids. Initially we have synthesized the different phenyliodonium coumarinate species (I–III).[31,32] They are electrophilic molecules with a positive charge at iodine, compensated by a internal negative charge that can be localized formally at the α-carbon or delocalized to a neighboring oxygen. Then we have carried out the palladium-catalyzed coupling reaction using $Pd(OAc)_2$ as catalyst and $P(t\text{-}Bu)_3$ as ligand to afford the compounds (**1–7**) with good yields.

By ^1H and ^{13}C NMR spectroscopy studies we have found out that the synthesized compounds (**1–7**) were presented in the 4-hydroxycoumarin tautomeric form. Therefore we have centered on their analysis at the physiologic pH conditions. In the biological inhibitory assays it was also used the appropriate solvent that guaranties the presence of this structure.

The MAO inhibitory activity of compounds **1–7** was evaluated in vitro by the measurement of the enzymatic activity of human recombinant MAO isoforms expressed in BTI insect cells infected with baculovirus.[34] Subsequently, the IC_{50} values and MAO-B selectivity indexes [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] for inhibitory effects of both new types of compounds and iproniazide (used as reference inhibitor) were calculated (Table 1).[34,35]

In the present Letter, we have studied the effect in the MAOI activity of the introduction of alkyl, alkoxy or halogen substituents in different positions of the 3-aryl-4-hydroxycoumarin nucleus. We have found that the simple 4-hydroxy-3-phenylcoumarin did not present MAOI activity (compound **1**). The introduction of a methoxy substituent in the *para* position of the 3-phenyl ring led to a compound **2** with inhibitory activity against MAO-B. So we take this compound as a point of reference for the subsequent modifications. Due to the fact that the 6 position of the coumarin skeleton is particularly relevant to the MAO-B inhibitor activity and selectivity,[36,37] we have studied the effect of the introduction of a methyl group or a chloro atom in this position. The introduction of a methyl group (compound **3**) slightly improved the activity. However, the replacement for a chloro atom at the same position decreases the activity (compound **4**). In addition, when we analyze the second series (compounds **5–7**) where a chloro atom has been introduced at the *meta* position of the *p*-methoxy-3-phenyl ring, the MAO-B inhibitory activity, in most of the cases, improves. In this case, if we introduce a methyl group in the 6 position (compound **6**) the MAOI-B activity did not change, whereas if the substituent is a chloro atom (compound **7**) the MAO-B inhibitory activity considerably improves. Therefore compound **7** is more active against MAO-B isoenzyme than the

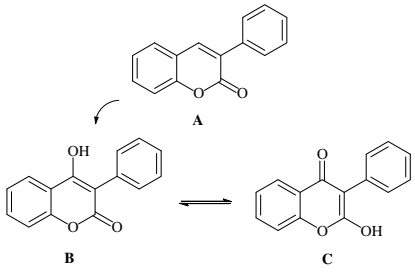
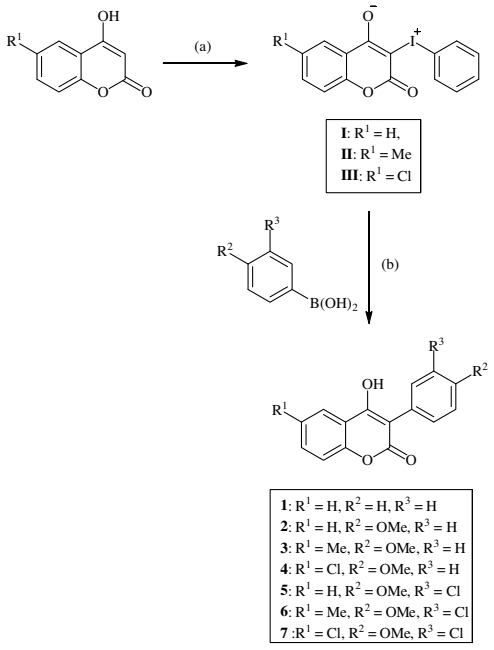


Figure 1. Chemical structures of the 3-phenylcoumarin (A), 4-hydroxy-3-phenylcoumarin (B) and 2-hydroxyisoflavone (C).



Scheme 1. Reagents and conditions: (a) $PhI(OAc)_2$, Na_2CO_3, H_2O, rt, 14 h; (b) $Pd(OAc)_2$, $P(t\text{-}Bu)_3$, LiOH, DME/H_2O, rt, 24–48 h.

Table 1
MAO-A and MAO-B inhibitory activity results for the synthesized compounds **1–7** and reference inhibitor

Compounds	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Selectivity index
1	[a]	[a]	—
2	[a]	69.59 ± 4.70	>1.4[b]
3	[a]	32.04 ± 2.16	>3.1[b]
4	[a]	[a]	—
5	[a]	9.26 ± 0.63	>10[b]
6	[a]	42.68 ± 2.88	>2.3[b]
7	[a]	2.79 ± 0.19	>36[b]
Iproniazide	6.56 ± 0.76	7.54 ± 0.36	0.87
R-(−)-Deprenyl	68.73 ± 4.21	$17x10^{-3} \pm 1.9 \times 10^{-3}$	4.043

All IC_{50} values shown in the table are expressed as means ± SEM from five experiments. Level of statistical significance:
[a] Inactive at 100 μM (highest concentration tested).
[b] Values obtained under the assumption that the corresponding IC_{50} against MAO-A is the highest concentration tested (100 μM).

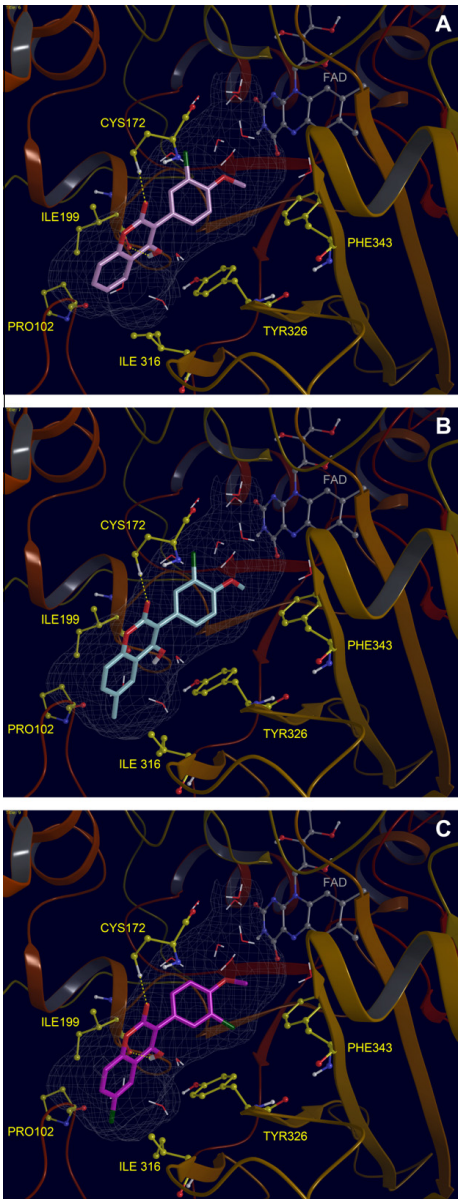


Figure 2. Best docking poses retrieved for compounds **5** (a), **6** (b) and **7** (c) into the MAO-B (PDB code: 2V60). Coumarins are represented in tube with carbon atoms colored in plum for **5**, turquoise for **6** and purple for **7**. Hidden ribbons for a better visualization from residues Glu159 to Glu179 (excluding Cys172). MAO-B binding site displayed as gray mesh style. Interacting residues and FAD cofactor labeled in ball and stick, with carbon atom colored in yellow and gray respectively. Water molecules depicted in wire rendering. H-bonds displayed in yellow dot line. Nonpolar hydrogens are omitted.

iproniazide but not than the *R*-(−)Deprenyl, used as reference compounds. None of the described compounds showed MAO-A inhibitory activity for the highest concentration tested (100 μM).

A computational approach, through docking calculations, was used to better understand which are the most energetically preferred orientations adopted by compounds **5**, **6** and **7** inside the MAO-B binding pocket.

High resolution crystal structure of hMAO-B (PDB code 2V60)[1] was taken as target for docking experiments. Protein Preparation Wizard of Maestro 9.1[38] was used to set-up protein before run calculations. With this helpful tool co-crystallized ligand was removed, water molecules beyond 5 Å from the ligand were deleted, hydrogen atoms were added and then minimized using OPLS_2005 force field. Compounds **5**, **6** and **7** were prepared with LigPrep[39] tool using MMFFs force field. The QM-Polarized Ligand Docking (QPLD)[40] protocol was used for docking calculations. In a first step, ligands were docked into the known[26] MAO-B binding site using Glide[41] standard precision (SP); the initial charges were calculated by semiempirical methods and 8 best poses for each ligand were retained. In the second step the Quantum Mechanical (QM) treatment of charges, calculated by Jaguar[42], allowed to take into consideration the polarization of the charges in the ligand by the protein. The final step consisted to redock the ligand, with improved charges, with Glide extra precision (XP) mode, retaining the 4 best poses for each ligand. With the aim of minimizing the protein–ligand complexes and return the estimate binding free energy for each ligand, the 4 output complexes derived from QPLD were subjected to MacroModel-eMBrAcE[43] minimization. With this flexible-receptor tool all residues with a distance not exceeding 5Å from the ligand were allowed to move freely, while residues outside this shell were frozen. The complexes were minimized using OPLS_2005 force field. Results were retrieved using Energy difference mode which calculates separately the energy associated first on the receptor, than on the ligand and finally on the complex using the follow equation:

$$\Delta E = E_{\text{complex}} - E_{\text{ligand}} - E_{\text{protein}}$$

The most stable binding solution for compounds **5**, **6** and **7** shows the same pattern (Fig. 2).

The entrance cavity of binding site is occupied by the hydroxycoumarin moiety leaving the 3-arylcoumarin rings directed toward FAD cofactor. Cys172 plays a significant role for the complex stabilization, forming an H-bond with carbonyl oxygen of all the three coumarins. The higher activity showed by **5** and **7**, compared to **6**, could be partially due to involvement of another H-bond in the binding mode; in this case only **5** and **7** form an H-bond between the 4-hydroxy group and Ile199. Furthermore, 3-arylcoumarin rings of **5**, **6** and **7** share a π–π stacking interaction with Tyr326. Interestingly, the *p*-methoxy group occupies the same position in the three coumarins, while the position adopted by the chloro atom in the 3-phenyl ring appears to be unimportant for the modulation of MAO-B inhibitor activity, because it assumes an opposite orientation in the two most actives compounds **5** and **7**. Good van der Waals and electrostatic interactions with Pro102, Ile316 and Phe343 were also observed for all the docked molecules.

As conclusion, these preliminary results allow us a better understanding of the molecular fragments that are essential to maintain and/or improve the MAO activity and selectivity. These findings encourage us to continue the efforts towards the optimization of the pharmacological profile of this structural moiety as an important scaffold for the potential treatment of ND.

Acknowledgements

This work was partially supported by the Ministerio de Sanidad y Consumo (FIS PS09/00501) and Xunta da Galicia

(PGIDIT09CSA030203PR and 10PXIB203303PR). This work was also partially supported by MIUR and Cagliari University (National Project "Stereoselezione in Sintesi Organica metodologie ed Applicazioni") and by CINMPIS and FIRB. S.S. thanks Regione Sardegna for the PhD grant (PR-MAB-A2009-613). M.J.M. thanks to Fundação para a Ciência e Tecnologia for the PhD grant (SFRH/BD/61262/2009). S.V.R. thanks to Ministerio de Educación y Ciencia for a PhD grant (AP2008-04263).

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33. *General procedure for the preparation of 3-aryl-4-hydroxycoumarins*: a degassed solution of appropriated phenyl boronic acid (2.2 equiv) and P(*t*-But)$_3$ (27 mL) in DME and H_2O (4:1, 12.5 mL) was added to a mixture of iodonium ylide (0.55 mmol), LiOH/H_2O (3 equiv) and $Pd(OAc)_2$ (6.2 mg) under argon at room temperature. After being stirred at the same temperature for 24–48 h. The resulting mixture was purified by FC (hexane/ethyl acetate, 7:3) to give the desired compound.
3-(3′-Chloro-4′-methoxyphenyl)-4-hydroxycoumarin (**5**). It was obtained with yield 80%. Mp: 298–300 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.94 (s, 3H, OCH_3), 7.21 (s, 1H, H2′), 7.28–7.49 (m, 3H, H6, H8, H5′), 7.56–7.83 (m, 2H, H7, H6′), 7.98 (d, 1H, H5, *J* = 7.6), 15.7 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 56.2, 105.0, 112.8, 116.6, 116.9, 120.7, 124.2, 124.4, 125.6, 131.5, 132.7, 132.8, 152.7, 154.3, 161.0, 162.3. MS *m*/*z* (%): 302 (M$^+$, 100), 182 (64), 121 (95). Anal. Calcd for $C_{16}H_{11}ClO_4$: C, 63.48; H, 3.66. Found: C, 63.45; H, 3.69.
3-(3′-Chloro-4′-methoxyphenyl)-4-hydroxy-6-methylcoumarin (**6**). It was obtained with yield 80%. Mp: 301–304 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.45 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.14–7.24 (m, 1H, H8), 7.25–7.37 (m, 3H, H7, H2′, H5′), 7.40–7.52 (m, 1H, H6′), 7.80 (s, 1H, H5), 15.4 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 20.9, 56.6, 105.0, 112.8, 116.4, 120.8, 123.8, 125.6, 129.0, 131.5, 132.7, 133.6, 133.6, 150.8, 154.3, 160.9, 162.4. MS *m*/*z* (%): 316 (M$^+$, 59), 182 (80), 135 (100), 97 (36), 83 (37), 71 (51), 57 (91). Anal. Calcd for $C_{17}H_{13}ClO_4$: C, 64.46; H, 4.14. Found: C, 64.46; H, 4.09.
6-Chloro-3-(3′-chloro-4′-methoxyphenyl)-4-hydroxycoumarin (**7**). It was obtained with yield 62%. Mp: 321–323 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.90 (s, 3H, OCH_3), 7.0–7.57 (m, 3H, H8, H2′, H5′), 7.60–7.84 (m, 2H, H7, H6′), 8.0 (s, 1H, H5), 15.7 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 56.8, 113.0, 119.0, 121.1, 123.6, 125.5, 128.7, 131.7, 132.6, 132.8, 135.2, 136.1, 151.5, 154.6, 160.2, 162.2. MS *m*/*z* (%): 338 (M$^+$, 59), 336 (100), 182 (75), 155 (88), 142 (26), 57 (31). Anal. Calcd for $C_{16}H_{10}Cl_2O_4$: C, 57.0; H, 2.99. Found: C, 57.05; H, 3.05.
34. Determination of human monoamine oxidase (hMAO) isoform activity. The effects of the tested compounds on hMAO isoform enzymatic activity were evaluated by a fluorimetric method. Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing the tested drugs in several concentrations and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity [165 pmol of *p*-tyramine/min (hMAO-A: 1.1 μg protein; specific activity: 150 nmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min/mg protein; hMAO-B: 7.5 μg protein; specific activity: 22 nmol of *p*-tyramine transformed/min/mg protein)] were placed in the dark fluorimeter chamber and incubated for 15 min at 37 °C. The reaction was started by adding (final concentrations) 200 μM Amplex® Red reagent, 1 U/mL horseradish peroxidase and 1 mM *p*-tyramine. The production of H_2O_2 and, consequently, of resorufin was quantified at 37 °C in a multidetection microplate fluorescence reader (FLX800™, Bio-Tek® Instruments, Inc., Winooski, VT, USA) based on the fluorescence generated (excitation, 545 nm, emission, 590 nm) over a 15 min period, in which the fluorescence increased linearly. Control experiments were carried out simultaneously by replacing the tested drugs with appropriate dilutions of the vehicles. In addition, the possible capacity of the above tested drugs for modifying the fluorescence generated in the reaction mixture due to non-enzymatic inhibition (e.g., for directly reacting with Amplex® Red reagent) was determined by adding these drugs to solutions containing only the Amplex® Red reagent in a sodium phosphate buffer. The specific fluorescence emission (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from vials containing all components except the hMAO isoforms, which were replaced by a sodium phosphate buffer solution.
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Journal of Medicinal Chemistry

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Synthesis and Electrochemical and Biological Studies of Novel Coumarin−Chalcone Hybrid Compounds

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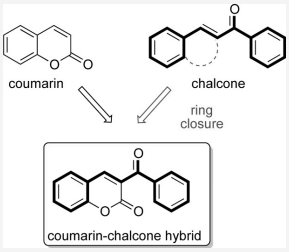
ABSTRACT: A series of novel hydroxy-coumarin−chalcone hybrid compounds **2a−i** has been synthesized by employing a simple and efficient methodology. An electrochemical characterization using cyclic voltammetry and ESR spectroscopy were carried out to characterize the oxidation mechanism for the target compounds. The antioxidant capacity and reactivity were determined by ORAC and ESR assays, respectively. Biological assays were assessed to evaluate the cytotoxicity and cytoprotection capacity against ROS/RNS on BAEC. The results revealed that all tested compounds present ORAC values that are much higher than other well-known antioxidant compounds such as quercetin and catechin. Compound **2e** showed the highest ORAC value (14.1) and also presented a low oxidation potential, good scavenging capacity against hydroxyl radicals, low cytotoxicity, and high cytoprotective activity.



INTRODUCTION

Free radicals and reactive oxygen and nitrogen species (ROS and RNS) are atoms, molecules, or ions possessing at least one unpaired electron; therefore, they are highly reactive with a wide range of other different molecules. They are constantly generated and maintained in balance in biological systems through metabolic processes, and they play important roles in a variety of normal biochemical functions such as cell signaling, apoptosis, gene expression, ion transport, and pathological processes.[1,2]

It is accepted that ROS/RNS play different roles in vivo. Some of these roles are positive and are related to their involvement in energy production, phagocytosis, regulating cell growth, intercellular signaling, and the synthesis of biologically important compounds.[3] However, the overproduction of ROS/RNS can have damaging effects on many molecules. ROS/RNS are capable of oxidizing cellular proteins, nucleic acids, and lipids because they are very small and highly reactive. For example, lipid peroxidation is a free-radical-mediated propagation of oxidative damage to polyunsaturated fatty acids involving several types of free radicals, and termination occurs through enzymatic means or by free-radical scavenging by antioxidants.[4,5] Therefore, the excess of ROS/RNS causes a disturbance in the prooxidant−antioxidant balance in favor of the former, leading to potential damage, which is known as oxidative stress.[1,6,7] Oxidative stress considerably contributes to the pathogenesis of inflammatory and cardiovascular diseases, cancer, diabetes, Alzheimer's disease, autism, and aging among others.[8−13]

The consumption of natural antioxidants, such as flavonoids, supports the endogenous antioxidant defense system. Both dietary and enzymatic antioxidants are components of interrelated systems that interact with each other to control ROS production and thereby ensuring adequate defenses against oxidative stress.[14,15]

In this sense, many studies concerning the antioxidant capacity of phenolic compounds of different types (e.g., flavonoids, coumarins, and tanins) have been established, and their physicochemical properties have been correlated with their antioxidant properties.[16] In general, free-radical scavenging, redox potential, and the antioxidant activity of these classes of compounds mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules, although it is also affected by other factors.[17] Therefore, low oxidation potentials, the number of hydroxyl groups present, and the existence of electron-donating groups and/or the stabilization of a secondary radical via delocalization through conjugated systems play a key role in the antioxidant activity.[18−21]

Received: April 16, 2013
Published: July 16, 2013

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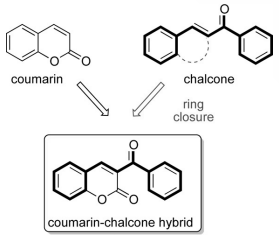
Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. Because of their ability to inhibit low-density lipoprotein (LDL) oxidation, flavonoids have demonstrated unique cardioprotective effects.[22,23] The protective effects of flavonoids in biological systems are attributed to their capacity for transferring electrons to radicals, chelating metal catalysts,[24] activating antioxidant enzymes,[25] reducing alpha-tocopherol radicals,[26] and inhibiting oxidases.[27]

Chalcones (α-phenyl-β-benzoylethylene) are prominent secondary-metabolite precursors of flavonoids and isoflavonoids in plants. Chemically, they consist of two aromatic rings linked by a three-carbon α,β-unsaturated system and are known to exhibit an impressive array of biological properties.[28−30] Chalcones have attracted much research attention because of their pharmaceutical potential resulting from their radical-scavenging,[31,32] antitumor,[33,34] anti-inflammatory,[35] and neuroprotective properties.[36] Thus, it is reasonable to explore the radical-scavenging properties of the hydroxyl derivatives of chalcones.

However, coumarins (2*H*-chromen-2-one) are a large family of compounds of both natural and synthetic origin that are important because of the pharmacological activities that these kind of compounds display,[37] such as antimicrobial,[38] monoamine oxidase (MAO) inhibition,[39−41] antitumor,[42] adenosine receptor antagonist,[43] and antioxidant[17] among others.

Therefore, coumarin and chalcones represent promising scaffolds in the medicinal chemistry realm. Taking into account the previously mentioned features[18,19] and with the aim of finding new chemical structures with antioxidant activity, we have designed a new scaffold in which the chalcone fragment is partially incorporated in the coumarin moiety. The resulting coumarin−chalcone hybrid fixes the trans-conformation of the chalcone scaffold by sharing the double bond of the pyrane ring of the coumarin nucleus (Scheme 1).

Scheme 1. Structural Rational Design of Coumarin−Chalcone Hybrid Compounds

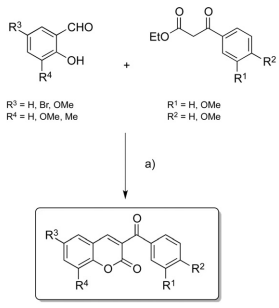


CHEMISTRY

Coumarin derivatives **2a−i** were efficiently synthesized in two steps,[40] which are briefly described as follows: (i) the synthesis of methoxy-3-benzoylcoumarins **1a−i** and (ii) the synthesis of hydroxy-3-benzoylcoumarins **2a−i**. These steps are outlined in Scheme 2.

On the basis of the widely used Knoevenagel condensation reaction for the preparation of coumarin derivatives,[37,44] we used an efficient one-step synthesis to generate methoxy-3-benzoylcoumarin precursors **1a−i**. These compounds were prepared in good yields (74−98%) using the appropriately substituted salicylaldehyde and the corresponding β-ketoester in the presence of piperidine in ethanol under reflux for 2−5 h, obtaining the desired compound as a precipitate that was separated by filtration and further purified by recrystallization in MeOH/DCM. Final compounds **2a−i** were then synthesized by the hydrolysis of the corresponding methoxy precursors **1a−i** by employing an excess of a Lewis acid, BBr_3, in DCM at 80 °C in a Schlenk tube for 48 h followed by treatment with MeOH and purification by flash chromatography of the crude product (69−98%).

Scheme 2. Synthesis of Methoxylated (1a−i) and Hydroxylated (2a−i) 3-Benzoylcoumarin Derivatives[a]



[a]Reagents and conditions: (a) piperidine, EtOH, reflux, 2−5 h; (b) BBr_3, DCM, 80 °C, 48 h.

ELECTROCHEMICAL STUDY

Cyclic Voltammetry. The electrochemical properties of hydroxy-coumarin−chalcone derivatives **2a−i** were studied by cyclic voltammetry, employing an electrochemical cell with three electrodes and different scan rates between 0.050 and 0.75 V* s^{-1}. Under these conditions, all derivatives exhibited one (compounds **2a−g**) or two (compounds **2h** and **2i**) anodic peaks without the corresponding cathodic peak (Figure 1). The anodic peak potentials (Epa I and Epa II) obtained at a 0.75 V* s^{-1} scan rate are listed in Table 1.

Characterization of Coumarin−Chalcone Radicals by ESR Spectroscopy. To confirm the formation of semiquinone radicals from compounds **2a−i**, an electron spin resonance (ESR) spectroscopy study was performed. For compounds **2a−d** and **2g**, the spin trap PBN was used. This nitrone derivative is a diamagnetic compound that, in the presence of free radicals, produces a spin adduct (paramagnetic species), increasing the half-life of the radicals species.[45] The experiment was carried out by electrolysis of the derivatives to control the potential in situ using DMSO as the solvent and TBAP as the supporting electrolyte at room temperature. Next, the PBN spin trap was added. In the absence of the PBN spin trap, an ESR spectrum was observed for derivatives **2e**, **2f**, **2h**, and **2i**. Compound **2e**, with two hydroxyl groups, evidenced a symmetric ESR

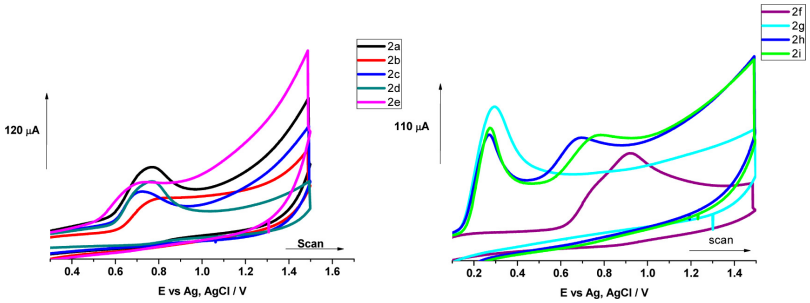


Figure 1. Cyclic voltammograms of the hydroxy-coumarin−chalcone derivatives **2a−i** in phosphate buffer, pH 7.4, at 0.75 V* s^{-1}.

Table 1. Oxidation Potential for Compounds 2a−i[a]

compound	Epa I (V)	Epa II (V)
2a		0.77
2b		0.79
2c		0.72
2d		0.77
2e		0.74
2f		0.92
2g	0.30	
2h	0.27	0.69
2i	0.27	0.77

[a]Values obtained at a scan rate of 0.75 V*s^{-1}.

spectrum with five lines (Figure 2a). Compound **2f** showed a hyperfine pattern with two lines (Figure 2b) and was simulated and characterized by two doublets resulting from two nonequivalent hydrogen atoms.

ORAC-FL Assay. The capacity for peroxyl radical scavenging by coumarin−chalcone hybrids was assessed using the oxygen-radical absorbance capacity (ORAC) assay. This methodology gives an index relative to a standard molecule (Trolox), which is a hydrosoluble analog of vitamin E. The ORAC values obtained for all derivatives are shown in Table 2. All derivatives presented an ORAC index higher than well-known antioxidants quercetin and catechin.[46] Compound **2e** presented the highest ORAC value of the studied series (14.1).

Antioxidant Reactivity by ESR Assay. The reactivity against a mixture of radical species for all of the studied coumarin derivatives was carried out by adapting the H_2O_2/NaOH/DMSO/DMPO system[47] using a noncatalytic and competitive fenton reaction in which the spin trap DMPO and the antioxidant molecule competes for the hydroxyl, superoxide anion, and methyl radicals.

Four wide hyperfine lines were observed in the control measure (labeled as "blank", indicating the absence of the tested and antioxidant molecules; Figure 3) because of the

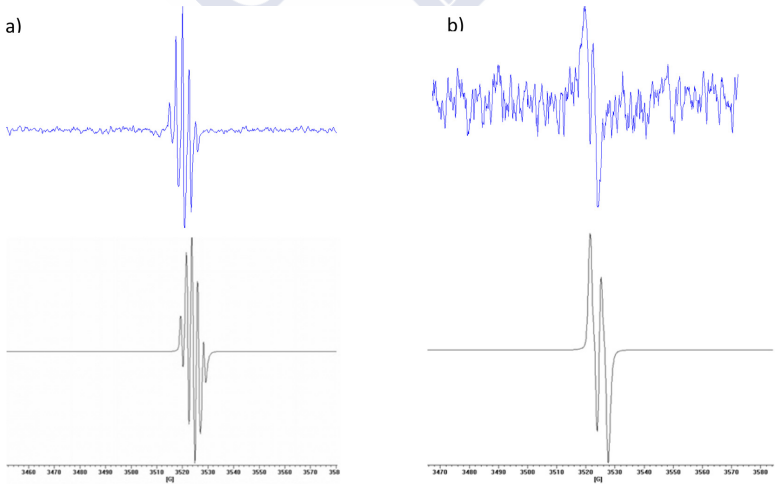


Figure 2. Experimental (blue) and simulated (black) ESR spectra for compounds **2e** (a) and **2f** (b). Simulated hyperfine splitting constants (hfscs) were a_H = 3.57 and 1.34 G for compound **2e** and a_H = 2.35, 2.35 (two equivalent hydrogen atom), and 1.75 G for compound **2f**.

Table 2. ORAC Values and the Percentage of Radicals Scavenging for Compounds 2a–i[a]

compound	ORAC value	radical scavenging (%)
2a	9.2 ± 0.4	26
2b	11.5 ± 0.4	39
2c	9.5 ± 0.3	28
2d	10.9 ± 0.3	45
2e	14.1 ± 0.3	58
2f	8.3 ± 0.3	65
2g	13.0 ± 0.4	100
2h	8.1 ± 0.6	75
2i	8.8 ± 0.5	100
Trolox	1.0 ± 0.2	31
quercetion	7.3 ± 0.2[b]	
catechin	6.8 ± 0.2[b]	

[a]Measured by ESR spin trapping in competition with DMPO. [b]Data obtained from ref 46.

mixture of the DMPO–OH, DMPO–CH_3, and DMPO–$O_2^{\bullet-}$ adducts that formed.

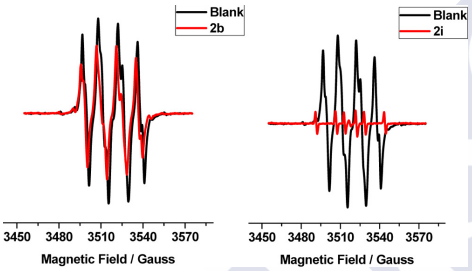


Figure 3. ESR spectra for the reactivity assays of compounds **2b** and **2i**.

BIOLOGY

Cytotoxicity Assays. The cytotoxicity of compounds **2a–i** on bovine aortic endothelial cells (BAEC) was assessed and evaluated over a concentration range of 0–50 μM by employing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay.[47] The results are shown in Table 3 for concentrations of 10, 20, and 50 μM for each compound studied.

Table 3. Cytotoxicity of Compounds 2a–i tested at Three Different Concentrations on BAEC[a]

compound	10 μM	20 μM	50 μM
2a	76 ± 7	60 ± 6	44 ± 5
2b	88 ± 2	95 ± 2	86 ± 1
2c	95 ± 3	93 ± 9	104 ± 6
2d	87 ± 7	82 ± 5	101 ± 8
2e	68 ± 1	94 ± 5	93 ± 10
2f	96 ± 3	101 ± 1	93 ± 4
2g	102 ± 4	100 ± 5	92 ± 5
2h	49 ± 2	68 ± 2	63 ± 2
2i	64 ± 2	23 ± 2	71 ± 6

[a]The results are expressed as the percentage of cell viability (n = 3).

Cytoprotection Assays: ROS/RNS Scavenging Activity in Cell Systems. *Hydrogen Peroxide.* To evaluate the cytoprotection against H_2O_2, derivatives presenting high cell-viability values (compounds **2b–g**) were selected. These compounds also presented high antioxidant capacity in ORAC assays and showed notable hydroxyl-radical scavenging activity.

BAEC were incubated with two different concentrations of each derivative (10 and 20 μM) for 24 h in a 24-well plate followed by treatment with 1 mM H_2O_2 for 2 h. Cell viability was then quantified by the MTT reduction assay (Figure 4a).

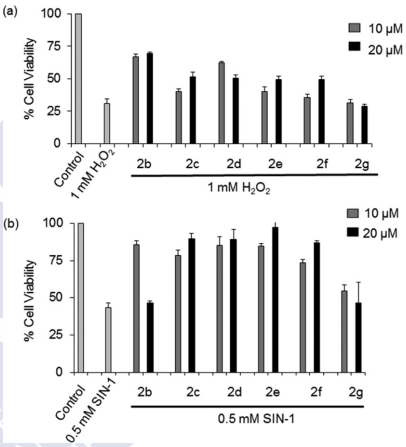


Figure 4. Cytoprotection of compounds **2b–g** at 10 and 20 μM against 1 mM H_2O_2- (a) and 0.5 mM SIN-1-induced (b) cytotoxicity of bovine aortic endothelial cells using MTT assay.

Peroxynitrite. The cytoprotective property of the compounds against peroxynitrite in BAEC was investigated by MTT assay. Peroxynitrite was generated in the cells using 3-morpholino-sydnonimine (SIN-1).[48] Figure 4b shows the results for the cytoprotection assays against peroxynitrite for compounds **2b–g**.

Theoretical Evaluation of ADME Properties. To correlate better the druglike properties of the coumarin–chalcone hybrid compounds, the lipophilicity, expressed as the octanol/water partition coefficient and herein called log *P*, as well as other theoretical calculations such as the topological polar surface area (TPSA), the number of hydrogen-bond acceptors, and the number of hydrogen-bond donors were calculated using the Molinspiration property program.[49] The theoretical prediction of the absorption, distribution, metabolism, and excretion (ADME) properties of all compounds is summarized in table 4.

RESULTS AND DISCUSSION

Taking into account the importance of the hydroxyl substituents in the antioxidant properties,[17,50] we have focused this study on hydroxy-coumarin–chalcone hybrids. All coumarin derivatives described in this Article (compounds **1a–i** and **2a–i**) were efficiently synthesized according to the simple and generalized experimental procedure outlined in

Table 4. Theoretical Prediction[a] of the ADME Properties of Compounds 2a−i

compound	log P	molecular weight	TPSA (Å^2)	n−OH acceptors	n−OHNH donors	volume (Å^3)
2a	2.92	266.25	67.51	4	1	227.00
2b	2.89	266.25	67.51	4	1	227.00
2c	3.70	345.15	67.51	4	1	244.88
2d	3.32	280.28	67.51	4	1	243.56
2e	2.42	282.25	87.74	5	2	235.01
2f	3.89	345.15	67.51	4	1	244.88
2g	3.21	361.15	87.74	5	2	252.90
2h	2.16	298.25	107.97	6	3	243.03
2i	2.92	377.15	107.97	6	3	260.92

[a]TPSA, topological polar surface area; n−OH, number of hydrogen acceptors; n−OHNH, number of hydrogen bond donors. The data was determined with the Molinspiration calculation software.

Scheme 2. Electrochemical, biological, and theoretical studies were carried out for final tested compounds **2a−i**.

From the experimental results obtained by cyclic voltammetry, we observed that under these conditions all derivatives exhibited one (compounds **2a−g**) or two (compounds **2h** and **2i**) characteristic anodic peaks without the corresponding cathodic peak (Figure 1), indicating an irreversible oxidation process as a result of the oxidation of one hydroxyl group in any of the aromatic rings, forming the semiquinone radical.

The anodic peak potentials (Epa I and Epa II) obtained at a 0.75 V* s^{-1} scan rate are listed in Table 1. Monohydroxyl derivatives **2a**, **2c**, and **2d**, which contain a hydroxyl substituent at the para position of the benzoyl moiety, presented an anodic peak potential in the same positive potential range (Epa II = 0.77, 0.72, and 0.77 V, respectively). The same positive potential in derivatives **2a** and **2d** indicates that the methyl substituent at position eight of the coumarin core in **2d** is not relevant in the oxidation process. In contrast, the bromine group in derivative **2c** seems to provoke a slight decrease in the anodic peak potential (Epa II = 0.72 V). The same effect is observed for compound **2e** (Epa II = 0.74 V) that, in addition to the hydroxyl group present in the benzoyl moiety, also bears an additional hydroxyl group in the coumarin core. Compounds **2b** and **2f**, with a hydroxyl group in the coumarin moiety, presented oxidation potentials that shifted to more positive values (0.79 and 0.92 V, respectively), disfavoring the oxidation process. This effect is more pronounced for compound **2f** because of the interaction through intramolecular hydrogen bonding between the OH group in position eight and the oxygen atom of the pyrone ring as well as the electron-withdrawing properties of this pyrone system.[51]

Compounds **2g**, **2h**, and **2i** showed a characteristic peak (Epa I) attributed to the catechol moiety. This oxidation occurred at a low potential (0.30, 0.27, and 0.27 V, respectively) because of the electron-donating effect of the hydroxyl group at the 3′ position of the benzoyl moiety as well as the favorable quinone formation as the final product in these derivatives. In addition to this low-potential oxidation peak, compounds **2h** and **2i** showed an additional second peak that was assigned to the hydroxyl group at position eight of the coumarin core.

The data obtained for the characterization of the coumarin−chalcone radicals by ESR spectroscopy showed that in the absence of the spin trap *N*-*tert*-butyl-α-phenylnitrone (PBN), the oxidation of compounds **2a−d** and **2g** gave no ESR signal. However, in the presence of PBN, a hyperfine pattern with six lines was observed, corresponding to the formation of a carbon-centered semiquinone spin adduct ($a_H \approx 13.5$ and 2.3 G were found for all compounds).[52] Compound **2e**, with two hydroxyl groups, evidenced a symmetric ESR spectrum with five lines (Figure 2a), and it was interpreted as a semiquinone radical in which the hyperfine pattern corresponds to one triplet assigned to two equivalent hydrogen atoms with a_H = 2.37 G and two doublets corresponding to one non equivalent hydrogen atom with hyperfine constants a_H = 2.35 and 1.75 G.

Compound **2f** showed a hyperfine pattern with two lines (Figure 2b) and was simulated and characterized by two doublets resulting from two nonequivalent hydrogen atoms with a_H = 1.34 and 3.57 G. For the radical species of compound **2h**, the ESR spectrum (data not shown) was simulated by two doublets attributed to two nonequivalent hydrogen atoms with a_H = 4.35 and 2.37 G. Finally, for the radical species of compound **2i**, the hyperfine pattern was interpreted and simulated by four doublets resulting from four nonequivalent hydrogen atoms with hyperfine coupling constants a_H = 2.82, 2.93, 2.06, and 1.83 G as well as one triplet with a_H = 3.37 G resulting from two equivalent hydrogen atoms.

The antioxidant capacity of compounds **2a−i** was studied by ORAC-FL assays. From the data obtained, which is summarized in Table 2, we observed that the difference between the ORAC values could be related to the substituents present in these derivatives. Derivatives **2a** and **2b**, with one hydroxyl group at the para position of the benzoyl moiety and at the six position of the coumarin scaffold, respectively, presented ORAC values of 9.2 and 11.5, respectively. Compounds **2c** and **2d**, with a hydroxyl group in the same position as in compound **2a** but with an additional bromine substituent in position six of the coumarin or a methyl substituent at the position eight, respectively, presented ORAC values of 9.5 and 10.9, respectively. Derivative **2e**, which combines the substitution pattern of compounds **2a** and **2b**, was found to be the compound with the highest ORAC value (14.1).

However, by structurally comparing compounds **2c** and **2g**, we observed that the only difference is the presence of an additional hydroxyl group at the 3′ position of the benzoyl moiety in compound **2g**, forming a catechol function. This modification increases the antioxidant capacity in compound **2g** (ORAC = 13.0). ORAC values of around 8 were observed for compounds **2f**, **2h**, and **2i**. In addition to the catechol function, compounds **2h** and **2i** present an additional hydroxyl group at position eight of the coumarin ring compared to structurally related compound **2g**. This additional hydroxyl group could form a hydrogen bond with the oxygen atom of the pyrone ring, disfavoring the transfer of the hydrogen atom.

The antioxidant reactivity of compounds **2a−i** was studied by an ESR assay. With this method, it was observed that the

intensity of the spectrum decreased in the presence of the tested coumarin−chalcone hybrid compounds that were added to the system (red line, Figure 3). This behavior was observed in all derivatives **2a−i**, and the percent of the radicals ($CH_3^•$, $OH^•$, $O_2^{•-}$) scavenging activity is represented in Table 2. The scavenging values obtained could be related to the number and position of the hydroxyl substituents. Compounds **2g** and **2i** showed a different hyperfine pattern, which was attributed to the formation of carbon-centered spin adducts (Figure 3), demonstrating that the secondary semiquinone radical for each compound was trapped by the DMPO spin trap and the radical scavenging percentages were close to 100.

In general, the MTT assay only provides qualitative trends for the cytotoxicity of the compounds and the cytoprotective properties. The cytotoxicity of compounds **2a−i** on BAEC using different concentrations (10, 20, and 50 μM) is shown in Table 3. Derivatives **2b−g** showed cell viabilities between 86 and 100% up to the maximum concentration of 50 μM. Compounds **2a**, **2h**, and **2i** showed significant toxicity at 50 μM, whereas compound **2a**, which was the most toxic, resulted in only a 44% viability. The varying degrees in the toxicity of the compounds can be accounted for by several factors, such as the differences in their compartmentalization properties, the nature and concentration of ROS produced, and their ability to scavenge radicals and/or initiate pro-apoptotic signaling pathways.

Because compounds **2a, 2i,** and **2h** demonstrated significant toxicity, they were not used for the cytoprotection assays. However, compounds **2b−g**, because of their low toxicity, were examined at lower doses of 10 and 20 μM to test their cytoprotection against hydrogen peroxide (H_2O_2) or peroxynitrite ($ONOO^-$) generated from SIN-1, and the data are shown in Figure 4 panels a and b, respectively. Compounds **2b** and **2d** showed the highest cytoprotection against H_2O_2 compared to all compounds, which presented around 40% cytoprotection at 10 μM. At 20 μM, compounds **2c−2f** were cytoprotective against $ONOO^-$ by ∼90−95%, whereas compound **2b** imparted no protection at this concentration. Compound **2g** was not cytoprotective against both H_2O_2 and $ONOO^-$ at any of the concentrations tested. All of the studied compounds showed observable cytoprotection, with remarkable results for compounds **2b−f**. In our previous work,[53] it was shown that a lypophilic spin trap such as PBN protects BAEC from H_2O_2-induced toxicity, whereas more polar DMPO protects the cells more efficiently from $ONOO^-$. The studied compounds show a better cytoprotective effect against $ONOO^-$. However, because of the complexity of the mechanisms that are directly and indirectly involved in this assay, it is difficult to determine if a correlation exists between the cytoprotection and partition coefficients because these latter values were theoretically calculated. Moreover, the oxidation potentials may not show a direct correlation with the cytotoxicity/cytoprotection data because of other cellular events that are involved such as variations in the ability of the compounds to redox cycle, subcellular compartmentalization, degradation by cellular oxido-reductants, and the ability to induce (or suppress) nonradical-mediated pro-apoptotic pathways. Therefore, although we would like to determine if a direct correlation exists between the cytotoxicity and physical data, such as the partition coefficient and redox potentials, the complexity of the mechanisms involved in cell viability makes this correlation difficult to realize.[19,54,55]

From the data obtained for the theoretical evaluation of the ADME properties, one can notice that hybrid compounds **2a−i** do not break any point of the Lipinski's rule of five, making them promising leads for drug candidates.[56] TPSA and log *P* values are compatible with those described as a predictive indicator of a drug's capacity for membrane penetration.[57]

■ CONCLUSIONS

In this Article, we describe the synthesis of coumarin−chalcone hybrid compounds by employing the Knoevenagel reaction as a key step for the efficient preparation of a selected series. The electrochemical properties of the studied compounds proved to be excellent, presenting low oxidation potentials, very high ORAC values (all of them were higher than the reference compounds quercetin and catechin), and a high percentage of radical scavenging. In most cases, the compounds presented low cytotoxicity for BAEC in culture even at the highest concentration tested as well as good cytoprotective effects against H_2O_2 and $ONOO^-$ induced cell death. In addition, the whole series of compounds presented good theoretical ADME properties. Therefore, on the basis of these results, the synthesized coumarin−chalcone hybrid compounds can be considered to be promising scaffolds in the studied area.

It is worth noting that compound **2e** presented the highest ORAC value (14.1), good scavenging capacity, low cytotoxicity, and high cytoprotection values, especially against $ONOO^-$ induced cell death (almost 100% cell viability at 20 μM); therefore, it can be considered to be potential candidate as a lead compound for more thorough studies of its antioxidant properties.

■ EXPERIMENTAL SECTION

Chemistry. Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using TMS as the internal standard (chemical shifts are given in δ values, *J* is given in Hz). Mass spectra were obtained using a Hewlett-Packard 5988A spectrometer. Elemental analyses were performed using a PerkinElmer 240B microanalyser and were within ±0.4% of calculated values in all cases. Silica gel (Merck 60, 230−00 mesh) was used for flash chromatography (FC). Analytical thin layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm). The purity of compounds **1a−i** and **2a−i** was assessed by HPLC and was found to be higher than 95%. All of the chemical reagents employed in the synthesis process were obtained from Aldrich Chemical Co., Fluka, Across, or Merck (analytical reagent grade). All reactions were carried out under a deoxygenated and dry argon atmosphere unless otherwise indicated. Argon was dried by flowing it through $CaCl_2$ columns, NaOH stones, and P_2O_5.

*General Procedure for the Synthesis of Methoxy-3-benzoylcoumarins **1a−i**.*[40] To a solution of the appropriate β-ketoester (1 mmol) and the corresponding salicylaldehyde (1 mmol) in ethanol (3 mL) was added piperidine in a catalytic amount. The mixture was refluxed for 2−5 h. After completion (indicated by TLC), the reaction was cooled, and the precipitated was filtered and washed with cold ethanol and ether to afford the desired compound. The compounds were further recrystallized from $MeOH/CH_2Cl_2$.

*6-Bromo-3-(4′-methoxybenzoyl)coumarin (**1c**).* Yellow solid. Yield: 92%. mp 222−224 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.25 (s, 1H, H-4), 8.05 (s, 1H, H-5), 7.92 (d, *J* = 8.5 Hz, 2H, H-2′, H-6′), 7.84 (d, *J* = 8.7 Hz, 1H, H-7), 7.44 (d, *J* = 8.7 Hz, 1H, H-8), 7.05 (d, *J* = 8.5 Hz, 2H, H-3′, H-5′), 3.85 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.1, 164.5, 158.1, 153.5, 143.3, 135.9, 132.7, 131.9, 129.0, 128.3, 120.6, 119.0, 116.7, 114.6, 56.2. MS (EI) *m*/*z* (%):

359/361 (M^+, 28), 135 (100), 92 (19), 77 (20). Anal. Calcd for $C_{17}H_{11}BrO_4$: C, 56.85; H, 3.09. Found: C, 56.82; H, 3.07.

*8-Methyl-3-(4′-methoxybenzoyl)coumarin (**1d**).* White solid. Yield 93%. mp 250−252 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.34 (s, 1H, H-4), 7.91 (d, J = 8.7 Hz, 2H, H-2′, H-4′), 7.73−7.24 (m, 3H, H-5, H-6, H-7), 7.06 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 3.86 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.2, 164.4, 158.4, 152.9, 152.8, 144.7, 134.7, 132.3, 129.6, 127.7, 125.8, 124.7, 118.5, 114.6, 56.1, 15.0. MS (EI) m/z (%): 295 ([M + 1]$^+$, 19), 294 (M^+, 100), 135 (88), 77 (31). Anal. Calcd for $C_{18}H_{14}O_4$: C, 73.46; H, 4.79; Found: C, 73.39; H, 4.57.

*6-Methoxy-3-(4′-methoxybenzoyl)coumarin (**1e**).* Yellow solid. Yield 80%. mp 182−184 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84 (s, 1H, H-4), 7.71 (d, J = 8.9 Hz, 2H, H-2′, H-6′), 7.13−6.94 (m, 3H, H-5, H-7, H-8), 6.77 (d, J = 8.9 Hz, 2H, H-3′, H-5′), 3.83 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.2, 164.0, 158.3, 155.9, 148.6, 144.2, 132.3, 128.9, 127.3, 121.1, 118.9, 117.6, 114.2, 111.6, 55.9, 55.8. MS (EI) m/z (%): 311 ([M + 1]$^+$, 29), 310 (M^+, 92), 135 (100), 92 (22), 77 (27). Anal. Calcd for $C_{18}H_{14}O_5$: C, 69.67; H, 4.55. Found: C, 69.45; H, 4.33.

*6-Bromo-3-(3′,4′-dimethoxybenzoyl)coumarin (**1g**).* Yellow solid. Yield 98%. mp 223−224 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.23 (s, 1H, H-4), 8.05 (d, J = 2.4 Hz, 1H, H-5), 7.84 (dd, J = 8.8, 2.4 Hz, 1H, H-7), 7.56 (dd, J = 8.4, 2.0 Hz, 1H, H-6′), 7.52−7.38 (m, 2H, H-8, H-2′), 7.05 (d, J = 8.4 Hz, 1H, H-5′), 3.86 (s, 3H, 4′-OCH_3), 3.81 (s, 3H, 3′-OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 189.8, 157.7, 154.3, 153.2, 149.0, 142.8, 135.6, 131.6, 128.7, 127.9, 125.8, 120.4, 118.8, 116.4, 111.3, 111.1, 56.1, 55.9. MS (EI) m/z (%): 389/391 (M^+, 59), 165 (100), 71 (31). Anal. Calcd for $C_{18}H_{13}BrO_5$: C, 55.55; H, 3.37. Found: C, 55.54; H, 3.39.

*8-Methoxy-3-(3′,4′-dimethoxybenzoyl)coumarin (**1h**).* Pale-yellow solid. Yield: 95%. mp 238−240 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.28 (s, 1H, H-4), 7.54 (dd, J = 8.2, 2.1 Hz, 1H, H-6′), 7.46 (d, J = 2.1 Hz, 1H, H-2′), 7.43−7.27 (m, 3H, H-5, H-6, H-7), 7.04 (d, J = 8.2 Hz, 1H, H-5′), 3.93 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.2, 157.9, 154.1, 148.9, 146.6, 144.6, 143.5, 128.8, 127.1, 125.7, 124.9, 120.7, 119.0, 115.5, 111.1, 111.0, 56.3, 56.0, 55.8. MS (EI) m/z (%): 341 ([M + 1]$^+$, 25), 340 (M^+, 97), 165 (100), 137 (9), 77 (10). Anal. Calcd for $C_{19}H_{16}O_6$: C, 67.05; H, 4.74. Found: C, 67.06; H, 4.74.

*6-Bromo-8-methoxy-3-(3′,4′-dimethoxybenzoyl)coumarin (**1i**).* Pale-yellow solid. Yield 90%. mp 276−277 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.21 (s, 1H, H-4), 7.67−7.49 (m, 3H, H-5, H-7, H-6′), 7.46 (s, 1H, H-2′), 7.04 (d, J = 8.5 Hz, 1H, H-5′), 3.96 (s, 3H, 4′-OCH_3), 3.85 (s, 3H, 8-OCH_3), 3.80 (s, 3H, 3′-OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 195.3, 155.1, 149.9, 149.8, 148.2, 143.6, 138.1, 129.4, 128.9, 128.7, 126.0, 123.4, 119.0, 116.8, 112.6, 112.0, 59.0, 57.6, 56.7. MS (EI) m/z (%): 418/420 (M^+, 98), 165 (100), 77 (18). Anal. Calcd for $C_{19}H_{15}BrO_6$: C, 54.43; H, 3.61. Found: C, 54.43; H, 3.62.

*General Procedure for the Synthesis of Hydroxy-3-benzoylcoumarins (**2a−i**).*[40] To the corresponding methoxy-3-benzoylcoumarin (1 mmol), BBr_3 in DCM (20 mmol, 1M) was added in a Schlenk tube. The tube was sealed, and the reaction mixture was heated at 80 °C for 48 h. The resulting crude product was treated with MeOH and rotated to dryness. The obtained precipitate was recrystallized from MeOH or purified by flash chromatography using hexane/ethyl acetate mixtures as the eluent to afford the desired hydroxy derivative.

*3-(4′-Hydroxybenzoyl)coumarin (**2a**).* White solid. Yield: 78%. mp 242−243 °C (mp lit. 239 °C).[58] ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.58 (bs, 1H, OH), 8.30 (s, 1H, H-4), 7.89−7.75 (m, 3H, H-5, H-2′, H-6′), 7.78−7.64 (m, 1H, H-7), 7.51−7.37 (m, 2H, H-6, H-8), 6.86 (d, J = 8.8, 2H, H-3′,H-5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.1, 163.4, 158.5, 154.3, 144.2, 133.6, 132.9, 129.9, 127.8, 127.5, 125.3, 118.7, 116.7, 115.8. MS (EI) m/z (%): 267 ([M + 1]$^+$, 20), 266 (M^+, 93), 237 (25), 121 (100), 93 (22), 65 (25). Anal. Calcd for $C_{16}H_{10}O_4$: C, 72.18; H, 3.79. Found: C, 71.83; H, 3.53.

*6-Bromo-3-(4′-hydroxybenzoyl)coumarin (**2c**).* Pale-yellow solid. Yield: 73%. mp 295−298 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.61 (bs, 1H, OH), 8.22 (s, 1H, H-4), 8.05 (d, J = 2.4 Hz, 1H, H-5), 7.93−7.73 (m, 3H, H-7, H-2′, H-6′), 7.45 (d, J = 8.8 Hz, 1H, H-8), 6.86 (d, J = 8.7 Hz, 2H, H-3′, H-5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.0, 163.6, 158.1, 153.4, 142.8, 136.2, 133.0, 131.8, 128.5, 127.6, 120.7, 119.0, 116.7, 115.9. MS (EI) m/z (%): 343/345 (M^+, 49), 121 (100). Anal. Calcd for $C_{16}H_9BrO_4$: C, 55.68; H, 2.63. Found: C, 55.30; H, 2.45.

*3-(4′-Hydroxybenzoyl)-8-methylcoumarin (**2d**).* White solid. Yield: 56%. mp 252−253 °C. ^{1}H NMR (300 MHz DMSO-d_6) δ 10.56 (bs, 1H, OH), 8.27 (s, 1H, H-4), 7.80 (d, J = 8.4 Hz, 2H, H-2′, H-6′), 7.63 (d, J = 7.5 Hz, 1H, H-5), 7.57 (d, J = 7.5 Hz, 1H, H-7), 7.30 (t, J = 7.5 Hz, 1H, H-6), 6.86 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 2.40 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.2, 163.4, 158.6, 152.6, 144.6, 134.6, 132.9, 127.8, 127.7, 127.2, 125.6, 124.8, 118.5, 115.9, 15.3. MS (EI) m/z (%): 281 ([M + 1]$^+$, 54), 280 (M+, 96), 252 (46), 251 (51), 120 (100), 93 (56), 65 (67). Anal. Calcd for $C_{17}H_{12}O_4$: C, 72.85; H, 4.32. Found: C, 72.67; H, 4.12.

*6-Hydroxy-3-(4′-hydroxybenzoyl)coumarin (**2e**).* Brown crystals. Yield: 65%. mp 301−303 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.58 (bs, 1H, 4′−OH), 9.86 (bs, 1H, 8-OH,), 8.21 (s, 1H, H-4), 7.79 (d, J = 8.8 Hz, 2H, H-2′, H-6′), 7.37−7.24 (m, 1H, H-5), 7.19−7.01 (m, 2H, H-7, H-8), 6.86 (d, J = 8.8 Hz, 2H, H-3′, H-5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.3, 163.4, 158.8, 154.4, 147.7, 144.0, 132.9, 127.8, 127.6, 121.6, 119.2, 117.6, 115.9, 113.7. MS (EI) m/z (%): 283 ([M + 1]$^+$, 9), 282 (M^+, 51), 121 (100), 93 (15), 65 (18). Anal. Calcd for $C_{16}H_{10}O_5$: C, 68.09; H, 3.57. Found: C, 68.23; H, 3.33.

*3-Benzoyl-6-bromo-8-hydroxycoumarin (**2f**).* Pale-yellow solid. Yield: 98%. mp 293−295 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.92 (s, 1H, OH), 8.30 (s, 1H, H-4), 7.93 (dd, J = 8.1, 1.4 Hz, 2H, H-2′, H-6′), 7.79−7.63 (m, 1H, H-5), 7.63−7.44 (m, 3H, H-3′, H-4′, H-5′), 7.34−7.24 (m, 1H, H-8). ^{13}C NMR (75 MHz, DMSO-d_6) δ 191.9, 157.9, 146.3, 144.8, 142.7, 136.3, 134.5, 130.0, 129.2, 127.8, 121.9, 121.8, 120.9, 116.2. MS (EI) m/z (%): 344/346 (M^+, 46), 105 (100), 77 (59). Anal. Calcd for $C_{16}H_9BrO_5$: C, 53.21; H, 2.51. Found: C, 52.97; H, 2.41.

*6-Bromo-3-(3′,4′-dihydroxybenzoyl)coumarin (**2g**).* Yellow solid. Yield: 72%. mp 264−266 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.14 (s, 1H, OH), 9.47 (s, 1H, OH), 8.19 (s, 1H, H-4), 8.05 (d, J = 2.1 Hz, 1H, H-5), 7.84 (dd, J = 8.8, 2.2 Hz, 1H, H-7), 7.45 (d, J = 8.8 Hz, 1H, H-8), 7.39−7.20 (m, 2H, H-2′, H-6′), 6.82 (d, J = 8.1 Hz, 1H, H-5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 189.6, 157.9, 153.3, 152.5, 145.9, 142.3, 135.7, 131.7, 128.4, 127.8, 124.5, 120.5, 119.0, 116.7, 116.3, 115.7. MS (EI) m/z (%): 360/362 (M^+, 87), 253 (47), 251 (45), 227 (14), 225 (18), 137 (100), 109 (44). Anal. Calcd for $C_{16}H_9BrO_5$: C, 53.21; H, 2.51. Found: C, 53.23; H, 2.52.

*8-Hydroxy-3-(3′,4′-dihydroxybenzoyl)coumarin (**2h**).* Bright-yellow solid. Yield: 81%. mp 277−279 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.35 (s, 1H, OH), 10.10 (s, 1H, OH), 9.47 (s, 1H, OH), 8.20 (s, 1H, H-4), 7.36−7.06 (m, 5H, H-5, H-6, H-7, H-2′, H-6′), 6.82 (d, J = 8.2 Hz, 1H, H-5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.2, 158.4, 152.3, 145.8, 145.0, 144.2, 142.8, 128.0, 127.4, 125.3, 124.2, 119.7, 119.5, 116.4, 115.7. MS (EI) m/z (%): 299 ([M + 1]$^+$, 8), 298 (M^+, 41), 155 (16), 137 (100), 91 (31). Anal. Calcd for $C_{16}H_{10}O_6$: C, 64.43; H, 3.38. Found: 64.43; H, 3.39.

*6-Bromo-8-hydroxy-3-(3′,4′-dihydroxybenzoyl)coumarin (**2i**).* Pale-yellow solid. Yield 69%. mp 300−302 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.89 (s, 1H, OH), 10.14 (s, 1H, OH), 9.48 (s, 1H, OH), 8.14 (s, 1H, H-4), 7.45 (d, J = 2.2 Hz, 1H, H-5), 7.38−7.12 (m, 3H, H-7, H-2′, H-5′), 6.81 (dd, J = 8.1, 3.0 Hz, 1H, H-6′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 189.3, 157.4, 152.0, 145.8, 145.5, 142.3, 141.9, 128.0, 127.4, 124.0, 121.1, 121.0, 120.5, 115.9, 115.8, 115.2. MS (EI) m/z (%): 376/378 (M^+, 100), 269 (30), 267 (24), 137 (80), 109 (74). Anal. Calcd for $C_{16}H_9BrO_6$: C, 50.95; H, 2.41. Found: C, 50.92; H, 2. 43.

Cyclic Voltammetry. Cyclic voltammetry was carried out using a potentiostat/galvanostat VersaSTAT 3 that was provided with the V3-Studio electrochemistry software package in 50 mM sodium phosphate buffer (pH 7.4) at room temperature using a three-electrode cell. A glassy-carbon electrode was used as the working electrode, a platinum wire, the auxiliary electrode, and Ag, AgCl/KCl (ca. 3.5 M), the reference electrode. All coumarin−chalcone hybrids were studied to 1 mM in 20% methanol as final concentration.

Characterization of Coumarin-Chalcone Radicals by ESR Spectroscopy. ESR measurements were carried out on an ESR spectrometer equipped with a high-sensitivity resonator at room temperature. Unless otherwise indicated, the instrument settings used for general spectral acquisition were microwave power, 10 mW, modulation amplitude, 1 G, receiver gain, 1.0×10^4 or 1.0×10^5, scan time, 21.5 s, time constant, 40.0 s, and sweep width, 120 G. The oxidation process was carried out by using 30% H_2O_2 and NaOH (25 mM) in the presence of chalcone−coumarin hybrids. Scans were integrated using the Bruker WINEPR v.2.11b software. The sample cells used were 50 μL glass capillary tubes. An automatic fitting program carried out the spectrum simulation. The hyperfine splitting constants were estimated to be accurate to within 0.05 G.

Radical-Generating System to Test Coumarin-Chalcone Hybrids. To the corresponding coumarin−chalcone hybrid compound (1.2 mmol) in dimethyl sulfoxide (20 μL), a hydrogen peroxide solution (50 μL, 30%) and a sodium hydroxide solution (50 μL, 25 mM) were added. The total volume of the resulting solution (300 μL) was completed with DMSO to achieve a final 4 mM solution of the tested compounds.

ORAC-FL Assay. The ORAC-FL assays were carried out on a Synergy HT multidetection microplate reader (Bio-Tek Instruments, Inc.) using white polystyrene 96-well plates. Fluorescence was read from the top, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by Gen 5 software. The oxygen radical absorbance capacity was determined as previously described[46] with slight modifications. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4) at a 200 μL final volume. FL (70 nM, final concentration) and coumarin−chalcone solutions in methanol with a range of concentration between 0.3 to 2 μM and 0.5 to 7 μM for the monohydroxy and dihydroxy derivatives, respectively, were placed in each well of the 96-well plate. This range of concentration was selected empirically to obtain a good separation between the fluorescence curves. This separation was crucial for more accurate data treatment.[59,60] The mixture was preincubated for 15 min at 37 °C before rapidly adding the AAPH solution (18 mM, final concentration). The microplate was immediately placed in the reader and automatically shaken prior to each reading. The fluorescence was recorded every 1 min for 120 min. A blank with FL and AAPH using methanol instead of the antioxidant solution as well as five calibration solutions using Trolox (0.5 to 2.5 μM) as a standard molecule were also used in each assay. The inhibition capacity was expressed as ORAC values, and it was quantified by employing eq 1. All reaction mixtures were prepared in triplicate, and at least three independent assays were performed for each sample.

$$\text{relative ORAC value} = \frac{[\text{AUC} - \text{AUC}^0]}{[\text{AUC}_{\text{trolox}} - \text{AUC}^0]} \frac{[\text{trolox}]}{[\text{derivative}]} \quad (1)$$

where AUC^0 = area under the curve blank; AUC = area under the curve derivative; $\text{AUC}_{\text{trolox}}$ = area under the curve Trolox (standard); [trolox] = Trolox concentration, molar; and [Derivative] = derivative concentration, molar

The AUC was calculated by integrating the decay of the fluorescence where F_0 is the initial fluorescence read at 0 min and F is the fluorescence read at a particular time. The AUC_{NET} corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. Data processing was performed using Origin Pro 8 SR2 (Origin Lab Corporation).

Antioxidant Reactivity by ESR Assay. The reactivity of all coumarin−chalcone hybrids against hydroxyl radicals was assessed using the noncatalytic Fenton method that was previously described.[56] In a typical experiment, 150 μL of dimethyl sulfoxide was mixed with 50 μL of NaOH (3 mM) followed by the addition of 50 μL of DMPO (30 mM) spin trap and 50 μL of hydrogen peroxide (30%). All derivatives were studied at a 4 mM final concentration and a 300 μL final volume. The mixture was positioned in the ESR cell, and the spectra were recorded after 5 min of the reaction.

Biological Studies. All chemicals and solvents used were of the highest analytical grade. For cell culture, all reagents or materials were purchased and used without further purification. Bovine aortic endothelial cells were purchased from Cell Systems (Kirkland, WA) and nonessential amino acids and Fetal Bovine Serum (FBS) were purchased from Gibco and Invitrogen, respectively.

Cell Culture. Cells were cultured in 75 cm^2 tissue-culture flasks using Dulbecco's modified Eagle's medium with 1 g/L of D-glucose and 4 mM L-glutamine and supplemented with 10% fetal bovine serum, 2.5 mg/L of endothelial cell growth supplement, and 1% nonessential amino acids in the absence of antibiotics at 37 °C in a humidified atmosphere of 5% CO_2 and 20% O_2. The medium was changed every 2 to 3 days, and the cells were subcultured once they reached 80−85% confluence.[51]

Cytotoxicity and Cytoprotection. Cytotoxicity assays in BAEC cells were performed for all coumarin−chalcone hybrids using the MTT reduction assay. BAEC were seeded in 24-well plates at a density ranging between 10^4−10^5 cells/mL per well. After the cells reached 60−70% confluence, they were incubated in the presence of 10 to 50 μM of the corresponding coumarin−chalcone derivatives for 24 h. After incubation, the medium was changed (2 mL) and 45 μL of a solution of MTT (5 mg/mL in DMEM supplemented with 0.5% FBS) was added to each well. The cells were incubated for another 2 h at 37 °C. The medium was then removed, and the wells were rinsed once with PBS. DMSO (0.6 mL) was added to each well at room temperature to solubilize the formazan crystals. The dissolved formazan was then transferred into 96-well culture plates, and the absorbance was measured at 570 nm using a UV−vis spectrophotometer.[57,58]

The percent of inhibition of cell viability was calculated using eq 2

$$\text{percent inhibition} = (1 - A_t/A_s)100 \quad (2)$$

where A_t and A_s are the absorbance of the sample solution and the solvent alone, respectively.

To assess the cytoprotective properties of the compounds against H_2O_2 and SIN-1, BAEC (10^4−10^5 cells/mL) were grown to 60−70% confluence in 24-well plates and each well was incubated with the selected derivative at 10 and 20 μM concentrations for 24 h. After incubation, the cells were washed with 1× PBS twice to remove the residual drug and exposed to 1 mM of H_2O_2 and 500 μM of SIN-1 for 2 h and 4 h, respectively. These time periods were chosen on the basis of the observed cell death (>50%) using the H_2O_2 and SIN-1 concetrations mentioned above. Finally, cell viability was evaluated by the MTT reduction assay and calculated using eq 2.

Statistical Analysis. The experimental results are expressed as the mean ± standard error of the mean (SEM) and are accompanied by the number (n) of observations. The differences between the groups were analyzed by student's t test. A p value lower than 0.05 was considered statistically significant.

Theoretical Evaluation of ADME Properties. The theoretical properties of log P, TPSA, n−OH, and n−OHNH were calculated by the methodology developed by Molinspiration using their software available on http://www.molinspiration.com/services/properties.html.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This project was partially supported by CONICYT-Chile project 24110059, Mecesup project UCH-0601, and personal funds of Spanish researchers. F.P.C. thanks CONICYT-Chile for her Ph.D. grant and Fulbright Fellowships for her doctoral stay. S.V.R. thanks the Ministerio de Educación y Ciencia for her FPU Ph.D. grant, AP2008-04263 (Spain). M.J.M. thanks

Fundação para a Ciência e Tecnologia for her Ph.D. grant, SFRH/BD/61262/2009 (Portugal).

■ ABBREVIATIONS

AAPH, 2,2′-azobis(2-methylpropionamidine)dihydrochloride; BAEC, bovine aortic endothelial cells; DCM, dichloromethane; DMEM, Dulbecco's modified Eagle's medium; DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; Epa, anodic peak potential; FL, fluorescein; MAO, monoamine oxidase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; ORAC, oxygen radical absorbance capacity; PBN, α-phenyl-*N*-*tert*-butylnitrone; RNS, reactive nitrogen species; SIN-1, 3-morpholinosydnonimine; TBAP, tetrabutylammonium perchlorate; TLC, thin layer chromatography; TPSA, topological polar surface area; trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

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CONCISE ARTICLE

Synthesis of coumarin–chalcone hybrids and evaluation of their antioxidant and trypanocidal properties†

Cite this: *Med. Chem. Commun.*, 2013, **4**, 993

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Received 18th January 2013
Accepted 22nd April 2013

DOI: 10.1039/c3md00025g

www.rsc.org/medchemcomm

Based on the observed biological activities of coumarins and chalcones, we have synthesized coumarin–chalcone hybrids with the aim of evaluating their antioxidant properties and trypanocidal activity against *Trypanosoma cruzi*, the parasite responsible for Chagas disease. All derivatives have been proved to be good antioxidants in spite of their moderate trypanocidal activity in the epimastigote stage (clone Dm28c). Based on these results, we can conclude that compounds **4** and **5** are potential candidates for *in vitro* studies of their antioxidant activity. These preliminary findings encourage us to the future structural optimization of these kinds of compounds.

1 Introduction

Chalcones (1,3-diaryl-2-propen-1-ones) are one of the major classes of naturally occurring compounds with widespread distribution in fruits, vegetables, spices, tea and soy based foodstuff and they have been subject of great interest for their remarkable pharmacological activities.[1,2] Chemically, they are open chain precursors of flavonoids and isoflavonoids, in which the two aromatic rings are linked by a three-carbon α,β-unsaturated carbonyl system. Chalcones have been reported to possess many useful properties, including antibacterial,[3,4] antimalarial,[5–7] antifungal,[8,9] antiviral[10,11] and anti-inflammatory[12–14] properties.

On the other hand, coumarins (2*H*-1-benzopyran-2-ones) are another family of naturally occurring compounds widely distributed in plants. These kinds of molecules of both natural and synthetic origin, have also attracted considerable interest due to their pharmacological activities,[15] for example, their enzyme inhibitory[16,17] antitumor[18,19] and antiviral[20,21] activities as well as their anti-inflammatory[22,23] and antioxidant[22,24–26] properties.

Chagas disease is caused due to the *Trypanosoma cruzi* (*T. cruzi*) parasite and it represents a major health problem in several countries of Latin America and in the southern United States. The only two available drugs (nifurtimox and benznidazole) are limited and suffer from several drawbacks such as poor efficacy, toxicity, resistance, and multiple side effects.[27,28] During the course of *T. cruzi* infection and disease development, ROS can be produced as a consequence of tissue destruction caused by toxic secretions of the parasite, immune-mediated cytotoxic reactions, and secondary damage in the myocardium. Therefore, interventions with antioxidant compounds, that reduce the generation or the effects of ROS, may exert beneficial effects in preventing or arresting oxidative damage.[29,30] Therefore, there is an urgent need for the discovery of new, more effective, and safer drugs for human use.

Antioxidants are a class of substances presenting a wide range of different chemical structures. They are able to decrease or prevent the oxidation of other sensitive molecules through different mechanisms like chelation of active metal ions, free radicals scavenging or inhibition of pro-oxidant enzymes.[31] Free radicals and reactive oxygen species (ROS) are atoms, molecules or ions possessing unpaired electrons, which are highly reactive with a wide range of other different molecules. They are constantly generated and eliminated in the biological system through metabolic processes and play important roles in a variety of normal biochemical functions, cell signaling apoptosis, gene expression, ion transport and abnormal pathological processes.[32,33] The overproduction of ROS can have damaging effects on many molecules. For example, ROS producing oxidative stress may contribute to Chagas disease progression.[34]

It is also known that the antioxidant properties of chalcones are quite dependent on the two aryl structures,[25,35] that is, the substituents on the two aryl rings of the chalcone moiety and their substitution patterns. Especially, the hydroxyl substituent

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3md00025g

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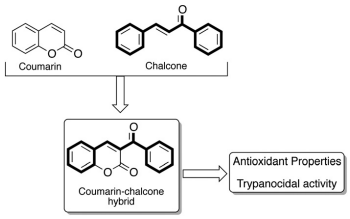


Fig. 1 Structural approach employed for the design of new coumarin–chalcone hybrid compounds.

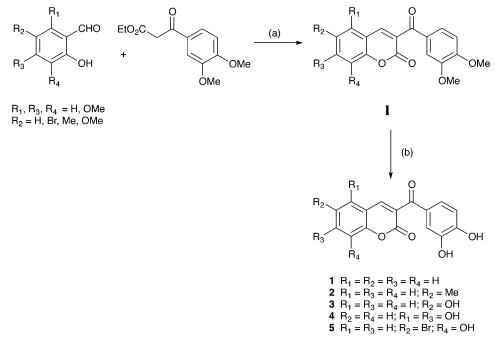
is one of the key groups that enhance the antioxidant activity of chalcone mainly due to its easy conversion to phenoxy radicals through the hydrogen atom transfer (HAT) mechanism. This phenoxy radical formation may be essential for the antioxidant properties, which are assessed primarily as radical scavenging potential of phenolic chalcones. In fact, the hydroxyl substituent is widespread among chalcones from natural sources.

Due to the potential antioxidant property and trypanocidal activity of the chalcone and coumarin moieties,[36,37] in the present work a series of coumarin–chalcone hybrids (Fig. 1) were synthesized and their antioxidant capacity and trypanocidal activity were evaluated. The chalcone moiety in the prepared molecules is included in the coumarin scaffold so that the geometry of the chalcone remains as a *trans* isomer due to the double bond position of the pyrone ring.

2 Chemistry

In order to achieve the final products, the synthesis of the hydroxy-3-benzoylcoumarins was carried out in two steps, shown in Scheme 1 and briefly described as follows: (a) synthesis of methoxy-3-benzoylcoumarins and (b) hydrolysis of the previous compounds to afford the final hydroxyl-3-benzoylcoumarins (**1–5**).

The methoxy-3-benzoylcoumarins, with a general structure **I** were efficiently synthesized with 78–94% yield by a Knoevenagel reaction treating the appropriate salicylaldehyde and ethyl 3,4-dimethoxybenzoylacetate with piperidine in ethanol at reflux for 2–6 h. The resulting methoxy derivatives were treated with boron tribromide in DCM at 80 °C in a Schlenk tube for 48 h to give the corresponding hydroxy derivatives **1–5** with 69–94% yield.



Scheme 1 Preparation of coumarin–chalcone hybrids. *Reagents and conditions*: (a) piperidine, EtOH, reflux, 2–6 h and (b) BBr_3, DCM, 80 °C, 48 h.

3 Electrochemical study

In order to evaluate the antioxidant behavior of the five compounds of this family, cyclic voltammetry, the Oxygen Radical Absorbance Capacity (ORAC FL-PGR) and the Electron Spin Resonance (ESR) studies were carried out.

3.1 Cyclic voltammetry

The electrochemical properties of hydroxyl coumarin–chalcone derivatives were studied by cyclic voltammetry. Fig. 2 shows a cyclic voltammogram at a glassy carbon electrode (GCE) immersed in DMSO/75 mmol L^{-1} phosphate (pH 7.4) buffer 30/70 media containing 1 mmol L^{-1} of compound **3** for several scan rates (v). Under these experimental conditions, two oxidation processes (peaks I and II) were observed. In general, it has been proposed that the charge transfer process at peak I corresponds to the oxidation of the catechol substituent, while the process at peak II comprises oxidation reaction involving the hydroxyl groups present in the coumarinic ring.

Results of the cyclic voltammetry, reported in Table 1 show that coumarin–chalcone hybrids present low oxidation potentials.

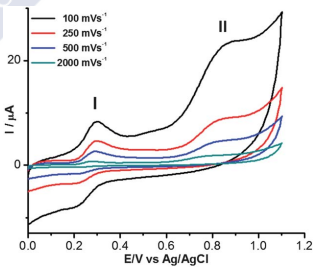


Fig. 2 Cyclic voltammogram for 1 mmol L^{-1} compound **3** in DMSO/75 mmol L^{-1} phosphate (pH 7.4) buffer 30/70 media at a GCE for v = 100, 250, 500, and 2000 mV s^{-1}.

Table 1 Oxidation potential peaks obtained by cyclic voltammetry

Compounds	E_{pa}[a] (mV)
1	322
2	449
3	312
4	337
5	247

[a] First oxidation peak potential at a scan rate of 2000 mV s^{-1}.

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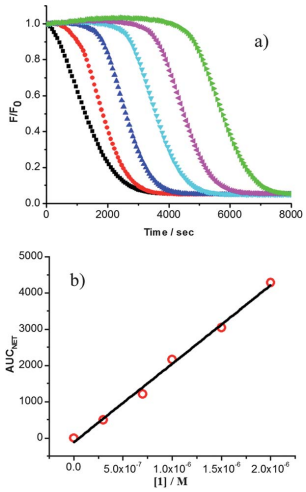

Fig. 3 (a) Kinetic profile of fluorescein consumption, AAPH mediated, in the presence of compound **5**. F_0 is the fluorescence in the absence of the compound and F the fluorescence in the presence of compound **5** and (b) graph of AUC_{NET} (net area under the curve) *versus* concentration of compound **5**.

3.2 ORAC assays

The capacity of scavenging peroxyl radicals was studied employing the ORAC method. In this assay, Trolox and 2,2′-azo-bis(2-amidinopropane) dihydrochloride (AAPH) were used as a reference antioxidant and a peroxyl radical source, respectively.[38] This assay evaluates the capacity of antioxidants (or of their complex mixtures) to inhibit the bleaching of a target molecule (probe) induced by peroxyl radicals.

The oxidation occurs due to exposition of the target molecule, fluorescein (FL)[39] or pyrogallol red (PGR),[40] to the peroxyl radical, leading to decay of fluorescence emission or decrease of PGR absorbance with time.

The consumption of FL is commonly inhibited, even by antioxidants of low reactivity, throughout kinetic profiles characterized by the presence of induction times. Fig. 3a shows the results of AAPH mediated fluorescein oxidation in the absence and presence of increasing concentration of compound **5** in phosphate pH 7.4. Therefore, ORAC-FL values would be more influenced by the stoichiometry of the reaction (defined as the number of radicals that each additive molecule can remove) than by the reactivity of the additives. Fig. 3b shows that the area under the curve (AUC_{NET}) of the kinetic profiles for compound **5** was linearly related to the concentration of the additive.

This behavior was observed for all compounds studied. However, the consumption of PGR by reactive antioxidants is inhibited without generating induction times.[41,42] This characteristic profile is observed for all the studied compounds, as well as for Trolox (Fig. 4).

The absence of induction times in the protective kinetic profiles of PGR would imply that the ORAC-PGR index is more related to the reactivity of the antioxidants than to stoichiometric factors. Taking into account the characteristics of the ORAC-FL and ORAC-PGR assays, it has been recently proposed that both methods could be considered as rendering complementary indexes.[42] Results of this study are expressed as ORAC-FL and ORAC-PGR values and are tabulated in Table 2.

All experiments were carried out in triplicate, and the data represent the mean values (±S.D.).

Table 2 ORAC-FL and ORAC-PGR values and the scavenging hydroxyl radical (%)

Compound	ORAC-FL	ORAC-PGR	% Scavenging
1	4.37 ± 0.22	[a]	84.6 ± 5.3
2	4.95 ± 0.14	[a]	82.1 ± 7.1
3	6.02 ± 0.12	[a]	84.8 ± 7.3
4	8.32 ± 0.27	0.52 ± 0.05	84.2 ± 6.4
5	8.51 ± 0.32	1.17 ± 0.10	90.9 ± 8.2
Quercetin	7.28 ± 0.22[c]	—	—
Catechin	6.76 ± 0.22[c]	—	—
DHMcoumarin[b]	3.3[d]		
Helichrysetin	4.4[e]		

[a] Inactive compound. [b] 6,7-Dihydroxy-4-methylcoumarin. [c] Data collected from ref. 39. [d] Data collected from ref. 49. [e] Data collected from ref. 50.

3.3 ESR assays

In order to study the antioxidant reactivity of all the hydroxylated coumarin–chalcone hybrids, we have adopted a

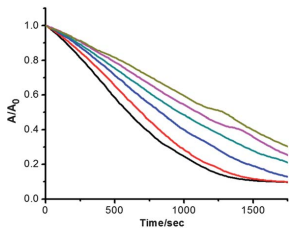

Fig. 4 Time-course of the consumption of PGR, AAPH mediated, in the presence of increasing concentration of Trolox.

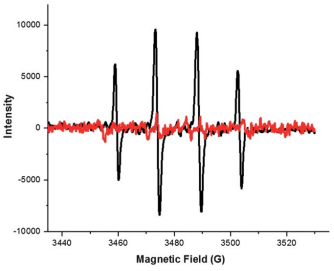

Fig. 5 ESR spectra for adduct DMPO–OH without an antioxidant molecule (black) and in the presence of compound **5** (red).

non-catalytic and competitive Fenton system.[23] Electron spin resonance (ESR) in combination with spin trapping techniques was employed to further verify that the tested compounds possess the ability to scavenge hydroxyl radicals, measuring thus, the percentage of scavenging of the compounds, summarized in Table 2. DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide) reacts with hydroxyl radicals to generate the spin resonance signal, and ESR can quantify this spin resonance signal. The ESR spectrum shows four hyperfine lines due to the DMPO–OH adduct formation. The coumarin–chalcone hybrids compete with DMPO for hydroxyl radicals. Thus, the ESR signal will diminish as presented in Fig. 5 for compound **5**.

4 Pharmacology

4.1 Trypanocidal activity

Studies on trypanocidal activitywere carried out in order to investigate a possible relationship between the antioxidant capacity of the compounds and their antiparasitic activity. Trypanocidal activity was evaluated against the *T. cruzi* epimastigote stage (clone Dm28c). It was measured through the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay,[43] widely used for *in vitro* measurement of the metabolic activity or viability of cell cultures. After incubation time, we determined the number of viable parasites by measuring absorbance at 570 nm in a multiwell reader (Asys Expert Plus©, Austria). Untreated parasites were used as controls (100% of viability). Results are reported as the percentage of non-viable epimastigotes regarding the control.

Results of two different concentrations of the synthesized compounds (10 and 100 μmol L^{-1}) are reported in Table 3.

5 Theoretical evaluation of ADME properties

To better correlate the drug-like properties of the coumarin–chalcone hybrid compounds the lipophilicity, expressed as the octanol/water partition coefficient and herein called log *P*, as well as other theoretical calculations such as the TPSA, the number of hydrogen acceptors and the number of hydrogen bond donors were calculated using the Molinspiration property program. The ADME properties of the studied compounds were calculated using the Molinspiration property program, and results are summarized in Table 4.[44]

Table 3 Trypanocidal activity results for the coumarin–chalcone hybrids and nifurtimox (Nfx)

Cmpd	% Trypanocidal activity at 10 μmol L^{-1}	% Trypanocidal activity at 100 μmol L^{-1}
1	23.8 ± 0.4	38.7 ± 2.1
2	27.9 ± 0.3	47.9 ± 1.0
3	18.9 ± 0.4	46.4 ± 0.8
4	[a]	[a]
5	9.5 ± 0.2	24.1 ± 0.7
Nfx	52.5 ± 2.2	100 ± 3.2

[a] Inactive at concentration tested.

Table 4 Theoretical structural properties of the coumarin–chalcone hybrids[a]

Compd	log *P*	TPSA (Å^2)	*n*-OH acceptors	*n*-OHNH donors	Volume (Å^3)
1	2.43	87.74	5	2	235.01
2	2.85	87.74	5	2	251.58
3	1.93	107.97	6	3	243.03
4	1.63	128.20	7	4	251.05
5	2.92	107.97	6	3	260.92

[a] TPSA, topological polar surface area; *n*-OH, number of hydrogen acceptors and *n*-OHNH, number of hydrogen bond donors. The data were determined with Molinspiration calculation software.

6 Results and discussion

Taking into account the importance of the hydroxyl substituents in the antioxidant properties,[26,45] we have focused the study on hydroxylated derivatives of the coumarin–chalcone hybrid compounds, which contain a catechol fragment in the benzoyl ring at the 3 position of the coumarin.

According to the data obtained in the cyclic voltammetry, all derivatives exhibited two anodic peaks without the corresponding cathodic peak, indicating an irreversible oxidation process as a result of the oxidation of one hydroxyl group in any of the aromatic rings, forming the semiquinone radical. We have thus firstly investigated the influence of the scan rate (v) in order to access in more detail the characteristics of electrochemical and chemical reactions that take place at the electrode/solution interface. The current ratio I_{pc}/I_{pa} progressively increases as a function of v, corroborating an EC-type mechanism[46] associated with peak I. It is known that the antioxidant capacity is conceivably related to the electrochemical behavior, being indicative that a low oxidation potential corresponds to a high antioxidant power.[47] In our case, this fact could be explained as mainly due to the strong electron donating effect of the catechol group, present in all the synthesized derivatives, which can form stable radicals in which the remaining *o*-OH group stabilizes the radical as a result of the intramolecular hydrogen bonding.[48]

Considering the ORAC-FL assays, we observed that the highest ORAC-FL values were found for compounds **4** and **5** (8.32 and 8.51 respectively). The results obtained are better than for quercetin (7.28) and catechin (6.76), flavonoids currently used as reference compounds,[39] as well as other simple coumarins, such as the 6,7-dihydroxy-4-methylcoumarin (DHMcoumarin) studied by our group[49] or chalcones collected from bibliographic sources such as helichrystein.[50] It is also worth mentioning the activity of compound **3** (6.02), comparable to catechin.

The different ORAC-FL values can be related to the substituents existing in the coumarin moiety and their pattern of substitution. In addition, the presence of a benzoyl substituent improves the electron delocalization with respect to the coumarin skeleton, influencing ORAC values.

Compound **1** is the structurally simplest compound, presenting a catechol group in the benzoyl ring, with no substituents on the coumarin skeleton. This feature could explain why

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compound **1** presents the lowest ORAC value (4.37) of all the studied compounds, but still being 4 times more active than the employed reference compound Trolox. If we introduce a methyl group at position 6 (compound **2**), we observe a slight increase in the ORAC-FL index (4.95) compared to compound **1**. When analyzed the obtained ORAC-FL values of compounds **1–3** based on the chemical structure and pattern of substitution, we observed that, among other features, the higher the electron donating effect of the substituent, the more active the compound. Compound **3** containing a hydroxyl at position 6, increases its ORAC value to 6.02 compared to the simplest synthesized compound **1**.

Compounds with the highest activities (**3–5**), with ORAC values 6.02, 8.51 and 8.32 respectively, all present, in addition to the catechol group in the benzoyl ring, at least one hydroxyl group that increases the electronic density, favoring the hydrogen atom transfer (HAT) mechanism. Compound **4**, with two hydroxyl groups in the coumarin moiety, showed a considerable increase in the ORAC-FL value (8.32) compared to compound **3**, in which only one hydroxyl substituent is present in the coumarin ring. In any case, the highest ORAC-FL value was found for compound **5**, in which one hydroxyl group is present at position 8 and a bromide atom is present at position 6 of the coumarin. Therefore, it has been found that the presence, the number and the position of substituents with different electronic effects on the coumarin skeleton are important structural factors that contribute to increase the antioxidant capacity of these coumarin–chalcone hybrids.

Regarding the ESR assays, whose results are reported in Table 2 we observed accuracy with the obtained ORAC values and the radical scavenging activity, as high as 80% for all tested compounds.

On the other hand, results reported in Table 3 have shown that all compounds present moderate trypanocidal activity with exception of compound **4** that resulted to be inactive at the concentration tested. However, these compounds have less activity than the positive control nifurtimox, the drug currently used against *T. cruzi* in the treatment of Chagas disease.

From the data obtained for the theoretical evaluation of the ADME properties, one can notice that all the hybrid compounds **1–5** do not break any point of the Lipinski's rule of five, making them promising leads for drug candidates.[51] Topological polar surface area (TPSA) and log *P* values are compatible with those described as a predictive indicator of the drug capacity of membrane penetration.[52]

It is noteworthy that compounds with the higher trypanocidal activities were those with lower ORAC values. These data can be taken into account for future studies on the mechanism of action of this kind of drug against *T. cruzi*.

7 Experimental

7.1 General methods

Starting materials and reagents were obtained from commercial suppliers and were used without purification. Melting points (mp) are uncorrected and were determined with a Reichert Kofler thermopan or in capillary tubes in a Buchi 510 apparatus. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.4 MHz) spectra were recorded with a Bruker AMX spectrometer using DMSO-d_6 or $CDCl_3$ as solvent. Chemical shifts (δ) are expressed in parts per million (ppm) using TMS as an internal standard. Coupling constants *J* are expressed in hertz (Hz). Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets) and m (multiplet). Mass spectrometry was carried out with a Kratos MS-50 or a Varian MAT-711 spectrometer. Elemental analyses were performed by a Perkin-Elmer 240B microanalyzer and were within $\pm$0.4% of calculated values in all cases. The analytical results were $\geq$95% purity for all compounds. Flash Chromatography (FC) was performed on silica gel (Merck 60, 230–400 mesh) and analytical TLC was performed on precoated silica gel plates (Merck 60 F254). Organic solutions were dried over anhydrous sodium sulfate. Concentration and evaporation of the solvent after reaction or extraction was carried out on a rotary evaporator (Büchi Rotavapor) operating at reduced pressure.

7.2 Synthesis

7.2.1 General procedure for the synthesis of hydroxy-3-benzoylcoumarins (1–5). To a solution of the appropriate β-ketoester (1 mmol) and the corresponding salicylaldehyde (1 mmol) in ethanol (5 mL) was added piperidine in a catalytic amount. The reaction mixture was refluxed for 2–6 h and after completion (followed by TLC), the reaction mixture was cooled and the precipitate was filtered and washed with cold ethanol and ether to afford the corresponding methoxy-3-benzoylcoumarin compounds with a general structure **I**. The appropriate methoxy derivative compound (1 mmol) was dissolved in DCM (1 mL) and BBr_3 in DCM (20 mmol, 1 M) was added in a Schlenk tube. The tube was sealed, and the reaction mixture was heated at 80 °C for 48 h. The resulting crude product was treated with MeOH and rotated to dryness. The obtained precipitate was recrystallized in MeOH or purified by flash chromatography using hexane–ethyl acetate mixtures as an eluent, to afford the desired hydroxy derivatives (Scheme 1).

7.3 Cyclic voltammetry

Cyclic voltammetry was carried out using a potentiostat/galvanostat VersaSTAT 3 provided with a V3-Studio electrochemistry software package, in 50 mM sodium phosphate buffer (pH 7.4), at room temperature, using a three electrode cell, a glassy carbon electrode was used as a working electrode, a platinum wire as an auxiliary electrode and Ag, AgCl/KCl (*ca.* 3.5 M) as a reference electrode. All coumarin–chalcone hybrids were studied to 1 mM in 20% methanol as final concentration.

7.4 Determination of ORAC

The ORAC analyses were carried out on a Synergy HT multi detection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, USA), using white polystyrene 96-well plates, purchased from Nunc (Denmark). The consumption of the probe molecules, FL or PGR, associated with its incubation in the presence of AAPH, was estimated from fluorescence (*F*) and absorbance (*A*) measurements, respectively. PGR consumption was evaluated from its absorption intensity (*A*) decrease at

540 nm, while FL consumption was evaluated from its decrease in the fluorescence intensity (F, excitation: 485/20 nm; emission: 528/20 nm). The plate reader was controlled by Gen 5 software. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and a final volume of 200 μL. FL (40 nM, final concentration) or PGR (70 μM, final concentration) and coumarin–chalcone hybrid solutions in methanol with a range of concentrations between 0.3 μM and 2 μM were placed in each well of 96-well plate. The mixture was preincubated for 15 min at 37 °C, before rapidly adding the AAPH solution (18 mM and 0.1 M for FL and PGR final concentration, respectively). The microplate was immediately placed in the reader and automatically shaken prior to each reading. The fluorescence and absorbance were recorded every 1 min for 120 min. A blank with FL or PGR and AAPH using methanol instead of the antioxidant solution and five calibration solutions using Trolox (0.5 μM to 2.5 μM for Fl and 50 μM to 250 μM for PGR) as antioxidant were also used in each assay. The inhibition capacity was expressed as ORAC-FL and ORAC-PGR values, and is quantified by integration of the area under the curve (AUC-NET). All reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample. The area under the fluorescence decay curve (AUC) was calculated integrating the decay of the fluorescence where F_0 is the initial fluorescence read at 0 min and F is the fluorescence read at time. The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. The ORAC-PGR values were calculated like we indicated previously. Data processing was performed using Origin Pro 8 SR2 (Origin Lab Corporation, USA).

7.5 ESR spectroscopy

To a solution of 100 μL of NaOH (50 μL, 4 mM), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) (50 μL, 30 mM) and hydrogen peroxide (50 μL, 30%) was added the antioxidant compound (50 μL, 3 mM in DMF). The resulting mixture was placed in an EPR cell, at room temperature (25 °C) and the spectrum was recorded after five minutes of reaction in the X band (9.68 GHz) using a Bruker ECS 106 spectrometer with a rectangular cavity and 50 kHz field modulation.

7.6 Determination of trypanocidal activity

Trypanocidal activity was evaluated against the *T. cruzi* epimastigote stage (clone Dm28c) and it was measured through the MTT assay,[43] using 0.22 mg mL^{-1} phenazine metosulfate (as electron carrier). The *T. cruzi* epimastigotes Dm28c strain, from our own collection (*Programa de Farmacología Molecular y Clínica, Facultad de Medicina, Universidad de Chile*) were grown at 28 °C in the Diamond's monophasic medium as reported earlier[53] but replacing blood by 4 μM hemin and adding fetal calf serum (5%). The coumarin–chalcone hybrids (10 and 100 μM in DMSO) were added to 3×10^6 parasites per mL in the RPMI 1640 culture medium (5% bovine fetal serum) for 24 h at 28 °C. The DMSO final concentration was less than 0.1% v/v. The same procedure was followed with nifurtimox as the positive control. The tetrazolium salt was added at a final concentration of 0.5 mg mL^{-1}, incubated at 28 °C for 4 h and then solubilized with sodium dodecyl sulfate (10%) in HCl (0.1 mmol L^{-1}) and incubated overnight. After incubation time, the number of viable parasites was determined by measuring the absorbance at 570 nm in a multiwell reader (Asys Expert Plus©, Austria). Untreated parasites were used as controls (100% of viability). Results are reported as the percentage of non-viable epimastigotes regarding the control. Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software, Inc. San Diego, CA). The data are expressed as mean ± S.D. The experimental data were analyzed by one-way analysis of variance (ANOVA), and differences between groups were assessed using the Tukey's post-test. The level of significance was set at $p < 0.05$, and all experiments were replicated three times.

7.7 Theoretical evaluation of ADME properties

The ADME properties of the studied compounds were calculated using the Molinspiration property program.[44] log P is calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors. Topological Polar Surface Area (TPSA) is calculated based on the methodology published by Ertl *et al.* as a sum of fragment contributions.[52] Oxygen and nitrogen centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood–brain barrier penetration. The method for calculation of molecular volume developed by Molinspiration is based on group contributions. These have been obtained by fitting the sum of fragment contributions to "real" 3D volume for a training set of about twelve thousand, mostly drug-like molecules. 3D molecular geometries for a training set were fully optimized by the semiempirical AM1 method.

8 Conclusions

In conclusion, we have confirmed the good antioxidant activity of the hydroxyl coumarin–chalcone hybrids **1–5**, two of them (compounds **4** and **5**) presenting better ORAC-FL values than the reference compounds quercetin and catechin. The use of a catechol group in the benzoyl ring and the presence of hydroxyl and bromine groups in the coumarin moiety make a big improvement in the antioxidant activity, compound **5** being the most active of the series under study. The results of the antioxidant assay using ESR showed significant reactivity against the hydroxyl radical for all tested compounds. A very interesting finding is that compounds **4** and **5** resulted to be very reactive and present good antioxidant capacity and scavenging percentage against hydroxyl and peroxyl radicals, as well as low oxidation potentials. Based on these results, we can conclude that compounds **4** and **5** are potential candidates for deeper studies of antioxidant activity. On the other hand, it is worth mentioning that molecules with higher trypanocidal activities showed a lower ORAC-FL index, which can be a key in the understanding of the mechanism of action of the compounds on the *T. cruzi* parasite.

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Acknowledgements

This work was partially supported by the Ministerio de Sanidad y Consumo (PS09/00501), Xunta de Galicia (PGIDIT09CSA030203PR) (Spain), FONDECYT (projects 1110029 and 1090078) and Anillo ACT112 (Chile). SVR thanks Ministerio de Educación y Ciencia for her PhD grant FPU (AP2008-04263). RFG gratefully acknowledges CONICYT-Chile for his PhD grant no. 24121574. MJM thanks Fundação para a Ciência e Tecnologia for her PhD grant (SFRH/BD/61262/2009).

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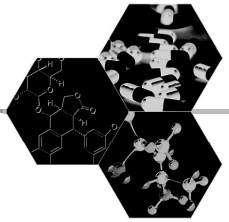

Synthesis and evaluation of antioxidant and trypanocidal properties of a selected series of coumarin derivatives

This article describes the preparation and characterization of a selected series of coumarin derivatives with the aim of evaluating their antioxidant properties and their activity against *Trypanosoma cruzi*, the parasite responsible for Chagas disease. All the derivatives demonstrated moderate trypanocidal activity in the epimastigote and trypomastigote stages (clone Dm28c), with Compound 3 presenting the highest trypanocidal activity of the entire series, displaying higher activity than nifurtimox, which was used as a reference compound. In addition to the trypanocidal activity, this compound proved to have a very interesting antioxidant profile, as well as no cytotoxicity. These preliminary findings encouraged the authors to study the future structural optimization of this scaffold.

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Coumarins (2H-1-benzopyran-2-one) are a family of natural compounds profusely distributed in the plant kingdom, which can be found in different plants, such as sweet woodruff, tonka bean and vanilla grass [1–3]. Derivatives of this well-known skeleton have been proven to display a wide range of important biological activities [1–3]. Differently substituted coumarins have already proved to be pharmacologically active as anticancer, anti-inflammatory, antimicrobial, vasorelaxant, cardioprotective and antioxidant agents [1–16]. Due to the role that coumarins can play in different areas, the synthesis, characterization and study of their derivatives are current topics of interest [17–24].

Different hydroxyl-substituted heterocyclic compounds, in which group hydroxycoumarins are included, are bioactive substances present in natural sources. They have been described as natural antioxidants and their presence contributes to prevent or minimize several oxidative processes [25]. They can protect cells from the oxidative damage caused by reactive oxygen species (ROS). In fact, the overproduction of free radicals has been related to cellular membrane and DNA damage, and indirectly with aging and oxidative stress-related diseases such as cancer, cardiovascular and neurodegenerative pathologies [26–28]. Antioxidants are capable of decreasing or preventing oxidation processes through different mechanisms, such as scavenging free radicals, inhibition of pro-oxidant enzymes or chelation of transition metal ions [29]. Therefore, the research and characterization of new bioactive hydroxy-substituted compounds has intensified over the last few years, either for the development of nutraceutics or drugs.

Production of ROS and the consecutive oxidative stress are factors that contribute to **Chagas disease (American trypanosomiasis)** progression [30]. This disease has its origins in a protozoan parasite known as ***Trypanosoma cruzi*** [31]. Chagas disease is a significant health problem in Central and South America, and it is one of the most relevant emerging parasitic diseases in developed countries [32,33]. The parasite presents a complex and multistage life cycle. In its development and infection it obligatory involves four different stages [34,35]. The infection is transmitted to humans predominantly by an insect vector, blood transfusions or between mother and fetus. During the life cycle of *T. cruzi* and its infection process, ROS can be produced. The overproduction of these species is a consequence of the destruction of different tissues, mainly caused by the parasite's toxic secretions, secondary damage in the myocardium and immune-mediated cytotoxicity. Therefore, intervention with antioxidant compounds that are capable of reducing the generation, or devastating effects, of ROS, will exert beneficial effects in protecting the cells from oxidative damage [36,37]. Natural products have played an important role in the therapy of this parasitic disease, but at the moment only nifurtimox and benznidazole drugs are the currently in use. These compounds are very unspecific, presenting high toxicity, low efficacy, high levels of resistance and multiple side effects [38,39]. In fact, this therapy is usually ineffective in chronic stages of the disease [32,40].

10.4155/FMC.13.147 © 2013 Future Science Ltd ISSN 1756-8919

Key Terms

Coumarins (2H-1-benzopyran-2-one): Family of compounds that present in their structure a benzene ring attached to a pyrone one.

Chagas disease (American trypanosomiasis): Pathology caused by a protozoan parasite called *Trypanosoma cruzi*. This disease causes 10–12 million infections and approximately 25,000 deaths/year.

***Trypanosoma cruzi*:** Flagellated protozoan parasite, the main insect vector of which is the *Triatomine infestans*.

Oxygen radical absorbance capacity: Antioxidants are a class of molecules responsible for protecting a fluorescent molecule (fluorescein) from the oxidative degeneration. The level of antioxidant-mediated protection can be quantified using the reference antioxidant Trolox® (a vitamin E analog) as standard.

Electron spin resonance: A technique useful for the study of compounds with unpaired electrons.

Therefore, the discovery of new and more effective drugs that are well tolerated and safer, is a topic of major interest.

Some coumarins proved to be important molecules in the regression of the described pathology. Previous studies described that they act by inhibiting GAPDH (enzyme commission number 1.2.1.12), an important protein in the trypanosomatids glycolytic pathway [41–43]. In addition, antioxidant properties were also attributed to the coumarin skeleton [44–46]. Based on this previous information related to the pharmacological activities of coumarins, in the present work a selected series of coumarin derivatives were synthesized and studied as antioxidant and trypanocidal agents **(Figure 1; 1–5)**.

Experimental

■ Chemistry

General methods

Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using tetramethylsilane as a internal standard (chemical shifts in δ-values, J in Hz). Elemental analyses were performed using a Perkin-Elmer 240B microanalyser and were within ±0.4% of calculated values in all cases. Silica gel (Merck 60, 230–00 mesh) was used for flash chromatography. Analytical thin layer chromatography was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm).

General methodology to prepare 3-aminocoumarins **2** & **3**

The commercially available 3-nitrocoumarin (2.5 mmol) was dissolved in ethanol and a catalytic amount of Pd/C was added to the mixture. The solution was stirred, at room temperature, under a H_2 atmosphere, for 5 h. After completion of the reaction, the mixture was filtered to eliminate the catalyst. The obtained crude was then purified by flash chromatography (hexane/ethyl acetate 9:1) to give the desired 3-aminocoumarin **2** or **3**.

General procedure for the preparation of 3-substituted coumarins **4** & **5**

The previously prepared 3-aminocoumarin **(2)** or 3-amino-4-hydroxycoumarin **(3)** (1 mmol) was dissolved in dichloromethane (9 ml), then pyridine (1.1 mmol) was added and the mixture was cooled to 0°C. 2-chloroacetyl chloride (1.1 mmol) was added drop-wise at this temperature, and the mixture was stirred overnight at room temperature. The batch was evaporated and purified by column chromatography (hexane–ethyl acetate 9:1) to give the desired 2-chloro-*N*-(coumarin-3-yl)acetamide **4** or **5**, respectively.

■ Oxidation potential determination by CV

CV was carried out using a Metrohm 797 VA instrument with a 797 VA Stand onvertor and a 797 VA Processor in DMSO/75 mM phosphate (pH 7.4) buffer 30/70 DMSO/ (~1.0×10^{-3} M) at room temperature with KCl (~0.1 M), using a three-electrode cell. A hanging drop mercury electrode was used as the working electrode, a platinum wire as the auxiliary electrode, and saturated calomel as the reference electrode.

■ Oxygen radical absorbance capacity-fluorescence

The **oxygen radical absorbance capacity** (ORAC) studies were carried out on a Synergy™ HT multidetection microplate reader, from Bio-Tek Instruments, Inc. (VT, USA), using white polystyrene 96-well plates, purchased from Nunc (Roskilde, Denmark). Fluorescence was read from the top, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by Gen 5 software. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and 200 µl final volume. Fluorescence (FL; 40 nM, final concentration) and coumarin solutions in methanol (range of concentration between 0.3 and 2 µM) were placed in each well of the 96-well plate. The mixture was preincubated for 15 min at 37°C, before rapidly adding the 2,2′-azo-bis(2-amidinopropane) dihydrochloride (AAPH) solution (18 mM, final concentration). The microplate was immediately placed in the reader and automatically shaken prior to each reading. The fluorescence was recorded every 1 min for 120 min. A blank with FL and AAPH using methanol instead of the antioxidant solution was used in each assay. Five calibration solutions using Trolox® (0.5–2.5 µM) as the antioxidant were also used in each assay. The inhibition capacity was expressed as ORAC-FL values and it was quantified by integration of the AUC_{NET}. All reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample. The area under the fluorescence decay curve (AUC) was calculated integrating the decay of the fluorescence,

where F_0 is the initial fluorescence read at 0 min and F is the fluorescence read at time. The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. Data processing was performed using Origin Pro 8 SR2 (Origin Lab Corporation, MA, USA).

■ Hydroxyl radical scavenging assay using electron spin resonance spectroscopy

Reactivity of all the coumarin derivatives against the hydroxyl radical was investigated using the non-catalytic Fenton type method. The mixture was put in on **electron spin resonance** (ESR) cell and the authors recorded the spectrum after five minutes of reaction. ESR spectra were recorded in the X band (9.7 GHz) using a Bruker ECS 106 spectrometer with a rectangular cavity and 50-kHz field modulation, equipped with a high-sensitivity resonator at room temperature. Spectrometer conditions were: microwave frequency 9.81 GHz; microwave power 20 mW; modulation amplitude 0.91 G; receiver gain 59 db; time constant 81.92 msec; and conversion time 40.96 msec. The scavenging activity of each derivative was estimated by comparing the DMPO-OH adduct signals in the antioxidant-radical reaction mixture and the control reaction at the same reaction time, and is expressed as scavenging percent of hydroxyl radical. To prepare the samples, 150 µl of *N,N*-dimethylformamide and 50 µl of NaOH (3 mM) were mixed, followed by the addition of 50 µl of DMPO spin trap (30 mM final concentration) and finally 50 µl of hydrogen peroxide 30%. The mixture was put in an ESR cell and the spectrum was recorded after 5 min of reaction. All the compounds were studied in concentrations of 3 mM, 50 µl.

■ Cytotoxicity assay

The effect of drug treatments on RAW 264.7 cells was evaluated through the tetrazolium dye (MTT; 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay as a viability test [47]. Briefly, 10 µl of 5 mg/ml MTT plus 0.22 mg/ml phenazine metosulfate (electron carrier), were added to each well containing RAW 264.7 cell culture in 100 µl RPMI 1640 without phenol red. Compounds under study, dissolved in DMSO, were added to the culture media at the concentrations demonstrated in the figures and tables. DMSO final concentration was less than 0.25% v/v. After incubation for 4 h at 37°C, the generated water-insoluble formazan dye was dissolved by the addition of 100 µl of 10% w/v sodium dodecyl sulphate (SDS) in 0.01 M HCl. The plates were further incubated overnight at 37°C, and optical density of the wells was determined using a microplate reader (Asys Expert Plus©, Asys Hitach, Austria) at 570 nm. Under these conditions, the optical density is directly proportional to the viable cell number in each well. All experiments were performed at least three times and data reported as means and their standard deviations from triplicate cultures. Results are reported as the percentage of non-viable RAW 264.7 cells regarding the control.

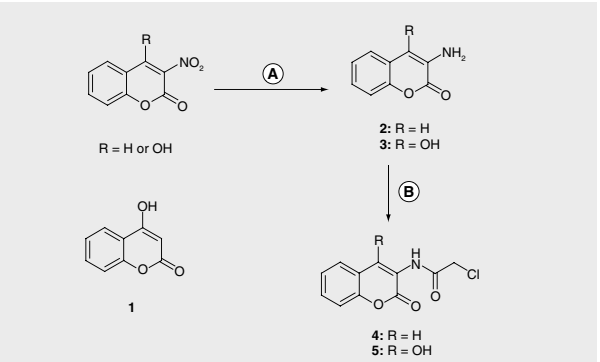

Figure 1. Synthesis of the described derivatives. Reagents and conditions: **(A)** H_2, Pd/C, ethanol, room temperature, 5 h; **(B)** $ClCOCH_2Cl$, pyridine, dichloromethane, 0°C to room temperature, overnight.

■ Determination of trypanocidal activity

Epimastigotes viability study

Trypanocidal activity was evaluated against the *T. cruzi* epimastigote stage (clone Dm28c). It was measured through the MTT assay [48] using 0.22 mg ml^{-1} phenazine metosulfate (as an electron carrier). *T. cruzi* epimastigotes (Dm28c strain), from the authors own collection (Programa de Farmacología Molecular y Clínica, Facultad de Medicina, Universidad de Chile, Santiago, Chile) were grown at 28°C in Diamond's monophasic medium, as reported earlier, but replacing blood with 4 µM hemin [49]. Fetal calf serum was added to a final concentration of 5%. In this colorimetric assay for testing the trypanocidal activity, the coumarin derivatives were dissolved in DMSO and were added to 3×10^6 parasites ml^{-1} at 10 µM final concentrations in RPMI 1640 culture medium for 24 h at 28°C.

Key Term

Cyclic voltammetry: A potentiodynamic electrochemical technique generally employed for the measurement and the study of the electrochemical properties of different compounds presented in solution.

DMSO final concentration was less than 0.1% v/v. Likewise, nifurtimox was added as a positive control. Tetrazolium salt was added at a final concentration of 0.5 mg ml^{-1}, incubated at 28°C for 4 h and then solubilized with 10% SDS/0.1 mM HCl and incubated overnight. After incubation, the number of viable parasites was determined by absorbance measures at 570 nm in a multiwell plate reader (Asys Expert Plus). Untreated parasites were used as controls (100% of viability). Results are reported as the percentage of non-viable epimastigotes regarding the control.

Trypomastigote viability study

Cell culture & in vitro infection with T. cruzi (Dm28c strain) trypomastigote

Vero cells were infected with Dm28c trypomastigotes at a 1:3 (cell:parasite) ratio. *T. cruzi* trypomastigotes were initially obtained from primary cultures of peritoneal macrophage from chagasic mice. Vero cells were cultured in 5% fetal bovine serum supplemented RPMI 1640 medium in humidified air with 5% CO_2 at 37°C. Vero cell cultures were then infected with trypomastigotes and incubated at 37°C in humidified air and 5% CO_2 for 5–7 days. After that time, the culture medium was collected, centrifuged at 500 × g for 5 min, and the trypomastigote-containing pellet was re-suspended in free-serum RPMI 1640 and penicillin–streptomycin at a final density of 1×10^7 parasites/ml. Trypomastigote viability assays were performed using the MTT reduction method as described previously. 1×10^7 parasites/ml were incubated in free-serum RPMI 1640 culture medium at 37°C over 24 h with or without the studied compounds. An aliquot of the parasite suspension was extracted and incubated in a 96-well flat-bottom plate and MTT was added at a final concentration of 0.5 mg/ml using 0.22 mg ml^{-1} phenazine metosulfate (as an electron carrier), incubated at 28°C during 4 h and then made soluble with 10% SDS–0.1 mM HCl and incubated overnight. Formazan formation was measured at 570 nm, with a reference wavelength at 690 nm, in a multiwell plate reader (Asys Expert Plus). Untreated parasites were used as controls (100% viability). Results are reported as the percentage of non-viable parasites regarding the control.

Amastigote viability study

Amastigotes were obtained with the same technique [48] used for trypomastigotes, but Vero cells were infected with Dm28c trypomastigotes at a 1:10 (cell:parasite) ratio, which induces cell rupture and release of amastigotes into the medium after 5 days. The culture medium was collected, centrifuged at 500 × g 5 min, and the amastigote-containing pellet was resuspended in free-serum RPMI 1640 and penicillin–streptomycin at a final density of 2×10^7 amastigotes/ml. Amastigote viability assays were performed using the MTT reduction method as described previously. 2×10^7 parasites/ml were incubated in free-serum RPMI 1640 culture medium at 37°C during 24 h with or without compound **4**. An aliquot of the parasite suspension was extracted and incubated in a 96 flat-bottom plate well and MTT was added at a final concentration of 0.5 mg/ml using 0.22 mg ml^{-1} phenazine metosulfate (as an electron carrier), incubated at 28°C during 4 h and then made soluble with 10% SDS–0.1 mM HCl and incubated overnight. Formazan formation was measured at 570 nm, with a reference wavelength at 690 nm, in a multiwell plate reader (Asys Expert Plus). Untreated parasites were used as controls (100% viability).

■ Statistical analysis

Statistical analyses were conducted using GraphPad Prism 5 software (GraphPad Software, Inc., [CA, USA]). IC_{50} were analyzed by nonlinear regression. The data are expressed as means ± SD. The experimental data were analyzed by one-way analysis of variance, and differences between groups were assessed using Tukey's post-test. The level of significance was set at $p < 0.05$. All experiments were done in triplicate and results correspond to means ± SD from at least three independent experiments.

Chemistry

Compound **1** is commercially available (Sigma-Aldrich, MO, USA). Coumarin derivatives **2–5** were efficiently synthesized following the protocol presented in **Figure 1** [50–53]. Starting from different substituted commercially available 3-nitrocoumarins, the 3-aminocoumarins **2 & 3** were afforded. They were prepared in ethanol, with Pd/C as catalyst, under a H_2 atmosphere. The final products were then purified by recrystallization in ethyl acetate to give the desired 3-aminocoumarins, in 95% yield. The acylation reaction of the 3-aminocoumarins **2** or **3** with the conveniently substituted acid chloride (2-chloroacetyl chloride), in pyridine, afforded the 2-chloro-*N*-(coumarin-3-yl)acetamides **4 & 5**, in 76 and 83% yields, respectively. The general reaction conditions are detailed in the 'Experimental' section.

Antioxidant capacity

The evaluation of the antioxidant properties of the studied compounds **(I–5)** was performed towards two different types of ROS: peroxyl and hydroxyl radicals. **Cyclic voltammetry** (CV), ORAC and ESR were the techniques employed to obtain the desired results.

■ CV

The electrochemical properties of coumarin derivatives were studied by CV. Results of the CV for compounds **I–5** are reported in **Table I,** and demonstrated that the coumarin derivatives present a variety of oxidation potentials depending on their substitution pattern.

In these experimental conditions, a single oxidation process (peak I) was observed. In general, it has been proposed that the charge transfer process at peak I corresponds to the oxidation of the hydroxyl or amine substituent.

The cyclic voltammogram of compound **5** is illustrated in **Figure 2**. It was recorded at a glassy carbon electrode immersed in DMSO/75 mM phosphate (pH 7.4) buffer 30/70 media containing 1 mM of compound **5**, for several scan rates (v).

■ ORAC assays

The peroxyl radical scavenging activity of the synthesized coumarins was evaluated by the ORAC method [54]. This assay uses a fluorescence-based technology (ORAC-FL) and allows a relative antioxidant index to be obtained by using Trolox, a hydrosoluble vitamin E derivative, as a reference. The compound AAPH was used as the peroxyl radical source. This assay evaluates the capacity of antioxidants to inhibit the bleaching of a target molecule (probe) induced by peroxyl radicals. The oxidation occurs due to exposition of the target molecule, FL, to the peroxyl radical, leading to an oxidation process reflected as a decay of fluorescence emission through time. In ORAC assays, the loss of fluorescence of FL generally corresponds to an induction time and is reliant on the antioxidant capacity of a compound. In fact, it refers to the time in which the FL is protected against the oxidative damage of peroxyl radicals, and this behavior is associated to a competitive reaction between the radical and the antioxidant [48,55]. ORAC data takes into account the induction time, the initial rate and the range of total antioxidant inhibition in one value [55].

Results expressed as ORAC-FL values are presented in **Table 2**. All the obtained ESR results for the percentage scavenging of the hydroxyl radical are also illustrated in **Table 2**.

Table 1. Oxidant potentials obtained by cyclic voltammetry for compounds 1–5.

Compounds	Epa† (mV)
1	1.33
2	0.80
3	0.50
4	1.10
5	0.64

†*First oxidation peak potential at scan rate of 2000 mVs^{-1}.*

The consumption of FL is commonly inhibited, even by antioxidants of low reactivity, throughout kinetic profiles. **Figure 3A** illustrates the results of AAPH-mediated FL oxidation in the absence and presence of an increasing concentration of compound **2** in phosphate pH 7.4, and demonstrates that the area under the curve (AUC_{NET}) of the kinetic profiles for compound **2** was linearly related to the concentration of the additive. **Figure 3B** illustrates the results of AAPH-mediated FL oxidation in the absence and presence of an increasing concentration of compound **3** in phosphate pH 7.4 and demonstrates that the AUC_{NET} of the kinetic profiles for compound **3** was linearly related to the concentration of the additive.

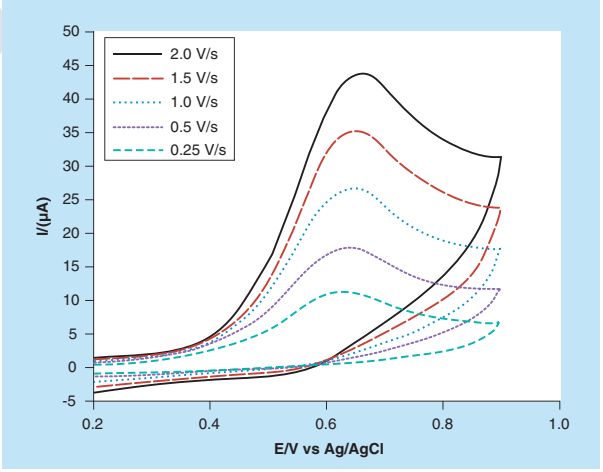

Figure 2. Cyclic voltammogram for 1 mM compound 5, in DMSO/75 mM phosphate (pH 7.4) buffer 30/70 media at a glassy carbon electrode for V = 250, 500, 1000, 1500, 2000 mVs^{-1}.
This figure can be viewed in full color at: www.future-science.com/doi/full/10.4155/FMC.13.147

Table 2. Oxygen radical absorbance capacity, fluorescein values and percentage scavenging of hydroxyl radical of the studied and reference compounds.

Compounds	ORAC-FL	Percentage scavenging†
1	4.200 ± 0.110	81.2 ± 7.3
2	0.820 ± 0.030	20.5 ± 2.0
3	4.360 ± 0.120	56.8 ± 4.0
4	0.065 ± 0.006	35.3 ± 3.2
5	1.810 ± 0.040	23.7 ± 2.8
Trolox	1	31.0 ± 2.5
6,7-dihydroxy-4-methylcoumarin	3.3‡	–

All the experiments were carried out in triplicate, and the data represent the mean values (± standard deviation). The ORAC-FL studies were carried out in 75 mM sodium phosphate buffer (pH 7.4) with coumarin solutions in methanol (range of concentration between 0.3 and 2 µM).
†The scavenging activity of hydroxyl radicals effect was calculated as follows: $[(A_0–A_x)/A_0] \times 100$, where A_x and A_0 are the double-integral electron spin resonance for the first line of samples in the presence (3 mM final concentration) and absence of test compounds, respectively.
ORAC-FL: Oxygen radical absorbance capacity-fluorescein.
‡Data collected from [62].

Kinetic profiles without induction time were observed for all the studied compounds. Increasing the concentration of the coumarin derivatives, the consumption of FL is inhibited. Results of this study are expressed as ORAC-FL values and are tabulated in **Table 2**.

■ ESR assays

To study the antioxidant capacity of all the synthesized coumarins towards hydroxyl radicals in depth, a non-catalytic and competitive-type Fenton system was performed, employing 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) as a spin trap [56]. In order to test the ability of the new compounds to scavenge hydroxyl radicals, ESR, in combination with spin-trapping techniques, was employed. The percentage of scavenging of the new compounds was measured and is summarized in **Table 2**. DMPO reacts with hydroxyl radicals to generate the spin resonance signal, which can be quantified by ESR. The ESR spectrum illustrates four hyperfine lines due to the DMPO-OH adduct formation. Coumarin derivatives compete with

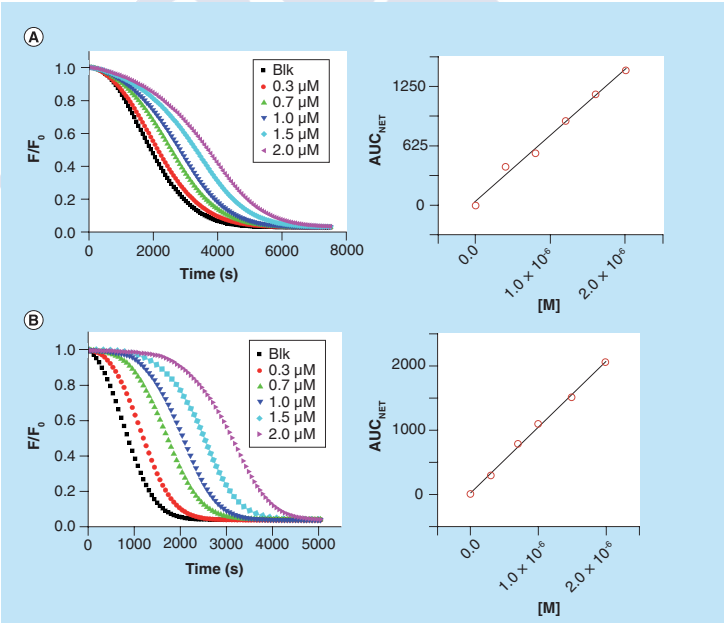

Figure 3. Kinetic profile of fluorescein consumption 2,2′-azo-*bis*(2-amidinopropane) dihydrochloride mediated in presence of compound 2 (A) and compound 3 (B). F_0 is the fluorescence in absence of the compound (Blk) and F the fluorescence in presence of the compound; graphic AUC_{NET} (net area under the curve) versus concentration of the compound.
This figure can be viewed in full color at: www.future-science.com/doi/full/10.4155/FMC.13.147

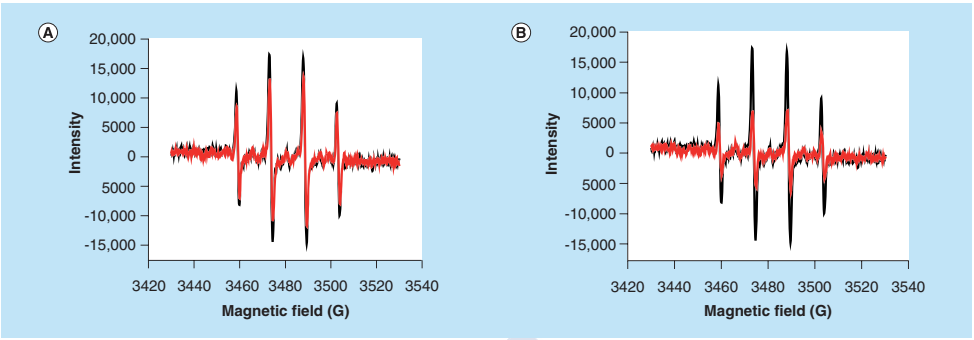

Figure 4. Electron spin resonance spectra obtained for the control (adduct 5,5-dimethyl-1-pyrroline-*N*-oxide without antioxidant molecule – black line) and for adduct 5,5-dimethyl-1-pyrroline-*N*-oxide. In the presence of **(A)** compounds **2** and **(B)** compound **3** (3 mM final concentration, light line).

DMPO for hydroxyl radicals, diminishing the ESR signal, as presented in **Figure 4**, for compound **2 & 3** (red line). ESR spectrum obtained in the control assay (DMPO + *N,N*-dimethylformamide + NaOH + H_2O_2) presents four hyperfine lines, due to the DMPO-OH adduct formation, as illustrated in **Figure 4** (black line). For each putative antioxidant coumarin compound, ESR spectra were also acquired to check their capacity of scavenging hydroxyl radicals.

The intensity of the spectra decreases when the coumarin derivatives were added into the system. This type of response was observed for all derivatives, reflecting different percentage of the hydroxyl radical scavenging activity **(Table 2)**.

Pharmacology

Cytotoxicity and trypanocidal studies were carried out in order to investigate a potential relationship between the antioxidant capacity demonstrated by the new compounds and their antiparasitic activity. Cytotoxicity and trypanocidal activities for all the synthesized compounds were evaluated using MTT assays [57], and the results are reported in **Table 3**.

■ Cytotoxicity in macrophages

To investigate *in vitro* cytotoxicity of the synthesized compounds, cultured macrophages (murine RAW 264.7 cells) were exposed to nifurtimox and to the coumarin suspensions at 10 and 100 µM for 24 h. Compound **4** was evaluated at different concentrations (0.5, 1, 5, 10 and 15 µM) to determinate the IC_{50} values **(Figure 5)**. Results are reported in **Table 3**.

■ Trypanocidal activity

Epimastigotes viability

Trypanocidal activity for all synthesized compounds was evaluated against the *T. cruzi* epimastigote (clone Dm28c) stage. It was measured through the MTT assay, a widely used methodology for *in vitro* measurement of cellular viability and/or metabolic activity. *T. cruzi* epimastigote strain was grown at 28°C in Diamond's monophasic medium, as reported previously [57], but with the blood replaced with 4 µM of hemin. Fetal calf serum was added to a final concentration of 5%. After incubation, the number of viable parasites was determined by absorbance measured at 570 nm, with a reference wavelength at 690 nm, in a multiwell plate reader. As a control, untreated parasites samples were used (100% viability). Results are reported in **Table 3**

Table 3. *In vitro* cytotoxicity in murin RAW 264.7 macrophages (IC_{50}) and trypanocidal activity results in epimastigote and trypomastigote stages (10 µM) for the coumarin derivatives and nifurtimox (reference compound).

Compounds	IC_{50} (µM)†	Trypanocidal activity in epimastigote stage (%)‡	Trypanocidal activity in trypomastigote stage (%)‡
1	>100	48.7 ± 2.8	11.5 ± 0.9
2	>100	42.1 ± 2.1	8.6 ± 0.6
3	>100	65.7 ± 3.5	22.2 ± 2.1
4	2.70 ± 0.17	53.7 ± 3.2	69.4 ± 4.4
5	>100	10.0 ± 0.6	5.8 ± 0.5
Nifurtimox	>100	52.5 ± 2.2	36.9 ± 3.4

All the experiments were carried out in triplicate, and the data represent the mean values (± standard deviation).
†IC_{50} is the concentration that produces 50% of inhibitory effect.
‡Results are reported as the percentage of non-viable parasites regarding the control at 10 µM.

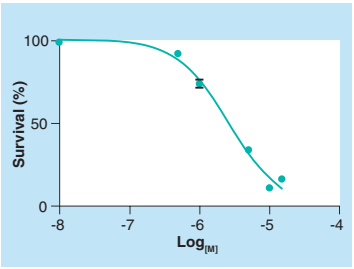

Figure 5. Dose–response curve of Compound 4 at different concentrations (0.5, 1, 5, 10 and 15 µM). Statistical analyses were conducted using GraphPad Prism 5 software (GraphPad Software, Inc. [CA, USA]). IC_{50} values were analyzed by nonlinear regression.

as the percentage of nonviable epimastigotes regarding the control.

Trypomastigote viability

Trypanocidal activity for all synthesized compounds was evaluated against the *T. cruzi* trypomastigote (clone Dm28c) stage. The trypomastigote stage, in mammals, is the disseminator of blood-borne infection. It was measured through the previously described MTT assay. Trypomastigotes were incubated in free-serum RPMI 1640 culture medium in humidified air with 5% CO_2 at 37 °C during 24 h with or without the studied compounds. After incubation time, the number of viable parasites was determined by absorbance measured at 570 nm, with a reference wavelength at 690 nm, in a multiwell plate reader. Results are reported as the percentage of non-viable trypomastigotes regarding the control in **Table 3**.

Discussion

The recent medicinal chemistry paradigms in drug design, namely the rational discovery of multitarget drugs, is a promising strategy to combat these types of multifactorial diseases, which promptedthe authors to look for new properties for the described coumarins. Based on this data, a new family of derivatives sharing the same scaffold and type of substituents was designed and synthesized looking for antioxidant and trypanocidal agents. Evaluation of the antioxidant activity of the described coumarins was performed towards different types of ROS: peroxyl and hydroxyl radicals. The electrochemical behavior of the compounds was also evaluated. The study of the electrochemical anodic behavior of the compounds was based principally on previous studies that related low oxidation potential to high antioxidant capacity [58]. CV, ORAC-FL and ESR reactivity assays were the techniques used to achieve the goals. In addition, the trypanocidal activity in epimastigote, trypomastigote and amastigote (Dm28c strain) stages was also evaluated.

According to the data obtained in the CV study, all the derivatives exhibited a single anodic peak without the corresponding cathodic peak, indicating an irreversible oxidation process as a result of the oxidation of a hydroxyl or amine group in the coumarin nucleus, forming a semiquinone radical. First investigated was the influence of the scan rate (v) in order to deeply assess the characteristics of the electrochemical and chemical reactions that take place at the electrode/solution interface. Results of the CV, reported in **Table 1**, demonstrated that coumarin derivatives with hydroxyl substituents in their structure displayed lower oxidation potential than those presenting amine groups. In spite of this, 4-hydroxycoumarin **(1)** reported the highest oxidation potential (1.33 mV). This exception is due to the fact that the oxidation of this coumarin derivative might not occur at the 4-OH group, but upon oxidation at the C3–C4 bond [59]. In the used experimental conditions, single oxidation processes (peak I) were observed for all compounds. It has been proposed that the charge transfer process at peak I corresponds to the oxidation of the hydroxyl or the amine substituent.

Compound **3**, presenting a hydroxyl group at position 4 and an amino one at position 3 of its skeleton, exhibited the lowest oxidation potential (0.50 mV). In addition, it presented the best ORAC-FL value of the whole series (4.36) and a percentage of hydroxyl radical scavenging of 56.8%. All the coumarin derivatives with a 4-OH substituent on their structure **(1, 3 & 5)** presented higher ORAC-FL values than the standard trolox, and compounds **3** (4.36) and **1** (4.20) higher than the 6,7-dihydroxy-4-methylcoumarin (3.3), used as a reference compound. In compounds **1 & 3** the presence of a hydroxyl group favored the hydrogen atom transfer mechanism. However, compound **5**, which presents the same electron-donating group, displayed a minor reactivity to the peroxyl radical. This could be explained due to the fact that the carbonyl substituent can form an intramolecular hydrogen bond with the

hydroxyl group at position 4, diminishing its ORAC-FL value. Compounds **2 & 4** (without hydroxyl groups at position 4) demonstrated low reactivity towards the peroxyl radical.

FIGURE 3A & B illustrate the result of the kinetic profile when the concentration of compounds **2 & 3**, in phosphate pH 7.4, was increased. In the same experimental conditions, a kinetic profile was observed without induction times for all the tested compounds. The absence of induction times in the kinetic profiles of FL would imply that the ORAC-FL index for these coumarin derivatives is more related to the antioxidants' reactivity than to stoichiometric factors [60]. The different ORAC-FL values can, therefore, be related to the substituents existent in the coumarin scaffold and their pattern of substitution.

Regarding the ESR assays, the results of which are reported in **TABLE 2**, it was observed that compounds **1 & 3**, which presented the best ORAC-FL values, also displayed the best reactivity towards hydroxyl radicals in the spin trapping assay (81.2 and 56.8%, respectively).

Regarding the biological assays, cytotoxicity and trypanocidal activity for all the synthesized compounds were evaluated and the results are reported in **TABLE 3**. Cytotoxicity assays, in murin RAW 264.7 macrophages, were carried out, and it was observed that four of the compounds present cytotoxic effects (IC_{50}) higher than 100 µM. Only compound **4** demonstrated cytotoxic effect with an IC_{50} value of 2.70 µM. In the study of the effect of the coumarin derivatives in the viability of the epimastigote (Dm28c) and trypomastigote (Dm28c) stages (10 µM), carried out using MTT assays, all the compounds presented activity.

It is known that the activity against one form of the parasite life cycle does not ensure similar activities against the others. Morphologic changes occur during the transformation between different stages of the parasite. In addition, important metabolic and macromolecular modifications take place along the life cycle. The sensibility of the different forms to the drugs is, therefore, modified [61]. The assayed compounds were significantly more active against the epimastigote form than the trypomastigote form of *T. cruzi*. These results are significant, since epimastigotes are present in the gut of the insect vector, but trypomastigotes are the infective forms in the mammalian host. The trypomastigote stage, in mammals, is the disseminator of blood-borne infection. In **TABLE 3** it is reported that the studied compounds present low trypanocidal activity in the trypomastigote stage, with the exception of compound **4**. This compound demonstrated better trypanocidal activity in both parasite forms (53.7 and 69.4%, respectively) than nifurtimox, used as reference compound. However, compound **4** presented the highest cytotoxic effect in murin RAW 264.7 macrophages. This means that the exposure to compound **4** resulted in a low selectivity index in RAW 264.7 cells. In addition, compound **4** was studied against the amastigote (Dm28c) stage, and presented a trypanocidal activity lower than the other parasite forms (26.6% at 10 µM). However, compound **4** was active for all stages of *T. cruzi*. compound **3** demonstrated low cytotoxicity in RAW 264.7 cells and presented interesting trypanocidal activity in epimastigote and trypomastigote stages (65.7 and 22.2%, respectively). In the epimastigote stage, compound **3** proved to have higher trypanocidal activity than nifurtimox (52.5%) and compound **1** presented close trypanocidal activity (48.7%). Based on the previously described results, it was concluded that compound **3** is the most interesting candidate for further studies.

Conclusion

In conclusion, the authors have confirmed the good antioxidant properties of the hydroxycoumarin derivatives **1, 3 & 5**, presenting better ORAC-FL values than standard Trolox. The presence of a 4-hydroxyl group in the coumarin scaffold resulted in a high improvement in the antioxidant activity, with compound **3** being the best of the series under study. The results of the antioxidant assay using ESR demonstrated reactivity against hydroxyl radical for all the tested compounds. An interesting finding is that the compounds with high antioxidant capacity determined by the ORAC-FL assay also presented high reactivity against the hydroxyl radical. Based on these results, it can be concluded that compounds **1 & 3** are potential candidates for further antioxidant capacity studies. In addition, the coumarin derivatives illustrated low cytotoxicity effect in murin RAW 264.7 cells. In the trypanocidal study, compounds **1, 3 & 4** presented high activity in epimastigotes stage and compounds **3 & 4** also displayed the highest trypanocidal activity in trypomastigote stage. Based on these results, it can be concluded that the joint presence of a hydroxyl group at position 4 and of an amino group at position 3

(compound **3** in relation to compounds **1 & 2**) increased the value of ORAC-FL and trypanocidal activity. The formation of the chloroacetamide derivative of compound **3 (5)** drastically decreased both properties. This effect was not observed in the same amidation of compounds **2 (4)**, which drastically decreased the ORAC-FL, but was more effective as a trypanocidal. The fact that compound **4** exhibited such low ORAC-FL and presented cytotoxicity led us to discard this skeleton in the process of identification of a new drug. Finally, compound **3** proved to be a good candidate for further studies due to the low cytotoxic effects and high trypanocidal activity, associated with an interesting antioxidative profile.

Future perspective

Chagas disease is a significant health problem in Central and South America, and it is one of the most significant emerging parasitic diseases in developed countries. Natural products have played a major role in the therapy of this parasitic disease, but at the moment only nifurtimox and benznidazole are the current drugs in use. These compounds are very unspecific, presenting high toxicity, low efficacy, high level of resistance and multiple side effects. Therefore, there is an urgent need for the discovery of new, more effective and safer drugs for human use. Based on the above-described results, compound **3** proved to be a good candidate for further studies due to the low cytotoxic effects and high trypanocidal activity, associated with an interesting antioxidative profile. This work is the starting point for the study of simple coumarins as trypanocidal compounds.

Financial & competing interests disclosure

This work was partially supported by FONDECYT (projects 1110029 and 1090078) and Anillo ACT112 (Chile), and personal founds of the Spanish researchers. RF Guíñez gratefully acknowledges CONICYT-Chile for his PhD scholarship (No. 21100132) and his PhD grant (No. 24121574). MJ Matos gratefully acknowledges Fundação para a Ciência e Tecnologia for her PhD grant (SFRH/BD/61262/2009). S Vazquez-Rodriguez gratefully acknowledges Ministerio de Educación y Ciencia for her FPU PhD grant (AP2008–04263). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Neglected diseases, such as Chagas disease, are a significant public health problem in Central and South America. Chagas, caused by *Trypanosoma cruzi*, is also one of the most relevant emerging parasitic diseases in developed countries. The relationship between trypanocidal activity and antioxidant capacity is a novel and innovative approach to the treatment of this pathology.
- A selected series of coumarins were efficiently synthesized in very good yields. These compounds are a promising scaffold against the described targets.
- Compounds **1** and **3**, which presented the best ORAC-FL values (4.20 and 4.36, respectively), also displayed the best reactivity towards hydroxyl radical in the spin trapping assay (81.2 and 56.8%, respectively). They proved to be very efficient antioxidants.
- In the trypanocidal study, compounds **1**, **3** and **4** presented high activity against the epimastigotes stage and compounds **3** and **4** also displayed the highest trypanocidal activity against the trypomastigote stage.
- The joint presence of a hydroxyl group at position 4 and of an amino group at position 3 (compound **3** in relation to compounds **1** and **2**) increased the value of ORAC-FL and trypanocidal activity. Therefore, compound **3** proved to be a good candidate for further studies due to the low cytotoxic effects and the high trypanocidal activity, associated with an interesting antioxidative profile.

References

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

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Bioorganic & Medicinal Chemistry 21 (2013) 3900–3906

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Remarkable antioxidant properties of a series of hydroxy-3-arylcoumarins ☆

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ARTICLE INFO

Article history:
Received 13 December 2012
Revised 26 March 2013
Accepted 3 April 2013
Available online 19 April 2013

Keywords:
3-Arylcoumarins
Hydroxyl derivatives
ORAC-FL assays
ESR assays
CV assays
Antioxidant capacity

ABSTRACT

In the present work we synthesized a series of hydroxy-3-arylcoumarins (compounds **1**–**9**), some of them previously described as MAO-B selective inhibitors, with the aim of evaluating their antioxidant properties. Theoretical evaluation of ADME properties of all the derivatives was also carried out. From the ORAC-FL, ESR and CV data it was concluded that these derivatives are very good antioxidants, with a very interesting hydroxyl, DPPH and superoxide radicals scavenging profiles. In particular compound **9** is the most active and effective antioxidant of the series (ORAC-FL = 13.5, capacity of scavenging hydroxyl radicals = 100%, capacity of scavenging DPPH radicals = 65.9% and capacity of scavenging superoxide radicals = 71.5%). Kinetics profile for protection fluorescein probe against peroxyl radicals by addition of antioxidant molecule **9** was also performed. Therefore, it can operate as a potential candidate for preventing or minimizing the free radicals overproduction in oxidative-stress related diseases.
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1. Introduction

Phenolic compounds are bioactive substances widely distributed in the vegetable kingdom. Generally, this group of compounds has one or more aromatic rings in their structure and one or more hydroxyl groups. They have been described to act as natural antioxidants and their presence contributes to prevent or minimize several types of oxidative processes.[1] Due to their antioxidant activity their ingestion is correlated with interesting benefits to health. Therefore, the research and characterization of new bioactive phenolic substances from diet has been intensified in the last years, either for the development of nutraceutics or medicines.[1] Due to their antioxidant properties they can protect cells from the oxidative damage of the reactive oxygen species (ROS). In fact, the overproduction of free radicals have been related to cellular membrane and DNA damage, and indirectly with aging and oxidative-stress related diseases like cancer, cardiovascular and neurodegenerative pathologies.[2] Therefore, antioxidants are very important for protecting the organisms from oxidative disorders, in which ROS are also involved.[3,4] Antioxidants are capable of decrease or prevent oxidation processes through different mechanisms, such as scavenging free radicals, inhibition of pro-oxidant enzymes or chelation of transition metal ions.[5]

An increasing number of reports suggested the involvement of oxidative stress in neurodegenerative diseases (ND), where the increased formation of ROS can contribute to neuronal damage and cell death.[3,4]

Suggestion has been made that the etiology of Parkinson's (PD) and Alzheimer's (AD) diseases may be closely linked to biochemical changes resultant from this oxidative stress.[6–8] Dopamine (DA) auto-oxidation naturally produces oxidative species and may contribute to ND such as PD and ischemia/reperfusion-induced damage. Monoamine oxidase (MAO) enzyme (particularly MAO-B) is responsible for metabolizing DA and plays an important role in oxidative stress through altering the redox state of neuronal and glial cells, leading to neuronal death.[9] Consequences are an over-production of MAO and non-MAO initiated hydrogen peroxide (H_2O_2) by proliferated reactive microglia and inability of neurons to dispose of H_2O_2 and other recative species like peroxyl radicals.[10] H_2O_2 produces highly toxic ROS, namely hydroxyl

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http://dx.doi.org/10.1016/j.bmc.2013.04.015

radical, by Fenton reaction that is catalyzed by iron and neuromelanin.[11] Concerning the mechanism of the clinical efficacy of MAO-B inhibitors in PD, the inhibition of DA degradation (a symptomatic effect) and also the prevention of the formation of neurotoxic DA degradation products, that is, ROS and DA derived aldehydes have been speculated.[12] The neuroprotective effect of rasagiline, a well-known MAO-B inhibitor, might be explained through multiple mechanisms, possibly due to reduction of DA catabolism with a subsequent increased activity on dopaminergic D_2 receptors and suppressing the action of ROS as well.[13] So, the possible mechanism of neuroprotection of MAO-B inhibitors may be related not only to MAO-B inhibition but also to induction and activation of multiple factors related with oxidative stress and apoptosis.[14]

Coumarins are a family of compounds widely distributed in the nature.[15] Due to their structural features, and biological properties, namely anticancer, anti-inflammatory, antioxidant, antithrombotic, vasorelaxant, antiviral and enzymatic inhibition agents, they have been ascribed as important building blocks in Organic Chemistry and Medicinal Chemistry.[16–23]

Recently, it was shown by our group that 3-substituted aryl coumarins are potent and selective MAO-B inhibitors.[24–30] In addition, it has been found that hydroxycoumarins are antioxidants scavenging ROS and/or chelating transition metals, exhibiting tissue-protective properties.[6,31–33] The complementarity of these activities for 3-arylcoumarins was not previously studied and described. The versatility of the used reactions allowed obtaining a family of compounds with hydroxyl and/or methyl substituents in different positions of the molecule. The election of these derivatives has considered the previously MAO-B inhibitory pharmacological evaluation and the low cost of the commercial reagents to begin with. Also, the influence of the substituents in the desired activity was taken into account.

2. Materials and methods

2.1. Chemistry

Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using TMS as internal standard (chemical shifts in δ values, J in Hz). Mass spectra were obtained using a Hewlett–Packard 5988A spectrometer. Elemental analyses were performed using a Perkin–Elmer 240B microanalyser and were within ±0.4% of calculated values in all cases. Silica gel (Merck 60, 230-00 mesh) was used for flash chromatography (FC). Analytical thin layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm).

2.1.1. General procedure for the preparation of methoxy-3-arylcoumarins

To a solution of the conveniently substituted *ortho*-hydroxybenzaldehyde (7.34 mmol) and the corresponding phenylacetic acid (9.18 mmol) in dimethyl sulfoxide (15 mL), *N,N′*-dicyclohexylcarbodiimide (11.46 mmol) was added. The mixture was heated at 110 °C for 24 h. Then, ice (100 mL) and acetic acid (10 mL) were added to the reaction mixture. After keeping it at room temperature for 2 h, the mixture was extracted with ether (3 × 25 mL). The organic layers were combined and washed with sodium bicarbonate solution (50 mL, 5%) and water (20 mL). Subsequently, the solvent was evaporated under vacuum and the dry residue was purified by flash chromatography (hexane/ethyl acetate 9:1), to give the desired methoxy-3-arylcoumarins.[23,28]

2.1.2. General procedure for the preparation of hydroxy-3-arylcoumarins

To a solution of a methoxy-3-arylcoumarin (0.50 mmol) in acetic acid (5 mL) and acetic anhydride (5 mL), at 0 °C, hydriodic acid 57% (10 mL) was added dropwise. The mixture was stirred under reflux, for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by crystallization (CH_3CN).[23,28,34]

2.1.2.1. 3-(3′,4′-Dihydroxyphenyl)-6-methylcoumarin (4). Yield: 92%; mp 199–200 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.43 (s, 3H, –CH_3), 6.43 (s, 1H, H-2′), 7.01 (d, J = 7.1 Hz, 1H, H-5′), 7.14 (d, J = 7.0 Hz, 1H, H-6′), 7.28–7.32 (m, 2H, H-7, H-8), 7.57 (d, J = 2.2 Hz, 1H, H-5), 7.83 (s, 1H, H-4), 10.40 (s, 2H, –OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.7, 100.1, 110.7, 111.6, 115.7, 119.4, 120.1, 127.3, 127.4, 132.1, 133.7, 138.8, 146.5, 146.8, 151.3, 161.6; EI MS m/z: 269 (13), 268 (M$^+$, 100), 241 (31), 240 (70), 239 (22), 165 (30), 125 (12), 111 (10); Anal. Calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.60; H, 4.49.

2.1.2.2. 3-(3′,4′-Dihydroxyphenyl)-8-methylcoumarin (5). Yield: 85%; mp 205–206 °C; ^{1}H NMR (300 MHz, DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 2.50 (s, 3H, –CH_3), 6.80 (d, $\boldsymbol{J}$ = 8.3 Hz, 1H, H-5′), 7.05 (dd, $\boldsymbol{J}$ = 8.2, $\boldsymbol{J}$ = 2.2 Hz, 1H, H-6′), 7.22 (d, $\boldsymbol{J}$ = 2.2 Hz, 1H, H-2′), 7.25 (d, $\boldsymbol{J}$ = 7.6 Hz, 1H, H-6), 7.44 (dd, $\boldsymbol{J}$ = 7.4, $\boldsymbol{J}$ = 1.0 Hz, 1H, H-7), 7.57 (dd, $\boldsymbol{J}$ = 7.6, $\boldsymbol{J}$ = 1.1 Hz, 1H, H-5), 8.09 (s, 1H, H-4), 9.09 (s, 1H, –OH), 9.24 (s, 1H, –OH); ^{13}C NMR (75 MHz, DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 14.9, 115.4, 116.0, 119.5, 119.9, 124.1, 124.6, 125.7, 126.1, 126.5, 132.2, 138.7, 144.8, 146.2, 150.9, 159.9; EI MS $\boldsymbol{m/z}$: 270 (12), 269 (76), 268 (M$^+$, 82), 241 (47), 240 (100) 239 (57), 211 (23), 166 (19), 165 (58), 152 (18), 139 (14), 125 (30), 111 (28), 82 (19); Anal. Calcd for $\boldsymbol{C_{16}H_{12}O_4}$: C, 71.64; H, 4.51. Found: C, 71.63; H, 4.49.

2.1.2.3. 3-(3′,5′-Dihydroxyphenyl)-8-methylcoumarin (6). Yield: 90%; mp 180–181 °C; ^{1}H NMR (300 MHz, DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 2.49 (s, 3H, –CH_3), 6.70 (s, 3H, H-2′, H-4′, H-6′), 7.26 (t, $\boldsymbol{J}$ = 7.6 Hz, 1H, H-6), 7.48 (d, $\boldsymbol{J}$ = 7.8 Hz, 1H, H-7), 7.62 (d, $\boldsymbol{J}$ = 7.7 Hz, 1H, H-5), 8.11 (s, 1H, H-4), 10.27 (s, 2H, OH); ^{13}C NMR (75 MHz, DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 15.3, 103.3, 107.2, 119.6, 124.5, 125.1, 126.8, 127.2, 133.1, 136.6, 140.9, 151.6, 158.5, 160.0; EI MS $\boldsymbol{m/z}$: 269 (21), 268 (M$^+$, 100), 241 (12), 240 (63), 239 (26); Anal. Calcd for $\boldsymbol{C_{16}H_{12}O_4}$: C, 71.64; H, 4.51. Found: C, 71.60; H, 4.50.

2.1.2.4. 3-(3′,4′,5′-Trihydroxyphenyl)-8-methylcoumarin (7). Yield: 82%; mp 189–190 °C. ^{1}H NMR (300 MHz, DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 2.49 (s, 3H, –$\boldsymbol{CH_3}$), 6.95 (s, 2H, H-2′, H-6′), 7.20 (t, $\boldsymbol{J}$ = 7.4 Hz, 2H, H-6, H-7), 7.38 (d, $\boldsymbol{J}$ = 7.4 Hz, 1H, H-5), 7.79 (s, 1H, H-4), 10.55 (s, 2H, –OH), 10.60 (s, 1H, –OH); ^{13}C NMR (75 MHz, DMSO-$\boldsymbol{d_6}$DMSO-$\boldsymbol{d_6}$): $\boldsymbol{\delta}$ 15.6, 106.1, 119.4, 124.2, 125.4, 125.7, 127.7, 130.4, 132.7, 135.6, 139.8, 146.7, 146.9, 160.6; EI MS $\boldsymbol{m/z}$: 285 (16), 284 (M$^+$, 100), 283 (84), 256 (32), 181 (10) 141 (10); Anal. Calcd for $\boldsymbol{C_{16}H_{12}O_5}$: C, 67.60; H, 4.25. Found: C, 67.61; H, 4.28.

2.2. Antioxidant assays

2.2.1. Oxygen radical antioxidant capacity-fluorescein (ORAC-FL)

The ORAC analyses were carried out on a Synergy HT multi detection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, USA), using white polystyrene 96-well plates, purchased from Nunc (Denmark). Fluorescence was read from the top, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by Gen 5 software. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and 200 μL final volume. FL (70 nM, final concentration) and hydroxy-3-arylcoumarin solutions in methanol with

a range of concentration between 0.3 and 2 μM were placed in each well of 96-well plate. The mixture was pre-incubated for 15 min at 37 °C, before rapidly adding the AAPH solution (18 mM, final concentration). The microplate was immediately placed in the reader and automatically shaken prior to each reading. The fluorescence was recorded every 1 min for 120 min. A blank with FL and 2,2′-azobis(2-methylpropionamidine)dihydrochloride (AAPH) using methanol instead of the antioxidant solution were used in each assay. Five calibration solutions using Trolox (0.5–2.5 μM) as antioxidant were also done. The inhibition capacity was expressed as ORAC values and is quantified by integration of the area under the curve (AUC_{NET}). All reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample. The area under the fluorescence decay curve (AUC) was calculated integrating the decay of the fluorescence where F_0 is the initial fluorescence read at 0 min and F is the fluorescence read at time. The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. Data processing was performed using Origin Pro 8 SR2 (Origin Lab Corporation, USA).

2.2.2. Hydroxyl radical scavenging assay using electron spin resonance (ESR)

Reactivity of all the hydroxy-3-arylcoumarin derivatives against the hydroxyl radical was investigated using the non-catalytic Fenton type method. ESR spectra were recorded in the X band (9.7 GHz) using a Bruker ECS 106 spectrometer with a rectangular cavity and 50 kHz field modulation, equipped with a high-sensitivity resonator at room temperature. Spectrometer conditions were: microwave frequency 9.81 GHz, microwave power 20 mW, modulation amplitude 0.91 G, receiver gain 59 db, time constant 81.92 ms and conversion time 40.96 ms. The scavenging activity of each derivative was estimated by comparing the DMPO-OH adduct signals in the antioxidant–radical reaction mixture and the control reaction at the same reaction time, and is expressed as scavenging percent of hydroxyl radical.

To prepare the samples, 150 μL of *N*,*N*-dimethylformamide (DMF) and 50 μL of NaOH (3 mM) were mixed, followed by the addition of 50 μL of 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) spin trap (30 mM final concentration) and finally 50 μL of hydrogen peroxide 30%. The mixture was put in an ESR cell and the spectrum was recorded after five minutes of reaction. All the compounds were studied to 4 mM final concentration (300 μL final volume).

2.2.3. DPPH radical scavenging assay using ESR

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging capacity[35–38] of the hydroxy-3-arylcoumarin derivatives was determined by an ESR spectrometry method.[39–43] Each hydroxy-3-arylcoumarin solution was mixed with DPPH stock solution to initiate the antioxidant–radical reaction. All the reaction mixtures contained 1.0 mM of DPPH and 1.0 mM of the studied compound. The control solution was prepared in absence of the studied compounds. Both DPPH and compounds solutions were prepared in acetonitrile. ESR signals were recorded after five minutes of reaction. Spectrometer conditions were: microwave frequency 9.81 GHz, microwave power 20 mW, modulation amplitude 0.95 G, receiver gain 59 db, time constant 81.92 ms and conversion time 40.96 ms. The scavenging activity of each compound was estimated by comparing the DPPH signals in the antioxidant–radical reaction mixture and the control reaction at the same reaction time, and was expressed as scavenging percent of DPPH.

2.2.4. Superoxide antioxidant assay using cyclic voltammetry (CV)

Cyclic voltammetry (CV) measurements were performed in a Metrohm 693VA instrument with a 694VA stand convertor and a 693VA processor, at room temperature, using a three-electrode cell. A glassy carbon (GC) electrode presenting an area of 0.03 cm^2 was used as the working electrode. The electrode surface was polished to a mirror finish with alumina powder (0.3 and 0.05 μM) before use and after each measurement. Platinum wire was used as auxiliary electrode and silver-silver chloride (Ag/AgCl, 3 M KCl) of Metrohm Company with a plastic tip was used as a reference electrode. The CV experiments were carried out in dimethyl sulfoxide (DMSO) of analytical grade (Sigma–Aldrich) with 0.1 M of tetrabutylammonium perchlorate (TBAP) as supporting electrolyte. Superoxide anion radical was generated in DMSO containing TBAP 0.1 M. The scan rate was kept 30 mV/s and potential window was −1.0 to −0.0 V. The atmospheric solubility of oxygen in DMSO was 2.1 mM.[44] The final concentration of each derivative (30 μM) was achieved by additions of the corresponding aliquot of stock solution (10 mL final volume). Finally, the antioxidant activity was assessed from the change in the cathodic current of the voltammograms in absence and present of the derivatives, using pertinent mathematical formulations.

2.2.5. Data statistical analysis

Statistical analyses were conducted using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA). The data are expressed as means ± SD. The experimental data were analyzed by one-way analysis of variance (ANOVA), and differences between groups were assessed using Tukey's post-test. The level of significance was set at $p < 0.05$, and all experiments were replicated 3 times.

3. Results

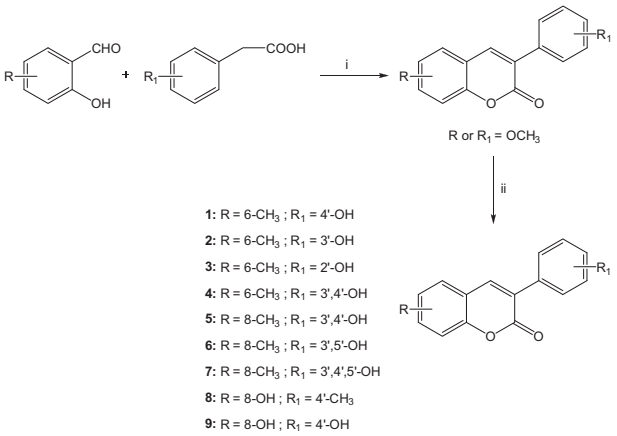
3.1. Chemical synthesis

Coumarin derivatives **1**–**9** were efficiently synthesized according to the protocol outlined in Scheme 1. The general reaction conditions and the characterization data of the new compounds were described in the experimental section. Perkin condensation of different *ortho*-hydroxybenzaldehydes with the adequate arylacetic acid, using *N*,*N*′-dicyclohexylcarbodiimide (DCC) as dehydrating agent,[28] afforded the methoxy-3-arylcoumarins. Hydroxyl derivatives were obtained from the above-mentioned methoxy substituted precursors by acidic hydrolysis, using hydriodic acid 57% in the presence of acetic acid and acetic anhydride.[28]

3.2. Antioxidant capacity assays

The evaluation of the antioxidant activity of the studied compounds was performed towards different types of reactive oxygen or nitrogen species—peroxyl, hydroxyl, superoxide and DPPH radicals. ORAC-FL, ESR and CV assays were the techniques used to obtain the desired results.

The peroxyl radical scavenging activity of the synthesized hydroxy-3-arylcoumarins was evaluated by the oxygen radical absorbance capacity (ORAC) method[45] This assay use a fluorescence-based technology (ORAC-FL) and allow obtaining a relative antioxidant index by using as reference trolox, a hydrosoluble vitamin E derivative. The exposition of the fluorophore, in this case fluorescein (FL) to the peroxyl radical lead to an oxidation process reflected as a decay of fluorescence emission through time. In ORAC assays, the loss of fluorescence of FL generally corresponds to an induction time and is reliant on antioxidant capacity of a compound. In fact, it refers to the time in which the FL is protected against the oxidative damage of peroxyl radicals and this behavior is associated to a competitive reaction between the radical and the antioxidant.[46,47] ORAC data take into account the induction time,



Scheme 1. Reagents and conditions: (i) DCC, DMSO, 110 °C, 24 h; (ii) HI 57%, AcOH, Ac_2O, reflux, 3 h.

Table 1
ORAC-FL index and hydroxyl, DPPH and superoxide radical scavenging data obtained for compounds **1**-**9**

Compd	ORAC-FL index	% Scavenging hydroxyl radicals[a]	% Scavenging DPPH radicals[a]	% Scavenging superoxide radicals[b]
1	6.1	100	27.7	17.4
2	6.7	5.2	3.4	17.5
3	8.4	100	10.6	25
4	5.3	6.05	28.3	28.9
5	5.7	100	66.7	77.3
6	5.5	75	11.2	22.6
7	5.3	100	100	76.5
8	6.3	16	28.6	17.4
9	13.5	100	65.9	71.5
Trolox	1.0	—	—	—
Quercetin	7.28[c]	—	20.0[d]	—
Catechin	6.76[c]	—	44.5[d]	—

[a] The scavenging activity of hydroxyl and DPPH radicals effect was calculated as follows: $[(A_0 - A_x)/A_0] \times 100$, where A_x and A_0 are the double-integral ESR for the first line of samples in the presence and absence of test compounds, respectively.
[b] The scavenging activity of superoxide radical effect was calculated as follows: [(*I*pc blank − *I*pc aox)/*I*pc blank] × 100, where *I*pc blank is cathodic current in the absence of the studied compounds and *I*pc aox is the cathodic current in the presence of the studied compounds.
[c] Data collected from Ref. 45.
[d] Data collected from Ref. 49.

initial rate and the range of total antioxidant inhibition in one value.[46]

Results expressed as ORAC-FL values are presented in Table 1. All the obtained ESR results for the% scavenging of the hydroxyl and DPPH radicals are also illustrated in Table 1. In addition, % of superoxide radical scavenging, performed by CV, are also represented in Table 1.

The ORAC-FL profile, intensity of fluorescence at 528 nm versus the incubation time was obtained for all derivatives. Compounds **3** and **9** (8.4 and 13.5, respectively) display the highest ORAC-FL indexes, comparing with the flavonoids quercetin and catechin that are very well known natural antioxidant compounds. Figure 1 shows the kinetic profile for protection of FL probe against peroxyl radicals obtained in presence of increasing concentrations of compound **9**. It was obtained a profile of fluorescence measure at 528 nm versus the incubation time at different concentration, for all derivatives.

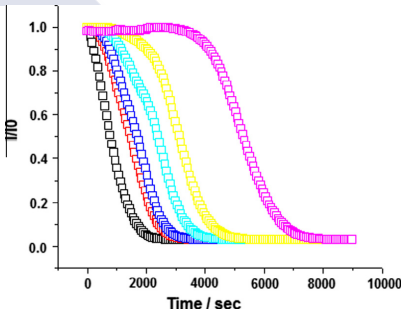


Figure 1. ORAC-FL profile (kinetic profile for protection FL probe against peroxyl radicals) for compound **9**.

In order to study the antioxidant reactivity of all the synthesized hydroxy-3-arylcoumarin derivatives towards hydroxyl radicals, a non-catalytic and competitive type Fenton system in which the DMPO spin trap was performed.[48] The ESR spin-trapping spectrum obtained in the control assay (DMPO+*N*,*N*-dimethylformamide + NaOH + H_2O_2) presents four hyperfine lines, due to the DMPO-OH adduct formation, as it is shown in Figure 2 (red line). For each putative antioxidant coumarin compounds ESR spectra were also acquired to check their capacity of scavenging hydroxyl radicals. The data obtained with compound **2** is depicted in Figure 2 (black line).

The intensity of the spectra decreases when the hydroxy-3-arylcoumarin derivatives were added into the system. For compound **9**, 100% of scavenging of hydroxyl radicals was obtained (Fig. 3—black line). This type of response was observed for all derivatives, reflecting different percentage of the hydroxyl radical scavenging activity (Table 1).

The stable free radical DPPH assay has been used for detecting the antioxidant activity in several chemical analyses.[35–38] Currently, DPPH assay is considered an easy and accurate method, appropriate for measuring the antioxidant capacity of fruits, vegetables, juices or extracts.[39] This is due to the electronic properties shared by DPPH and peroxyl radicals (the unpaired electron is delocalized through the pair of nitrogen or oxygen atoms, respec-

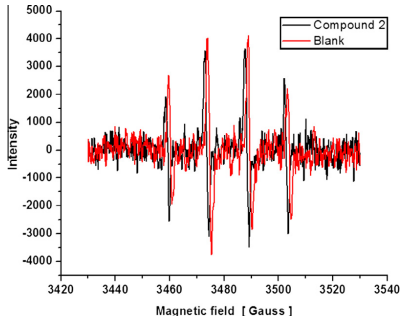


Figure 2. ESR spectra obtained for the control (adduct DMPO-OH without antioxidant molecule—red line) and for adduct DMPO-OH in the presence of compound **2** (black line).

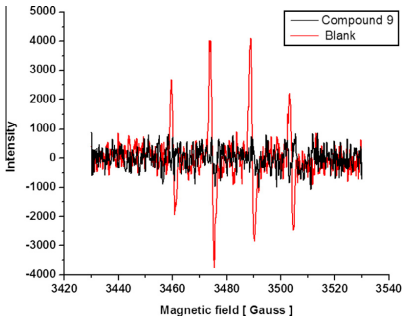


Figure 3. ESR spectra obtained for the control (adduct DMPO-OH without antioxidant molecule—red line) and for adduct DMPO-OH in the presence of compound **9** (black line).

tively), in such way that the reaction rate between DPPH and several antioxidants provides a good approximation for scavenging activities with lipid peroxyl radicals.[40,41] ESR spectra of DPPH in absence and presence of compounds **4**, **7** and **9** are showed in Figure 4.

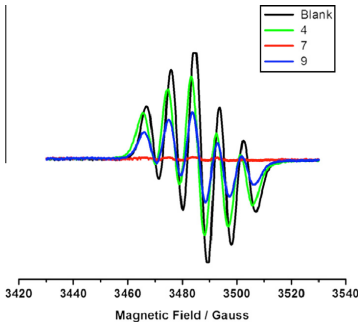


Figure 4. ESR signal from DPPH radical in absence (blank) and presence of compounds **4**, **7** and **9**.

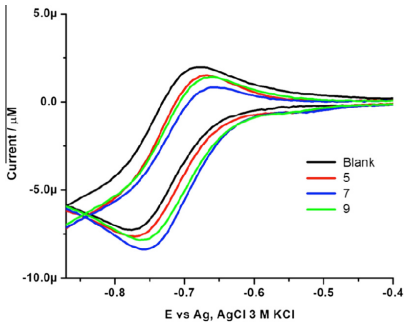


Figure 5. Cyclic voltammograms of superoxide radical in absence (blank) and presence of compounds **5**, **7** and **9** in DMSO + TBAP 0.1 M, on GC (working electrode) versus. Ag/AgCl, at room temperature, with scan rate of 30 mV/s.

Superoxide anion radical was generated by one electron reduction of the atmospheric molecular oxygen dissolved in DMSO at room temperature (25 °C). Then, the voltammetric performance of the nine compounds was studied, one by one, in DMSO and TBAP 0.1 M. The resultant CV responses for derivative **5**, **7** and **9**, and in absence of any derivative (blank) are represented in Figure 5.

3.3. Theoretical evaluation of ADME properties

In order to better understand the overall properties of the described compounds, the lipophilicity (expressed as the octanol/water partition coefficient and herein called log*P*), was calculated using the Molinspiration property calculation program.[50] The theoretical prediction of ADME properties (molecular weight, log*P*, number of hydrogen donors and acceptors) of all the compounds was carried out and is presented in Table 2.[51,52]

4. Discussion

In previous works, 3-arylcoumarin derivatives were described as potent and selective MAO-B inhibitors.[28] This family of compounds and their remarkable data on the selective inhibition of MAO-B isoenzyme and putative application for ND therapy were the inspiration for this work. In particular, compounds **1**–**3** were previously described as potent and selective MAO-B inhibitors, with IC_{50} values between 120 and 650 nM.[28] On the other hand the recent medicinal chemistry paradigms in the drug design, namely the rational discovery of multi-target drugs, as a promising strategy to combat this type of multifactorial diseases prompted us to look for other properties for this type of coumarins. Based on this data, a new family of derivatives sharing the same scaffold and type of substituents was designed and synthesized.

The evaluation of the antioxidant activity of the hydroxy-3-arylcoumarin compounds was performed towards different types of reactive oxygen or nitrogen species—peroxyl, hydroxyl, superoxide and DPPH radicals. ORAC-FL, ESR reactivity and CV assays were the techniques used to achieve the goals. From the obtained data, it was concluded that the antioxidant scavenging activity is related with the type of substituents presented in the 3-arylcoumarin skeleton.

Compound **9** was found to be the most interesting coumarin of the series. This compound has two hydroxyl groups in its structure, one at position 8 and another at position 4′ of the 3-arylcoumarin scaffold. The other compounds have structural combinations of two types of substituents (methyl and one, two or three hydroxyl groups). Compounds **1**, **3**, **5** and **7** have ORAC-FL values between

Table 2
Theoretical structural properties of the hydroxy-3-arylcoumarins derivatives **1–9**[a]

Compd	log*P*	Molecular weight	TPSA	*n*-OH acceptors	*n*-OHNH donors	Volume
1	3.68	252.27	50.44	3	1	224.57
2	3.66	252.27	50.44	3	1	224.57
3	3.89	252.27	50.44	3	1	224.57
4	3.19	268.27	70.67	4	2	232.59
5	3.17	268.27	70.67	4	2	232.59
6	3.11	268.27	70.67	4	2	232.59
7	2.88	284.27	90.90	5	3	240.61
8	3.92	252.27	50.44	3	1	224.57
9	2.99	254.24	70.67	4	2	216.03

[a] log*P*—octanol/water partition coefficient; TPSA–topological polar surface area; *n*-OH—number of hydrogen acceptors; *n*-OHNH—number of hydrogen bond donors. The data was determined with Molinspiration calculation software.[50]

5.3 and 8.4 and the higher scavenging activity towards hydroxyl radicals (100% – total scavenging). From these derivatives, compound **7** presented the best DPPH and superoxide radicals scavenging (100 and 76.5%, respectively). Compounds **1** and **3** have on their structure a methyl group at position 6 of the coumarin moiety and a hydroxyl group in the 3-aryl ring. The ORAC-FL indexes are 6.1 and 8.4, respectively, but the position of the hydroxyl group in the exocyclic aromatic ring seems to have no influence on the hydroxyl radical scavenging capacity (100%, in both cases). The DPPH radical scavenging values of these compounds are, respectively, 27.7% and 10.6% and superoxide radical scavenging values are, respectively, 17.4% and 25%. Their profiles are, therefore, very similar. Compounds **5** and **7** have a methyl group at position 8 of the coumarin moiety, and two or three hydroxyl groups, respectively, in the 3-aryl ring. Both ORAC-FL values (5.7 and 5.3, respectively), hydroxyl radical scavenging capacity (100%) and superoxide radical scavenging capacity (77.3% and 76.5%, respectively) are similar, proving that the presence of the extra hydroxyl group does not seem to significantly affect the peroxyl, hydroxyl and superoxide radicals scavenging activities. One the other hand, DPPH radical scavenging capacity is 66.7% for compound **5** and 100% for compound **7**. Compounds **2**, **4** and **8**, with ORAC-FL values between 5.3 and 6.7, presented the lowest hydroxyl scavenging capacity (between 5.2% and 16%). Compound **2** presented also the lowest DPPH radical scavenging capacity (3.4%) and compounds **1** and **8** the lowest superoxide radical scavenging capacities (17.4%). Comparing the data obtained for compound **4** (3′,4′-dihydroxy substituted) and for compound **1** (4′-hydroxy substituted), it can be concluded that the presence of two hydroxyl groups led to the decline in the ORAC-FL index (6.1–5.3) and the hydroxyl radical scavenging capacity (100%–6.05%). Comparing compound **4** with **2** (3′-hydroxy substituted), it is also noted a decrease in the ORAC-FL index (from 6.7 to 5.3), caused by the presence of two hydroxyl groups in the molecule. However, the hydroxyl radical scavenging capacity is almost the same (6.05% and 5.2%, respectively). Superoxide radical scavenging capacity is also similar (28.9 and 17.5, respectively). Compounds **4** and **5** have the same substitution pattern, changing only the position of the methyl group from 6 to 8. This modification strongly affects the hydroxyl radical scavenging capacity (from 6.05% to 100%), the DPPH radical scavenging capacity (from 28.3% to 66.7%) and superoxide radical scavenging capacity (from 28.9% to 77.3%). Compound **6**, with a methyl group at position 8 of the coumarin moiety and two hydroxyl groups at positions 3′ and 5′ of the 3-aryl ring, in spite of presenting an ORAC-FL value of 5.5 and low DPPH and superoxide radicals scavenging (11.2 and 22.6, respectively), evidence a significant tendency to scavenge hydroxyl radicals (75%).

As said before, the highest ORAC-FL values were found for compounds **3** and **9** (8.4 and 13.5, respectively). The results are very interesting comparing with the ORAC-FL values of catechin (6.76) and quercetin (7.28), very well known natural antioxidants. Comparing with trolox (ORAC-FL = 1.0), the interesting ORAC-FL values of compounds **3** and **9** make them promising antioxidant molecules. It is important to notice that compound **5**, **7** and **9** presented good trends against all the studied radicals.

From the obtained data, it is also remarkable that all the coumarin derivatives possess log*P* values compatible with those required to cross membranes. TPSA, described to be a predictive indicator of membrane penetration, is also found to be positive. In addition, it can be observed that no violations of Lipinski's rule (molecular weight, log*P*, number of hydrogen donors and acceptors) were found. This is important information about the promising potential of these derivatives.

All the synthesized compounds, in spite of presenting different chemical substituents, disclose interesting ORAC-FL values, in most cases accompanied by a remarkable ability to scavenge hydroxyl, DPPH and superoxide radicals. Therefore, the data acquired so far are relevant allowing proposing hydroxy-3-arylcoumarins as a valid scaffold for the design of novel antioxidants.

5. Concluding remarks

In conclusion, in the current work coumarins presenting very promising antioxidant profiles were described. Compound **9** proved to be the most interesting molecule of the whole series, with an ORAC-FL of 13.5, 100% of scavenging of hydroxyl radicals, 65.9% of scavenging of DPPH radicals and 71.5% of scavenging of superoxide radicals. This derivative has presented good antioxidant capacity towards different types of reactive oxygen or nitrogen species—peroxyl, hydroxyl, superoxide and DPPH radicals. Compound **3**, previously describe as very good selective MAO-B inhibitor, presented also a very interesting antioxidative profile. It is important to notice that compound **5** and **7** also presented good trends against all the studied radicals. In addition, it can be observed that no theoretical violations of Lipinski's rule were observed for all the studied derivatives. Therefore, the described compounds seem to present desirable ADME properties. Based on these results, it can be concluded that especially compounds **3** and **9** are potential candidates for a further optimization process and could be successfully employed in the prevention or minimization of the oxidative damage caused by overproduction of oxygen free radicals.

Acknowledgments

The current work was supported by Mecesup (Project UCH-0601), CONICYT-Chile (Project N 24110059), Fundação para a Ciência e Tecnologia (Project PTDC/QUI-QUI/113687/2009) and personal funds from the researchers. M.J. Matos thanks Fundação para a Ciência e Tecnologia (SFRH/BD/61262/2009) Ph.D. Grant, F. Pérez-Cruz thanks CONICYT-Chile PhD. grant, Becas-Chile and

Fulbright doctoral stay fellowships and S. Vazquez-Rodriguez thanks Ministerio de Educación y Ciencia (AP2008-04263) PhD. Grant.

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Research Paper

Chalcone-based derivatives as new scaffolds for *h*A_3 adenosine receptor antagonists

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Keywords
adenosine receptor; chalcone; coumarin-chalcone hybrids; structure–activity relationship

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Received November 9, 2012
Accepted December 19, 2012

doi: 10.1111/jphp.12028

Abstract

Objectives With the aim of finding new adenosine receptor (AR) ligands based on the chalcone scaffold, we report the synthesis of a new series of coumarin–chalcone hybrids and the pharmacological characterization of their actions at four subtypes of AR.

Methods The synthesized compounds **5–10** were characterized in radioligand binding (A_1, A_{2A} and A_3) and adenylyl cyclase activity assays (A_{2B}) to determine the affinity of the compounds for the four human AR (*h*AR) subtypes.

Key findings Coumarin–chalcone hybrids were found to be ligands with a novel structure, not reported thus far, that showed varying affinity and selectivity for AR subtypes.

Conclusions The coumarin–chalcone hybrids in which ring B of the chalcone scaffold was a thiophene (compounds **5** and **9**) were found to be the most potent compounds of the series. Compound **9**, in which ring A of the chalcone moiety was the phenyl ring of the coumarin, showed similar activity against *h*A_1, *h*A_{2A} and *h*A_3 ARs, while compound **5**, in which ring A of the chalcone was substituted by the benzopyrone ring of the coumarin moiety, showed similar activity only at the *h*A_3 AR and, therefore, was deemed to be selective (K_i (dissociation constant) = 5160 nM).

Introduction

Adenosine is a purine nucleoside produced by all metabolically active cells. It acts as an endogenous modulator controlling a wide range of physiological processes due to its interaction with four specific cell membrane G-protein-coupled receptors (GPCRs) classified as A_1, A_{2A}, A_{2B} and A_3 adenosine receptors (ARs).[1–3] Because of their ubiquitous presence in cells, ARs have been seen as promising targets in the field of medicinal chemistry. ARs are distributed along different tissues in mammalian systems and regulate diverse physiological functions by modulating cell signalling, being activated by endogenous adenosine and blocked by antagonists.[4,5] In the last two decades, a large number of ligands have been synthesized in the search for potent and selective agonists and antagonists for each AR subtype; selective A_{2B}AR agonists are among the most recently reported.[6] Targeting ARs has opened a new window for potential drug treatment of a variety of pathologies such as asthma, neurodegenerative disorders, cancer and inflammatory and ischaemic conditions.[7–12] More specifically, AR antagonists are involved in several pathological processes such as inflammation (A_{2A}),[13,14] heart and renal failure (A_1)[15] or neurological disorders like Parkinson's[16] and Alzheimer's disease (A_{2A} and/or A_1).[17]

AR antagonists developed recently present a chemical diversity, while the 1,3-dialkylxanthines are considered as derivatives of the classical scaffold.[18,19] Among the non-classical antagonists, the flavone and isoflavone derivatives have played a remarkable role, namely genistein, which has been described as a competitive antagonist at the A_1 AR in FRTL (thyroid) cells[20] or galangin, which was found to bind three subtypes of ARs, and it has been shown that this type of compound presents micromolar affinity for the A_3 AR.[21] (Figure 1)

Coumarins (chromone isosteres) and chalcones (flavone with an opened pyrone ring) are another class of benzopyran-related compounds of natural origin that

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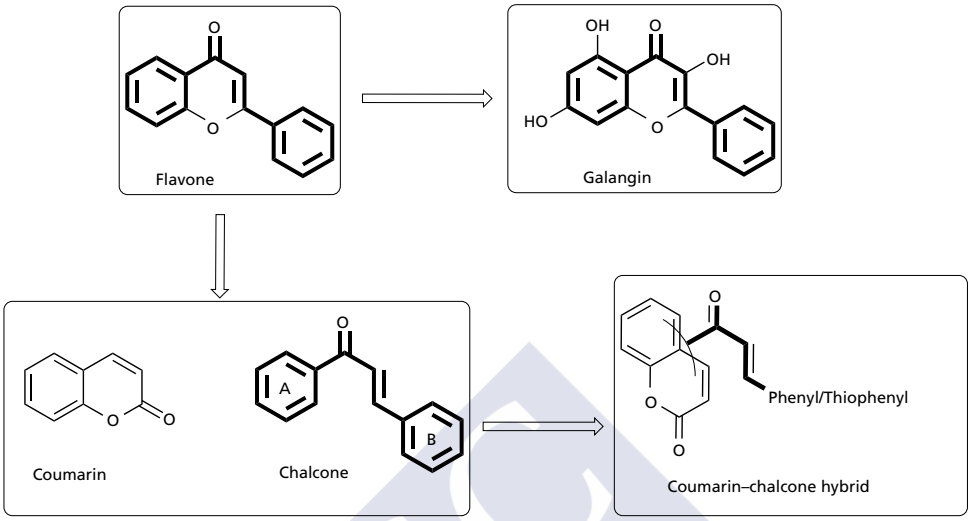

Figure 1 Rational design of the synthesized compounds based on flavonoid scaffolds.

present a wide range of pharmacological actions.[22–24] Coumarins have health value mainly due to their antimicrobial,[25] enzyme-inhibitory,[26–28] anticancer[29] and antioxidant[30] activity.

On the other hand, chalcones also present related biological activity as antimicrobial and anti-inflammatory,[24] anti-tumour,[29] antioxidant[31] or enzyme-inhibitory effects.[32] Thus, due to structural similarities between flavones, coumarins and chalcones, we decided to synthesize hybrid compounds bearing both scaffolds in the same molecule, and to evaluate their activity towards the four subtypes of human AR expressed in Chinese hamster ovary (CHO) cells. The designed compounds have the chalcone scaffold as the main structural core, and the different derivatives are mainly based on the substitution of one or both of the chalcone phenyl rings (A and/or B) for a coumarin and/or thiophene with different substituents (Figure 1). In addition, the drug-like properties of the hybrids were also evaluated.

Materials and Methods

Synthetic methodologies

Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using tetramethylsilane as internal standard (chemical shifts in δ values, J in Hz). Mass spectra were obtained using a Hewlett-Packard 5988A spectrometer. Elemental analyses were performed using a Perkin-Elmer 240B microanalyser and were within ± 0.4% of calculated values in all cases. Silica gel (Merck 60, 230–00 mesh) was used for flash chromatography. Analytical thin-layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm). The purity of compounds was assessed by high-performance liquid chromatography (HPLC) coupled at diode array detector (DAD) on a Thermo Quest Spectrasystem (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a P4000 pump, a UV6000 UV-Vis diode array detector and an SN4000 interface for operation via a personal computer. Instrument software ChromQuest 5.0 (Thermo Fisher Scientific) was used for data acquisition. Different analytical columns and mobile phases (all solvents were HPLC grade) were tested. The mobile phase was H_2O–CH_3CN (70:30) and an Eclipse xdb C18 column (5 µm particle size, 0.46 mm i.d., 25 cm length; Agilent Technologies) was used. The purity of the compounds was found to be higher than 95%.

Chemistry

The chalcone-based derivatives **5–10** were efficiently synthesized according to the protocol outlined in Figure 2.

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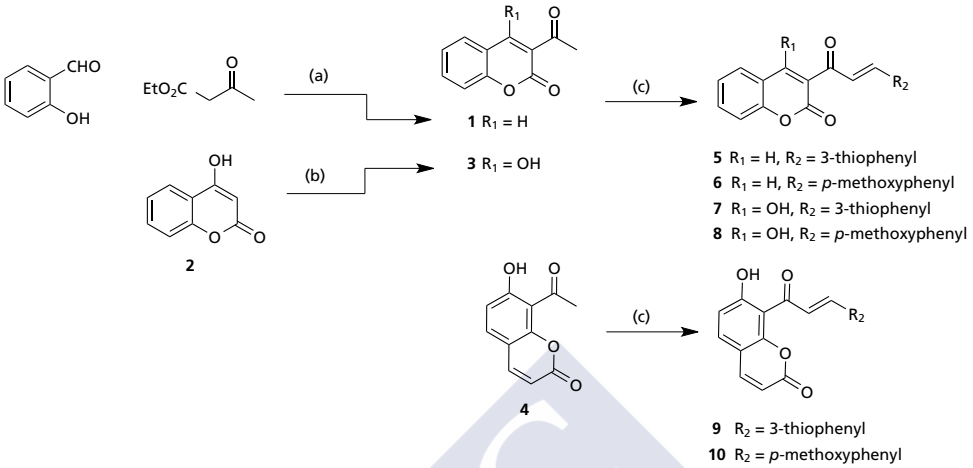

Figure 2 Protocol for synthesis of chalcone-based derivatives **5**–**10**. Reagents and conditions: (a) solvent-free, piperidine, rt, 10 min; (b) $POCl_3$, glacial acetic acid, reflux, 30 min; (c) appropriate aldehyde, EtOH, piperidine, reflux, 2–6 h.

Different starting materials were used in accordance with the substitution pattern of the final compounds. To synthesize compounds **5** and **6**,[33] we first prepared the 3-acetylcoumarin (**1**) by a Knoevenagel reaction using salicylaldehyde and ethyl acetoacetate without solvent and employing piperidine in a catalytic amount at room temperature.[34] To prepare compounds **7** and **8**,[35] 4-hydroxycoumarin (**2**) was used as the starting material. Acylation at the 3 position of compound **2**, using $POCl_3$ in glacial acetic acid under reflux, afforded precursor **3** (93%).[35] Compounds **9** and **10** were prepared from the commercially available 8-acetyl-7-hydroxycoumarin (**4**). Starting from precursors **1**, **3** and **4**, a Claisen-Schmidt condensation in EtOH, using piperidine as base, and under reflux mixed with the corresponding aromatic aldehydes, afforded the desired final compounds **5**–**10** in good yields (47–87%).

Synthesis of 3-(3-aryl)acryloylcoumarin (5–8) and 8-(3-aryl)acryloylcoumarin (9, 10) derivatives

The corresponding 3-acetylcoumarin (**1** or **3**, 1 mmol) or 8-acetylcoumarin (**4**) and the conveniently substituted aromatic aldehyde (1.1 mmol) were dissolved in EtOH (3 ml) and a catalytic amount of piperidine (0.05 ml) was added. The reaction mixtures were stirred for 2–6 h under reflux. After completion of reaction (followed by TLC), the solvent was evaporated under vacuum and the dry residue was purified by flash chromatography (hexane–ethyl acetate, 85:15) to give the desired products **5**–**10**.

Biological assays

The binding affinity of the compounds for the human AR subtypes hA_1, hA_{2A} and hA_3 was determined using radioligand competition experiments in CHO cells that had been stably transfected with the individual receptor subtypes.[36,37] The radioligands used were 1 nM [^{3}H]2-chloro-N6-cyclopentyladenosine (CCPA) for hA_1, 10 nM [^{3}H] N-ethylcarboxamidoadenosine (NECA) for hA_{2A} and 1 nM [^{3}H]2-(1-Hexynyl)-N-methyladenosine (HEMADO) for hA_3 receptors. Due to the lack of a suitable radioligand for hA_{2B} receptors the potency of antagonists at hA_{2B} receptor (expressed on CHO cells) was determined by inhibition of NECA-stimulated adenylyl cyclase activity.[36] The 50% inhibitory concentration (IC50) for inhibition of cAMP (cyclic adenosine monophosphate) production was determined and converted to a K_i value (dissociation constants) using the Cheng and Prusoff equation.[38] For all the tested compounds no measurable activity for the hA_{2B} AR (K_i > 10 000 nM) was detected.

Statistical methods

K_i values (dissociation constants) were determined in radioligand competition experiment with seven or eight different concentrations of test compound and each concentration was tested in duplicate. K_i values are given as geometric means of three independent experiments with 95% confidence intervals. For analysis of the competition curves the programme SCTFIT was used.[39]

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Theoretical evaluation of absorption, distribution, metabolism and excretion properties

The absorption, distribution, metabolism and excretion (ADME) properties of the studied compounds were calculated using the Molinspiration property programme.[40] LogP was calculated using the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors. Topological polar surface area (TPSA) was calculated based on the methodology published by Ertl *et al.* as a sum of fragment contributions.[42] Oxygen- and nitrogen-centred polar fragments were considered. Polar surface area (PSA) has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood–brain barrier penetration. The method for calculation of molecule volume developed at Molinspiration is based on group contributions. These have been obtained by fitting the sum of fragment contributions to 'real' three-dimensional (3D) volume for a training set of about 12 000, mostly drug-like molecules. 3D molecular geometries for a training set were fully optimized by the semi-empirical AM1 method.

Results

Structural identification

(*E*)-3-(3-(Thiophen-3-yl)acryloyl)coumarin (**5**): Yield 61%. Mp 146–148°C ^{1}H NMR (300 MHz, $CDCl_3$) δppm 8.57 (s, 1H, H-4), 7.87 (d, $J = 15.6$ Hz, 1H, H_β), 7.74 (d, $J = 15.7$ Hz, 1H, H_α), 7.69–7.60 (m, 3H, H-2', H-4', H-5'), 7.43 –7.29 (m, 4H, H-5, H-6, H-7, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δppm 186.5, 155.1, 148.1, 141.8, 138.4, 138.3, 134.2, 132.6, 130.7, 129.9, 126.9, 125.7, 124.9, 123.6, 118.6, 116.5. MS *m/z* (%) (ESI): 283 ($[M + 1]^+$, 8), 282 ($[M]^+$, 35), 254 (100), 226 (73), 137 (88), 109 (93). Anal. Calcd. for $C_{16}H_{10}O_3S$: C, 68.07; H, 3.57. Found: C, 68.11; H, 3.59

(*E*)-4-Hydroxy-3-(3-(thiophen-3-yl)acryloyl)coumarin (**7**): Yield 87%. Mp 192–194°C ^{1}H NMR (300 MHz, $CDCl_3$) δppm 8.25 (d, $J = 15.6$ Hz, 1H, H_β), 8.15–7.99 (m, 2H, H_α, H-5), 7.77–7.61 (m, 2H, H-2', H-5'), 7.52 (dd, $J = 5.2$, 1.3 Hz, 1H, H-4'), 7.43–7.23 (m, 3H, H-6, H-7, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δppm 192.5, 181.5, 160.1, 154.6, 140.5, 138.3, 135.9, 131.1, 127.1, 125.9, 125.6, 124.2, 122.1, 116.9, 116.2. MS *m/z* (%) (ESI): 299 ($[M + 1]^+$, 13), 298 ($[M]^+$, 68), 280 (38), 270 (46), 137 (100), 121 (99), 109 (86). Anal. Calcd. for $C_{16}H_{10}O_4S$: C, 64.42; H, 3.38. Found: C, 64.39; H, 3.32

(*E*)-7-Hydroxy-8-(3-(thiophen-3-yl)acryloyl)coumarin (**9**): Yield 47%. Mp 169–171°C. ^{1}H NMR (300 MHz, $CDCl_3$) δppm 14.10 (bs, 1H, OH), 8.16 (d, $J = 15.3$ Hz, 1H, H_β), 7.98 (d, $J = 15.1$ Hz, 1H, H_α), 7.80–7.59 (m, 2H, H-4, H-5), 7.59–7.44 (m, 2H, H-6, H-2'), 7.39 (dd, $J = 5.0$, 2.8 Hz, 1H, H-4'), 6.93 (d, $J = 8.7$ Hz, 1H, H-5'), 6.30 (d, $J = 9.6$ Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$) δppm 193.2, 167.8, 159.4, 144.3, 139.6, 138.3, 134.4, 130.4, 127.3, 125.9, 125.7, 115.8, 111.9, 111.0, 109.6. MS *m/z* (%) (ESI): 299 ($[M + 1]^+$, 11), 298 ($[M]^+$, 28), 203 (25), 189 (100), 84 (89). Anal. Calcd. for $C_{16}H_{10}O_4S$: C, 64.42; H, 3.38. Found: C, 64.44; H, 3.40.

(*E*)-7-Hydroxy-8-(3-(4-methoxyphenyl)acryloyl)coumarin (**10**): Yield: 68%. Mp 187–188°C. ^{1}H NMR (300 MHz, $CDCl_3$) δppm 14.00 (s, 1H, OH), 8.03 (d, $J = 15.4$ Hz, 1H, H_β), 7.82 (d, $J = 15.5$ Hz, 1H, H_α), 7.62–7.42 (m, 3H, H-4, H2', H6'), 7.34 (d, $J = 8.8$ Hz, 1H, H-5), 6.86–6.66 (m, 3H, H-6, H3', H-5'), 6.12 (d, $J = 9.5$ Hz, 1H, H-3), 3.70 (s, 3H, -OMe). ^{13}C NMR (75 MHz, $CDCl_3$) δppm 192.85, 167.84, 162.22, 159.47, 155.44, 146.39, 144.28, 134.17, 131.05, 127.55, 123.42, 115.71, 114.59, 111.88, 111.01, 109.70, 55.41. MS *m/z* (%) (ESI): 323 ($[M + 1]^+$, 24), 322 ($[M]^+$, 47), 321 (100), 293 (10), 134 (44). Anal. Calcd. for $C_{19}H_{14}O_5$: C, 70.80; H, 4.38. Found: C, 70.77; H, 4.35.

Binding affinity assays

The data obtained for the binding affinity assays from radioligand binding experiments for compounds **5**–**10** are

Table 1 The binding affinity of compounds **5**–**10** for the human adenosine receptor subtypes hA_1, hA_{2A} and hA_3

Compound	K_i (nM)		
	hA_1	hA_{2A}	hA_3
5	>30 000	>30 000	5 160 (3 000–8 900)
6	>10 000	>10 000	>10 000
7	>30 000	>30 000	31 500 (21 600–46 000)
8	>10 000	>10 000	>10 000
9	8 330 (7 510–9 230)	11 900 (7 790–18 300)	5 020 (3 260–7 730)
10	>10 000	>10 000	20 200 (14 600–28 100)

The binding affinity (K_i) for the human AR subtypes hA_1, hA_{2A} and hA_3 of compounds **5**–**10** was determined using radioligand competition experiments in Chinese hamster ovary (CHO) cells. Highest concentrations tested were different depending on the solubility of compounds under the respective assay conditions. Values are geometric means of three experiments and given with 95% confidence intervals in parentheses.

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Table 2 Theoretical structural properties of the coumarin–chalcone hybrids

Compound	log*P*	Molecular weight	TPSA (Å^2)	*n*-OH acceptors	*n*-OHNH donors	Volume (Å^3)
5	3.22	282.32	47.28	3	0	237.11
6	3.87	306.32	56.52	4	0	271.94
7	2.93	298.32	67.51	4	1	245.12
8	3.58	322.32	76.54	5	1	279.96
9	3.19	298.32	67.51	4	1	245.12
10	3.84	322.32	76.74	5	1	279.96

n-OH, number of hydrogen acceptors; n-OHNH, number of hydrogen bond donors; TPSA, topological polar surface area. The data was determined with Molinspiration calculation software.

summarized in Table 1. Compound **9** showed binding affinity to hA_1 and hA_{2A} ARs in the micromolar range, comparable with the prototypical antagonist theophylline.[4,36] Compounds **5** and **9** bound with affinity in the low micromolar range to hA_3AR, with compound **5** being the one that showed selectivity for this subtype.

Theoretical evaluation of ADME properties

To better correlate the drug-like properties of the coumarin–chalcone hybrid compounds, the lipophilicity, expressed as the octanol/water partition coefficient and herein called logP, as well as other theoretical calculations such as the TPSA, the number of hydrogen acceptors and the number of hydrogen bond donors were calculated using the Molinspiration property programme.[40] From the data obtained, it was noticed that all the hybrid compounds, **5**–**10**, not only had logP values compatible with those required to cross membranes but also they did not break any point of the Lipinski's rule of five. Theoretical prediction of ADME properties of all compounds is summarized in Table 2.

Discussion

With the aim of finding novel and selective AR ligands, we have synthesized chalcone- and coumarin-containing hybrids in which one or both phenyl groups of the chalcone scaffold either retains the two phenyl groups corresponding to an original chalcone structure (rings A and B, Figure 1), or is isosterically substituted by heteroaromatic rings (pyrone ring of the coumarin and/or thiophene).

The data obtained in the binding affinity assays are shown in Table 1. From all the synthesized derivatives, compounds **5**, **7** and **9** exhibit significant binding affinity in the low micromolar range for one or more AR. The common feature of compounds **5**, **7** and **9** is the presentation of a thiophenyl substituent as the key isosterical change in ring B of the chalcone. However, only compound **5** displays a noteworthy selectivity for the hA_3 AR (K_i = 5160 nM). It is interesting to note that compound **9**, when compared with compound **5**, shows similar A_3 affinity; however, it also exhibits similar affinity for A_1 and A_{2A} ARs whereas compound **5** is devoid of measurable affinity for these subtypes. The configuration with both A and B rings replaced seems to be favourable for high A_3 affinity and selectivity. Comparing compounds **5**, **7** and **9** suggests that the 4-hydroxyl group in compound **7** and the corresponding 7-hydroxyl group in compound **9**, respectively, might abolish subtype selectivity. Compound **9** presents a binding affinity for the hA_3 AR (K_i = 5020 nM) and also for hA_1 AR (K_i = 8330 nM) and hA_{2A} AR (K_i = 11 900 nM). The A_1 and A_{2A} affinities are thus comparable to the affinity of the classical naturally occurring antagonist theophylline. [36]

Comparison of the structurally related compounds **5** and **7**, reveals that the only difference between them is the absence or presence of a hydroxyl group at position 4, respectively. One can conclude that the presence of the hydroxyl group seems to cause a marked decrease in the binding affinity for the hA_3 AR (K_i = 5160 and 31 500 nM, respectively).

Comparing another pair of 8-substituted derivatives, compounds **9** and **10**, that differ in the substitution of ring B of the chalcone (compound **10** has the original phenyl ring of the chalcone while compound **9** presents a thiophenyl ring as a result of an isosteric change), one can observe that compound **9** shows affinity with no selectivity and compound **10** is less potent at all three receptor subtypes. Due to the limited affinity close to the detection limit the degree of selectivity cannot be determined. From these preliminary structure–activity relationships, one can conclude that substitution of ring B of the chalcone (Figure 1) by a thiophenyl ring (compounds **5**, **7** and **9**) favoured binding affinity towards the AR. However, hA_3 AR selectivity is attained when ring B of the chalcone is substituted by a thiophene ring and when ring A is substituted for the pyrone ring contained in the coumarin moiety (compound **5**).

On the other hand, looking at the theoretical evaluation of ADME properties (Table 2), it can be observed that no violations of Lipinski's rule of five (molecular weight, logP, number of hydrogen donors and acceptors) were found, making these hybrid compounds promising leads for drug candidates.[41] TPSA, described as being a predictive

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indicator of the drug capacity of membrane penetration, was also found to be positive.[42]

The remarkable results found for compounds **5** and **9** encourage us to continue our research looking for the optimization of these lead compounds with the aim of obtaining potent and selective chalcone-based hA_3 AR ligands.

Conclusions

Isosteric substitution of the phenyl rings of the chalcone moiety for one or two heteroaromatic rings (benzopyrone and/or thiophenyl) results in compounds (**5** and **9**) with binding affinity towards particular subtypes of ARs. A preliminary structure–activity relationship study of the synthesized derivatives allowed verification that hA_3 AR selectivity was achieved only when ring B of the chalcone was substituted for a thiophene and ring A was substituted for the pyrone ring included in the coumarin scaffold (compound **5**). Replacing ring B by a thiophenyl group but keeping a phenyl group in place of ring A, namely the benzene ring of coumarin (compound **9**), resulted in an increase of hA_1 and hA_{2A} AR affinity. As binding affinity remained similar for hA_3 AR, selectivity was, therefore, lost.

These findings encourage us to continue the efforts towards the optimization of the pharmacological profile for this type of hybrid as potent and selective ligands for the hA_3 ARs.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was partially supported by the Ministerio de Sanidad y Consumo (PS09/00501) and Xunta de Galicia (PGIDIT09CSA030203PR) (Spain) and by the Foundation for Science and Technology (FCT) project PTDC/QUI-QUI/113687/2009 (Portugal). S. Vazquez-Rodriguez thanks the Ministerio de Educación y Ciencia for the PhD FPU grant (AP2008-04263). M. J. Matos thanks Fundação para a Ciência e Tecnologia for the PhD grant (SFRH/BD/61262/2009).

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Molecules **2013**, *18*, 1394-1404; doi:10.3390/molecules18021394

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molecules

ISSN 1420-3049

www.mdpi.com/journal/molecules

Article

Synthesis and Structure-Activity Relationships of Novel Amino/Nitro Substituted 3-Arylcoumarins as Antibacterial Agents

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Received: 24 December 2012; in revised form: 15 January 2013 / Accepted: 17 January 2013 / Published: 24 January 2013

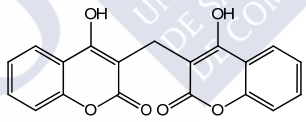
Abstract: A new series of amino/nitro-substituted 3-arylcoumarins were synthesized and their antibacterial activity against clinical isolates of *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) was evaluated. Some of these molecules exhibited antibacterial activity against *S. aureus* comparable to the standards used (oxolinic acid and ampicillin). The preliminary structure-activity relationship (SAR) study showed that the antibacterial activity against *S. aureus* depends on the position of the 3-arylcoumarin substitution pattern. With the aim of finding the structural features for the antibacterial activity and selectivity, in the present manuscript different positions of nitro, methyl, methoxy, amino and bromo substituents on the 3-arylcoumarin scaffold were reported.

Keywords: amino/nitro substituted 3-arylcoumarins; Perkin reaction; antibacterial assays; *S. aureus*; *E. coli*

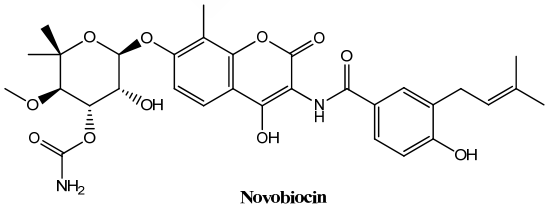
1. Introduction

Drug discovery is considered a complex and slow activity. However, with the intention of discovering new chemical entities in a new and more efficient way, medicinal chemistry researchers have developed new approaches and methods. Some of the new products are based on diversity molecules found and extracted from natural sources. Coumarins are a large family of compounds of natural or synthetic origin, associated with remarkable pharmacological activities [1,2]. They occur naturally in plants and microorganisms and approximately 1,000 coumarin derivatives have been isolated from over 800 species [2]. The fused heterocyclic framework of coumarins has been used as a prototype scaffold for the synthesis of a wide variety of analogues in order to study and improve their biological properties. Its structural variety is responsible for the important place they occupy in the realm of natural products and synthetic organic chemistry [2,3]. Some coumarins have been studied for their antimicrobial [4–7], cardioprotective [8], anticancer [9,10], antioxidative [11] and enzymatic inhibition properties [12–17]. Goth described the antibacterial properties of coumarins for the first time in 1945 when he studied the dicoumarol (Figure 1) [18]. Some other coumarin derivatives have proven to display antibacterial and antifungal activities [19–22]. A study of coumarin derivatives substituted on the pyrone ring indicated that 3-carboxyl derivatives show significant activities [23]. Different 4-substituted coumarins, such as 4-chlorocoumarins, exhibit also an interesting antimicrobial profile [24,25]. Novobiocin (Figure 1), chlorobiocin and coumermycin A_1 are important natural occurring antibiotics in which the coumarin nucleus is present in their skeleton [26].

Figure 1. Chemical structures of dicoumarol and novobiocin.



Dicoumarol



Novobiocin

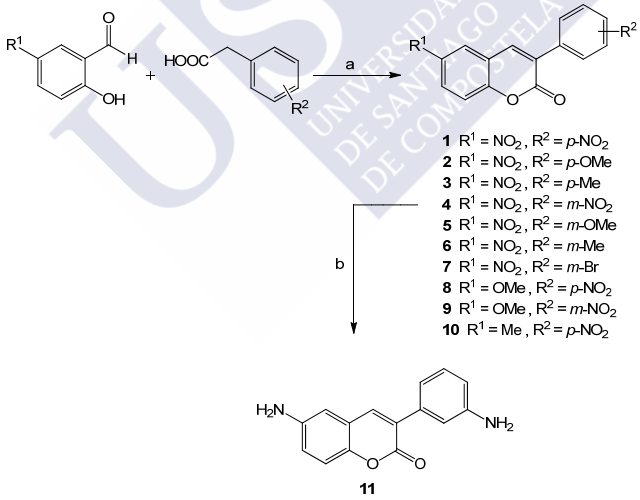
The most active of them is novobiocin, isolated from *Streptomyces niveus* and which is mainly active against Gram-positive bacteria. Although they antagonize the B subunit of the essential *E. coli* DNA gyrase supertwisting activity *in vitro* and the bacterial multiplication, they are not used in the clinic due to their relatively weak activity against Gram-negative bacteria, side effects and poor water solubility [4,26]. On the other hand, these three examples are antibiotics, which present activity against methicillin-resistant *S. aureus* (MRSA) [26]. The number of multidrug-resistant (MDR) bacteria is increasing and the Gram-positive MRSA are of particular importance. The ability of

S. aureus species to develop resistance to virtually all antibiotics is a major concern and the discovery of new antimicrobial agents is essential to combat this problem [27]. MRSA are often an important problem for severe infections in patients, especially if they are immunosuppressed, during long stays in hospitals [27]. The dramatic worldwide increase of dangerous infections by resistant and multi-resistant microbes makes, therefore, the search of new molecules and new chemical entities an important topic in medicinal chemistry [28,29]. As the ideal drug candidate has not been attained, an intensive search for new and innovative antimicrobials is still needed. Unfortunately, due to economic reasons, pharmaceutical companies have considerably decreased this effort in recent years [30].

2. Results and Discussion

Based on previous findings in the area [24,25], and in our experience with substituted 3-arylcoumarins [15,31,32], in the present work we proposed the synthesis and antibacterial evaluation of a series of different substituted amino/nitro 3-arylcoumarins (Scheme 1). With the aim of finding the structural features for the antibacterial activity and selectivity, we decided to explore the importance of the nature and position of small groups (methoxy, bromo, nitro, amino and methyl substituents) into both coumarin nucleus and 3-aryl ring (Scheme 1).

Scheme 1. Synthesis of 3-arylcoumarins (**1**–**11**).



Reagents and conditions: (a) NaH, acetic anhydride, r.t., 3 h; (b) H_2, Pd/C, ethanol, r.t., 3 h.

New derivatives **2**, **4**–**7** and **9**–**11**, as well as the already described compounds **1**, **3** and **8** [33–36], were efficiently synthesized according to the synthetic protocol outlined in Scheme 1. 3-Arylcoumarins **1**–**10** were synthesized in a dry Schlenk tube, in presence of sodium hydride and with acetic anhydride as solvent, at room temperature for three hours. The reaction mixture was purified by flash chromatography, using hexane/ethyl acetate as eluent in a proportion of 9:1. Starting from different commercially available substituted salicylaldehydes and the respectively substituted phenylacetic acids, we obtained ten derivates in good yields (60–75%). The derivative **11** was obtained

from **4**, in ethanol, with palladium/carbon as catalyst and under hydrogen atmosphere for three hours. The reaction mixture was purified by flash chromatography, using hexane/ethyl acetate as eluent, in a proportion of 9:1. We obtained the desired derivate in good yield (80%). Then, two different methodologies were employed for the antibacterial evaluation of these compounds: disk diffusion test and microdilution method. Clinical antimicrobial drugs–oxolinic acid and ampicillin–were used as positive controls. The inhibition zones for growth inhibitory effects of the new compounds and controls were measured in millimetres (Table 1, method A). The minimum inhibitory concentrations (MICs) of the new compounds and controls were measured in microgram/millilitre (Table 1, method B).

Table 1. *In vitro* antibacterial activity [a] of amino/nitro substituted 3-arylcoumarins **1**–**11** (**A**) expressed as inhibition zones (mm) [b] and (**B**) expressed as MICs (μg/mL).

Compounds	Method A (mm)		Method B (μg/mL)	
	S. aureus	***E. coli***	***S. aureus***	***E. coli***
1	19	NA	128	-
2	25	NA	64	-
3	22	NA	32	-
4	22	NA	32	-
5	25	NA	64	-
6	32	NA	8	-
7	14	NA	256	-
8	NA	NA	>512	-
9	NA	NA	>512	-
10	NA	NA	>512	-
11	18	NA	128	-
Oxolinic acid	26	31	2	<1
Ampicillin	32	23	2	8

[a] These results are average results of three experiments; [b] The compounds were used at the concentration of 100 μg/disk and the inhibition zones are stated in mm; NA = not active; diameter of inhibition zone ≤ 5 mm.

The obtained inhibition zones revealed that all the 6-nitro derivatives (compounds **1**–**7**) presented antibacterial activity against *S. aureus*, showing inhibition zones ranging between 14 and 32 mm. The amino derivative **11** presented activity against the same bacterial strain. The 3-arylcoumarins nitro substituted only in the 3-aryl ring (compounds **8**–**10**) were inactive against *S. aureus* in the *in vitro* disk diffusion test assays, independently of the relative position of this nitro group (*para* or *meta* positions). From the data, it is shown that compound **6** and ampicillin presented the same inhibition zone against *S. aureus* (32 mm) and a higher inhibition zone than the oxolinic acid (26 mm). Hence, the preliminary experimental data revealed that some of the tested compounds showed a very interesting activity profile against *S. aureus* when compared with the standards ampicillin and oxolinic acid. In addition, the different nature and position of the substituent in the coumarin scaffold seems to influence the antibacterial activity.

In order to deeply study the antibacterial activities and quantify the previously mentioned information, MICs of compounds **1**–**11** against *S. aureus* were also determined. This second methodology (method B) is used to clarify and refine the previously results obtained by method A, which could be influenced by differences in the diffusion of the compounds. This study was performed

against the same bacteria strain, by a microdilution assay with serial dilutions (from 512 to 1 μg/mL) of the synthesized and the reference compounds. MICs of compounds **1**–**11** against *E. coli* were not evaluated, taking into account the lack of inhibition zones in the previous disk diffusion test (inhibition zones ≤ 5 mm). Analysing the MICs results for *S. aureus*, it is clear that the presence of a nitro substituent at the six position of coumarin moiety (compounds **1**–**7**) is more beneficial than its presence only at *meta* or *para* positions of the 3-aryl ring (compounds **8**–**10**).

Compounds with nitro substituents either in the coumarin moiety and in the aryl group (compounds **1** and **4**) are not better than compounds where the nitro in the aryl ring is substituted for a methyl or a methoxy group (compounds **2** and **3** compared to compound **1**, and compounds **5** and **6** compared to compound **4**). However, compound **4** (MIC = 32 μg/mL), with the nitro substituent in *meta* position of the 3-aryl ring, is better than compound **1** (MIC = 128 μg/mL), with the same substituent in *para* position. The presence of a methyl group in the *meta* position is the best substitution when one nitro substituent is present at six position of the coumarin moiety (compound **6**, MIC = 8 μg/mL). In general, *meta* substitutions are more favourable than *para*, being the *meta*-methyl substituent the most active, followed by *meta*-nitro and finally *meta*-methoxy. There is one exception to this conclusion. The introduction of a bromo atom at the same position was the worst substitution of the studied 3-aryl-6-nitrocoumarins (compound **7**, MIC = 256 μg/mL). A *para*-methyl substituent in the 3-aryl ring (compound **3**, MIC = 32 μg/mL) is the best substitution in this *para* position. Therefore, the *para*-methyl substitution is better than the *para*-nitro and *para*-methoxy substitutions. Comparing the two positions in the 3-aryl ring, the substitutions by methyl groups are more favourable for the desired activity. The substitution of nitro by amino groups significantly decreases the activity of the described compounds (comparing compounds **4** and **11**).

In order to evaluate if there was a correlation between the activity and the lipophilicity, and to better understand the overall properties of the described compounds, logP (octanol/water partition coefficient) values were calculated using the Molinspiration property calculation program [37]. The results are presented in Table 2.

Table 2. LogP (octanol/water partition coefficient) values calculated for the amino/nitro substituted 3-arylcoumarins **1**–**11** [a].

Compounds	LogP
1	3,631
2	3,729
3	4,120
4	3,607
5	3,705
6	4,096
7	4,457
8	3,729
9	3,705
10	4,120
11	1,841

[a] The data was determined with Molinspiration calculation software [37].

Analysing the obtained data, it can be concluded that there are no linear correlations between the lipophilicity and the activity of the compounds. Compounds **5** and **6** are two of the best compounds against *S. aureus*, presenting intermediate, but different, logP values (3.705 and 4.096 respectively). The compounds with the lowest (compound **11**, 1.841) and the highest (compound **7**, 4.457) logP presented activity. Compound **9**, with the same logP as compound **5**, resulted to be inactive. Also, compound **10**, with the same lipophilicity profile as compound **3** (logP = 4.12), resulted to be inactive. Therefore, there are no clear correlation between activity and logP values. It is important to highlight that the calculated logP values for all the described compounds are lower than 5.

3. Experimental

3.1. Chemistry

Merck supplied the entire chemicals used in the synthesis. Melting points (mp) are uncorrected were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75.4 MHz) spectra were recorded with a Bruker AMX spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) using TMS as an internal standard. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets) and m (multiplet). Mass spectrometry was carried out with a Kratos MS-50 or a Varian MAT-711 spectrometer. Elemental analyses were performed by a Perkin-Elmer 240B microanalyzer and were within ±0.4% of calculated values in all cases. Flash Chromatography (FC) was performed on silica gel (Merck 60, 230–400 mesh); analytical TLC was performed on precoated silica gel plates (Merck 60 F254).

General Procedure for the preparation of 3-arylcoumarins **1**–**10**. In a 20 mL dry Schlenk tube, to a solution of the conveniently substituted salicylaldehyde (2.46 mmol) and the arylacetic acid (2.46 mmol), in acetic anhydride (6 mL), NaH (2.46 mmol) was added in small portions, and the reaction mixture was stirred for 3 hours, at room temperature. The obtained crude was filtered and washed with diethyl ether. The solid was then purified by flash chromatography (hexane/ethyl acetate 9:1) to give the desired coumarins **1**–**10**.

3-(4′-Methoxyphenyl)-6-nitrocoumarin (**2**). Pale yellow solid, 75% yield. Mp: 62–63 °C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.80 (s, 3H, OCH_3), 7.05 (d, 2H, H-3′, H-5′, J = 9.45 Hz), 7.62–7.69 (m, 3H, H-2′, H-6′, H-8), 8.36 (s, 1H, H-4), 8.73 (d, 2H, H-5, H-7, J = 2.93 Hz). ^{13}C-NMR ($CDCl_3$) δ (ppm): 55.9, 116.4, 119.7, 127.9, 128.4, 128.6, 129.2, 132.2, 132.5, 134.3, 139.1, 139.4, 151.5, 155.8, 161.2. ESI-MS *m/z*: 297 (M^+, 100). Anal. Calcd. for $C_{16}H_{11}NO_5$: C, 64.65; H, 3.73; Found: C, 64.67; H, 3.76.

3-(3′-Nitrophenyl)-6-nitrocoumarin (**4**). Pale yellow solid, 69% yield. Mp: 87–88 °C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.57–7.63 (m, 2H, H-8, H-6′), 7.71–7.77 (m, 1H, H-5′), 8.15 (s, 1H, H-4), 8.42-8.63 (m, 4H, H-4′, H-2, H-5, H-7). ^{13}C-NMR ($CDCl_3$) δ (ppm): 118.1, 120.2, 123.1, 123.3, 124.3. 124.5, 124.7, 129.4, 133.6, 135.1, 141.2, 144.6, 147.9, 159.2, 160.5. ESI-MS *m/z*: 312 (M^+, 100). Anal. Calcd. for $C_{15}H_8N_2O_6$: C, 50.70; H, 2.58. Found: C, 50.72; H, 2.60.

3-(3′-Methoxyphenyl)-6-nitrocoumarin (**5**). Pale yellow solid, 71% yield. Mp: 53–54 °C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.71 (s, 3H, OCH_3), 6.80 (s, 1H, H-2′), 7.17–7.22 (m, 3H, H-4′, H-5′, H-6′), 7.57 (d, 1H, H-8, J = 6.5 Hz), 7.77 (s, 1H, H-5), 8.19 (d, 1H, H-7, J = 6.5 Hz), 8.39 (s, 1H, H-4). ^{13}C-NMR ($CDCl_3$) δ (ppm): 55.4, 84.5, 112.4, 115.5, 122.0, 123.5, 125.8, 126.8, 129.5, 129.7, 136.9, 145.7, 153.2, 159.6, 168.7, 173.0. ESI-MS *m/z*: 297 (M^+, 100). Anal. Calcd. for $C_{16}H_{11}NO_5$: C, 64.65; H, 3.73. Found: C, 64.63; H, 3.70.

3-(3′-Methylphenyl)-6-nitrocoumarin (**6**). Pale yellow solid, 69% yield. Mp: 55–56 °C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.25 (s, 3H, CH_3), 6.90–7.30 (m, 5H, H-2′, H-4′, H-5′, H-6′, H-8), 7.75 (s, 1H, H-4), 8.44 (d, 1H, H-7, J = 2.8 Hz), 8.62 (d, 1H, H-5, J = 2.8 Hz). ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.5, 118.4, 123.3, 124.5, 124.6, 124.8, 125.9, 126.6, 128.3, 128.5, 132.7, 138.4, 141.1, 144.6, 159.3, 160.9. ESI-MS *m/z*: 281 (M^+, 100). Anal. Calcd. for $C_{16}H_{11}NO_4$: C, 68.32; H, 3.94. Found: C, 68.31; H, 3.92.

3-(3′-Bromophenyl)-6-nitrocoumarin (**7**). Pale yellow solid, 60% yield. Mp: 240–241 °C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.12–7.26 (m, 2H, H-4′, H-5′), 7.46–7.71 (m, 5H, H-2′, H-6′, H-5, H-7, H-8), 7.78 (s, 1H, H-4). ^{13}C-NMR ($CDCl_3$) δ (ppm): 117.9, 122.7, 123.1, 124.5, 124.6, 124.8, 127.9, 129.5, 129.7, 130.8, 134.6, 140.7, 144.6, 159.1, 161.1. ESI-MS *m/z*: 345 (M^+, 100). Anal. Calcd. for $C_{15}H_8BrNO_4$: C, 52.05; H, 2.33. Found: C, 52.01; H, 2.30.

6-Methoxy-3-(3′-nitrophenyl)coumarin (**9**). Pale yellow solid, 73% yield. Mp: 84–85 °C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.77 (s, 3H, OCH_3), 7.01–7.10 (m, 2H, H-5, H-7), 7.54–7.59 (m, 2H, H-5′, H-6′), 7.71 (d, 1H, H-8, J = 7.6 Hz), 8.06–8.09 (m, 2H, H-2′, H-4′), 8.15 (s, 1H, H-4). ^{13}C-NMR ($CDCl_3$) δ (ppm): 56.7, 115.0, 116.8, 122.3, 124.9, 128.7, 130.2, 134.2, 135.5, 137.2, 138.0, 143.3, 148.3, 156.8, 167.7, 172.7. ESI-MS *m/z*: 297 (M^+, 100). Anal. Calcd. for $C_{16}H_{11}NO_5$: C, 64.65; H, 3.73. Found: C, 64.63; H, 3.70.

6-Methyl-3-(4′-nitrophenyl)coumarin (**10**). Pale yellow solid, 75% yield. Mp: 100–101 °C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.36 (s, 3H, CH_3), 7.21 (d, 2H, H-7, H-8, J = 8.2 Hz), 7.38 (s, 1H, H-5), 7.63–7.70 (m, 2H, H-2′, H-6′), 7.99–8.20 (m, 2H, H-3′, H-5′), 8.38 (s, 1H, H-4). ^{13}C-NMR ($CDCl_3$) δ (ppm): 20.6, 122.6, 123.9, 125.1, 127.9, 130.2, 131.4, 136.4, 136.6, 136.8, 137.2, 148.1, 149.3, 170.2, 190.6. ESI-MS *m/z*: 281 (M^+, 98). Anal. Calcd. for $C_{16}H_{11}NO_4$: C, 68.32; H, 3.94. Found: C, 68.38; H, 3.99.

Procedure for the Preparation of 3-(3′-Aminophenyl)-6-Aminocoumarin (**11**)

The previously prepared 3-(3′-nitrophenyl)-6-nitrocoumarin (**4**, 2.46 mmol) was dissolved in ethanol (5 mL) and a catalytic amount of Pd/C was added to the mixture. The solution was stirred, at room temperature, under a H_2 atmosphere, for 3 h. After completion of the reaction, the mixture was filtered to eliminate the catalyst. The obtained crude was then purified by flash chromatography (hexane/ethyl acetate 9:1) to give the desired coumarin **11** as a white solid in 80% yield. Mp: 105–106 °C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.02 (s, 2H, NH_2), 6.50–6.55 (m, 1H, H-4′) 6.56 (s, 1H, H-2′), 6.69–6.73 (m, 1H, H-6′) 6.80 (dd, 2H, H-5, H-7, J = 7.8, J = 2.4 Hz), 7.30–7.38 (m, 2H, H-5′, H-8), 8.4 (s, 1H, H-4). ^{13}C-NMR ($CDCl_3$) δ (ppm): 114.9, 115.7, 116.7, 120.9, 123.6, 125.4, 127.2, 128.6,

133.7, 135.8, 136.2, 143.3, 145.5, 149.7, 161.9. ESI-MS *m/z*: 252 (M^+, 100). Anal. Calcd. for $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79. Found: C, 71.45; H, 4.82.

3.2. Biological Assays

Disk Diffusion Test: The antimicrobial activity of the compounds was assayed by the disk diffusion method, following the procedures of the Clinical and Laboratory Standards Institute (CLSI, 2006). The inoculum was prepared as a saline suspension of colonies from a 24 h Mueller Hinton Agar (MHA) (Cultimed, Barcelona, Spain) plate culture of the microorganisms, containing approximately 1×10^8 colony forming units (CFU)/mL (OD_{620} of 0,09). Colony counts on inoculum suspension were verified by the plate dilution method using MHA plates and counting the bacterial colonies produced. The entire surface of the MHA plate was inoculated by streaking with the swab containing the inoculum. Antimicrobial solution (concentration of 10000 μg/mL) was prepared using DMSO as solvent. Sterile disks of 6 mm diameter (Liofilchem, Roseto degli Abruzzi, Italy) embedded in the drug at a final concentration of 100 μg/disk were kept on agar surface. Sterile disks embedded with DMSO were used as a negative control. The plates were incubated at 37 °C for 24 h. Zones of inhibition were measured in millimeter.

Disk Minimum Inhibitory Concentrations (*MIC*s): The MICs were evaluated using the broth microdilution method, following the procedures of the Clinical and Laboratory Standards Institute (CLSI, 2006). Broth microdilution tests were performed with 96 sterile flat-bottom microtiter plates (Becton Dickinson Labware Europe, Madrid, Spain). Antimicrobials were serially diluted (512 to 1 μg/mL) in Mueller-Hinton Broth (MHB) (Cultimed) and then one hundred microliters of each dilution were deposited into each well. The inoculum was prepared by making a MHB suspension of colonies from a 24 h MHA (Cultimed) plate culture of the microorganisms, containing approximately 1×10^8 colony forming units (CFU)/mL (OD_{620} of 0,09). The inoculum was diluted 1:100 in MHB to yield 1×10^6 CFU/mL. Ten microliters of this suspension was inoculated into the each well. Colony counts on inoculum suspension were verified by the plate dilution method using MHA plates and counting the bacterial colonies produced. MHB with and without inoculum was used as growth-control and negative control, respectively. The plates were incubated at 37 °C for 24 h. The MIC was established comparing the amount of growth in the wells containing the antimicrobial agents with the amount of growth in the growth-control wells both with the unaided eye and by using a photometric device (OD_{620}).

4. Conclusions

In conclusion, in the present study it was shown that eight out of the eleven synthesized amino/nitro-substituted 3-arylcoumarins have inhibitory activity against *S. aureus*. MIC determinations proved that the tested compounds presented different profiles against *S. aureus* due to their substituents, being 3-(3′-methylphenyl)-6-nitrocoumarin (**6**) the best one. A nitro substituent at the 6-position of the coumarin moiety seems to be essential for the antibacterial activity of this kind of compounds. The introduction of an amino substituent seems to decrease the described activity. The pharmacological potential of these amino/nitro-substituted 3-arylcoumarins confirms that this scaffold can be effectively optimized into a candidate for the treatment of some bacterial infectious diseases.

Acknowledgments

Thanks are due to the Ministerio Español (PS09/00501), Xunta da Galicia (PGIDIT09CSA030203PR and PGIDT08MMA011200PR) and IN845B-2010/089 for the financial support. M.J.M. thanks to Fundação para a Ciência e Tecnologia for a PhD grant (SFRH/BD/61262/2009). S.V.R. thanks the Ministerio de Educación y Ciencia for a PhD grant (AP2008-04263).

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Sample Availability: Samples of the compounds are available from the authors.

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1140 *Medicinal Chemistry*, 2012, *8*, 1140-1145

Looking for New Targets: Simple Coumarins as Antibacterial Agents

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Abstract: The dramatic worldwide increase of dangerous infections by resistant and multi-resistant microbes makes the search of new molecules and new chemical entities an important topic in Medicinal Chemistry. As the ideal drug candidate has not been attained, an intensive search for new and innovative antimicrobials is still needed. A small series of 3-amino/nitrocoumarins without substitutions or substituted by methyl or methoxy groups at different positions were synthesized and evaluated for their antibacterial and antifungal activities against clinical isolates of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* strains. Some of these structurally simple molecules exhibited antibacterial activity. The preliminary SAR study showed that the antibacterial activity against *E. coli* and *S. aureus* was dependent on the kind and position of the substitution pattern at the coumarin moiety.

Keywords: Aminocoumarins, nitrocoumarins, coumarin synthesis, antibacterial assays, inhibition zones, minimum inhibitory concentrations, *S. aureus*, *E. coli*.

INTRODUCTION

In recent years great advances have been made in our ability to design and test new products derived from chemical synthesis. Some of these products are based on a diversity of molecules found and extracted from natural sources. Nitrogen-containing heterocyclic compounds are very interesting structural units for both the organic chemists and the biochemists [1]. Among other different heterocyclic compounds, coumarins are a large family of compounds associated with remarkable pharmacological activities. Their structural variety is responsible for the important place that they occupy in the natural products and synthetic organic chemistry realm [2,3]. Recent studies pay special attention to their anti-inflammatory, antioxidative, cardioprotective, anticancer, antimicrobial and enzymatic inhibition properties [4-15]. Novobiocin, chlorobiocin and coumermycin A_1 (Fig. **1**) are important natural occurring antibiotics, presenting the coumarin nucleus in their skeleton. This family of compounds has a 4-hydroxy-8-methyl/chlorocoumarin core etherified and aminated in the 7 and 3 positions, respectively. These compounds antagonize the B subunit of the essential *E. coli* DNA gyrase supertwisting activity *in vitro* and the bacterial multiplication [16]. They are not used in clinic because of their relatively weak activity against the Gram-negative bacteria, side effects and poor water solubility [17]. On the other hand, these three examples are antibiotics with activity against methicillin-resistant *S. aureus* (MRSA) [17]. MRSA are often an important problem for severe infections in patients, especially immunesuppressed people, during their stays in hospitals [18].

It has been proved that some other coumarin derivatives have other antibacterial and antifungal activities [19-21]. Different 4-substituted coumarins, such as 4-chlorocoumarins, exhibit an interesting antimicrobial profile [22,23]. Also, coumarin derivatives (benzopyrone moiety) could be considered structurally similar to quinolone drugs (benzopyridone moiety). These drugs have been widely used in clinic, with broad antimicrobial spectrum, including many encountered pathogens (Fig. **2**) [24]. This fact makes the study of quinolone-like compounds an interesting topic on this research area.

3-Nitrocouamrins and related compounds have been chosen as the subject for a QSAR study [25]. For those studies literature data containing results of microbiological activity screening of 33 coumarin derivatives against *C. albicans* and *S. aureus* have been selected. There are no more compounds in the literature related to this scaffold and these activities. Therefore, it seems to be a good area to explore.

The dramatic worldwide increase of dangerous infections by resistant and multi-resistant microbes makes, therefore, the search of new molecules and new chemical entities an important topic in Medicinal Chemistry [26,27]. As the ideal drug candidate has not been attained, an intensive search for new and innovative antimicrobials is still needed. Unfortunately, due to economic reasons, pharmaceutical companies have dropped considerably this effort in recent years [28].

RESULTS AND DISCUSSION

Based on previous findings, in the chemical structure of the novobiocin and their derivatives (Fig. **1**) and in our experience with 3-substituted coumarins [22,23,29,30], in

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1875-6638/12 $58.00+.00

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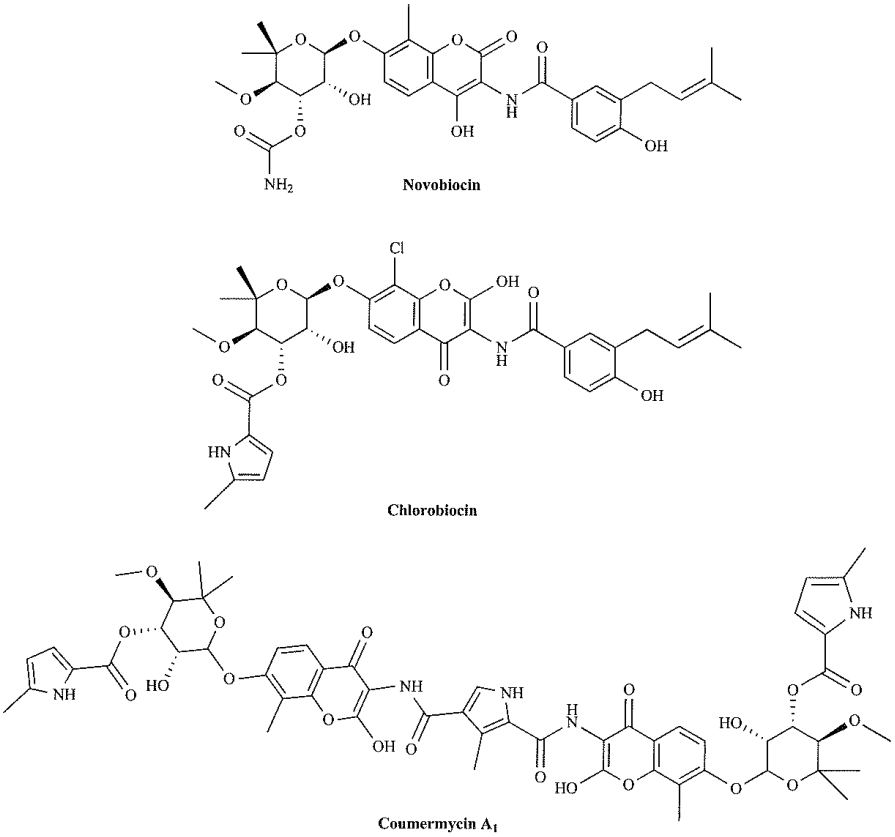


Fig. (1). Chemical structure of some coumarin derivatives with potent antimicrobial activity.

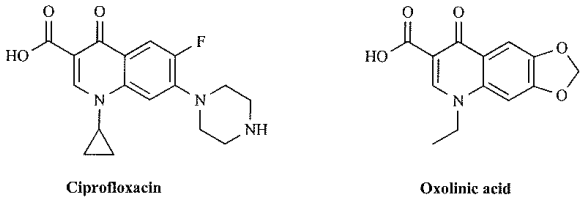
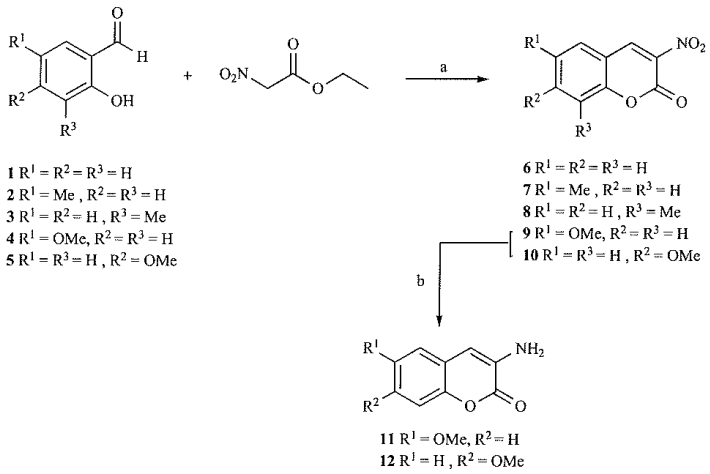


Fig. (2). Chemical structure of two quinolone derivatives.

rience with 3-substituted coumarins [22,23,29,30], in the present work we have proposed the synthesis and antimicrobial evaluation of different substituted 3-aminocoumarins (Scheme **1**). With the aim of mimetizing the novobiocin structure we have proposed the inclusion of a simple ether (methoxy group) in the 3-aminocoumarin moiety. Derivatives **11** and **12** were synthesized and evaluated. These two derivatives proved to have no activity against the tested bacteria. Therefore, we have centralized our work in the 3-nitrocoumarin scaffold. It was described that some nitrogen-containing moieties could increase significantly the antimicrobial efficiency and broaden their antimicrobial spectrum [20]. With the aim of finding out structural features for the antimicrobial activity and selectivity, we decided to explore also the importance of the nature and position of small groups (methoxy and methyl substituents) at 6, 7 or 8 position of the phenyl ring of the 3-nitrocoumarin moiety.

The coumarin derivatives **6-12** [31,32] were efficiently synthesized according to the synthetic protocol outlined in Scheme **1**. Starting from different commercially available salicylaldehydes and the ethyl nitroacetate, we obtained 3-



Scheme 1. *Reagents and conditions:* (a) acetic anhydride, NaH, r.t., 3 h, yields: 75-90 %; (b) H_2, ethanol, r.t., 5 h, yields: 95 %.

Table 1. ***In vitro*** **Antibacterial Activity[a] of 3-amino/nitro-coumarins 6-12 (A) Expressed as Inhibition Zones (mm).[b] (B) Expressed as MIC (μg/mL)**

Compounds	Method A (mm)		Method B (μg/mL)	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
6	16	12	32	64
7	15	12	16	64
8	15	11	32	64
9	13	NA	32	128
10	11	NA	32	256
11	NA	NA	> 256	> 256
12	NA	NA	> 256	> 256
Oxolinic acid	26	31	2	< 1
Ampicillin	32	23	2	8

[a] These results are average results of three experiments.
[b] The compounds were used at the concentration of 100 μg/disk and the inhibition zones are stated in mm.
NA = not active; diameter of inhibition zone ≤ 5 mm.

nitrocoumarins **6-10** in good yields (75-90 %). They were synthesized in a dry Schlenk tube, with acetic anhydride as solvent, in the presence of sodium hydride at room temperature for three hours. The reaction mixture was purified by flash chromatography, using hexane/ethyl acetate as eluent, in a 9:1 proportion. The 3-aminocoumarins **11** and **12** were prepared from the previously synthesized 3-nitrocoumarin (compounds **9** and **10**, respectively), in ethanol and using Pd/C as catalyst under H_2 atmosphere. The obtained products were purified by column chromatography (hexane/ethyl acetate 9:1) to give the desired aminocoumarins in a 95% yield.

Two different methodologies were employed in the antimicrobial studies: disk diffusion test and microdilution method. Clinical antimicrobial drugs –oxolinic acid (quinolone antibiotic, Fig. **2**) and ampicillin (*β*-lactamic antibiotic)– were used as positive controls. In the first case, the antibacterial and antifungal activities of the compounds **6-12** were evaluated *in vitro* by the measurement (in mm) of the inhibition zone of a Gram-positive bacterium (*S. aureus*), a Gram-negative bacterium (*E. coli*) and a yeast (*C. albicans*), using the disk diffusion test. The antibacterial and antifungal activities were tested at concentrations of 100μg/disk, in DMSO. The bacterial/fungal cultures were inoculated and incubated according to the described protocol. The inhibition zones values for growth inhibitory effects of the new compounds and controls were measured in millimetres (Table **1** – method A).

The obtained results revealed that the studied 3-aminocoumarins (compounds **11** and **12**) are inactive against all the tested strains in the *in vitro* assays, while the 3-nitrocoumarins (compounds **6-10**) can, in some cases, efficiently inhibit their growth. Compounds **7** and **8**, with a methyl group at 6 and 8 positions respectively, and compound **6**, without any substituents in the phenyl ring, are all active against both tested bacteria. Compounds **9** and **10**, with a methoxy group at 6 and 7 positions respectively, did not show any antimicrobial activity against *E. coli* by the first methodology. Also, they resulted to be less active against the *S. aureus* than the other nitro derivative compounds. In spite of the interesting results against the two tested bacteria, the entire series is inactive against the yeast *C. albicans*. The experimental data revealed that some of the tested compounds showed interesting activity against the two tested bacteria when compared with the standards ampicillin and oxolinic acid and that the different nature and position of the substituent in the coumarin scaffold changes the selectivity for both bacteria.

In order to deeply study the antibacterial activities and quantify the previously mentioned information, minimum inhibitory concentrations (MICs) of **6-12** against *S. aureus* and *E. coli* were also determined. This study was done against the same bacteria strains, by the microdilution method with serial dilutions (from 512 to 1 μg/mL) of the synthesized and the reference compounds. The MICs are summarized in table 1 – method B. MICs against *C. albicans* of compounds **6-12** were not evaluated taking into account the lack of inhibition zones in the previous disk diffusion test. Almost all the results are congruent with the ones found for the first methodology. The small differences between the two methodologies could be due to the experimental conditions. Some compounds have problems in their diffusion in agar. So, the first one is only a good preliminary orientation for the studies. Analyzing the MICs results, all the five nitro substituted studied compounds presented activity against the two tested bacteria. From the data we can infer that the studied compounds have higher antibacterial activity against *S. aureus* (16-32 μg/mL) than against *E. coli* (64-256 μg/mL), as we previously verified. Compounds **9** and **10**, with a methoxy substituent in the phenyl ring of the coumarin, were not selective against *S. aureus*, as it seems in the first study. However they resulted to be less active against *E. coli* (128 and 256 μg/mL, respectively) than compounds **6-8** (64 μg/mL), with a methyl substituent or without any substitution. The nature of the substituent seems to be important for the activity against *E. coli*. With this methodology, compound **7**, with a methyl group at position 6, proved to have half of the minimum inhibitory concentration against *S. aureus* than the other methyl substituted compound **8** and the unsubstituted compound **6**. The position of the methyl substituent seems to be relevant against *S. aureus*.

CONCLUSION

In conclusion, in the present study it was shown that all the synthesized 3-nitrocoumarins have inhibitory activity against *S. aureus* and *E. coli*. MICs determination proved that the tested compounds, against both bacteria species, presented different profiles due to the substituents. The effect against *E. coli* seems to depend on the type of substituent in the phenyl ring of the coumarin moiety (methyl > methoxy). The effect against *S. aureus* seems to depend on the position of the methyl substitution (position 6 > position 8). The pharmacological potential of these 3-nitrocoumarins confirms that this lead could be effectively optimized into a drug candidate for the treatment of some bacterial diseases. These findings have encouraged us to continue the efforts towards the optimization of the antimicrobial profile of these coumarins.

EXPERIMENTAL

General Methods

Melting points (mp) are uncorrected and were determined with a Reichert Kofler thermopan or in capillary tubes in a Buchi 510 apparatus. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.4 MHz) spectra were recorder with a Bruker AMX spectrometer. Chemical shifts (d) are expressed in parts per million (ppm) using TMS as an internal standard. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets) and m (multiplet). Mass spectrometry was carried out with a Kratos MS-50 or a Varian MAT-711 spectrometer. Elemental analyses were performed by a Perkin-Elmer 240B microanalyzer and were within ±0.4% of calculated values in all cases. Flash Chromatography (FC) was performed on silica gel (Merck 60, 230-400 mesh); analytical TLC was performed on precoated silica gel plates (Merck 60 F254).

General Methodology to Prepare 3-nitrocoumarins

In a 20 mL dry Schlenk tube, to a solution of the conveniently substituted salicylaldehyde (2.46 mmol) and ethyl nitroacetate (2.46 mmol), in acetic anhydride, NaH (2.46 mmol) was added, in small portions, and the reaction mixture was stirred for 3 hours, at room temperature. The obtained crude was filtered and washed with diethyl ether. The solid was then purified by flash chromatography (hexane/ethyl acetate 9:1) to give the desired coumarins (**6–10**).

8-Methyl-3-nitrocoumarin (**8**). It was obtained a white solid with a yield of 78 %. Mp: 147-148 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.49 (s, 3H, CH_3), 7.35 (t, 1H, H-6, J=7.6 Hz), 7.58 (dd, 1H, H-7, J=7.6, 1.6 Hz), 7.62 (dd, 1H, H-5, J=7.6, 1.5 Hz), 8.73 (s, 1H, H-4). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ (ppm): 15.32, 115.90, 125.49, 126.89, 128.33, 135.25, 137.44, 142.74, 153.21, 156.23. ESI-MS m/z: 206 (12), 205 (M^+, 100), 147 (13), 103 (54), 102 (12), 77 (30), 51 (14). Anal. Calcd. for $C_{10}H_7NO_4$: C, 58.54; H, 3.44. Found: C, 58.52; H, 5.39.

General Methodology to Prepare 3-aminocoumarins

The previously prepared 3-nitrocoumarin (2.46 mmol) was dissolved in ethanol and a calatytic amount of Pd/C was added to the mixture. The solution was stirred, at room temperature, under H_2 atmosphere, for 5 hours. After the completion of the reaction, the mixture was filtered to eliminate the catalyst. The obtained crude was then purified by flash chromatography (hexane/ethyl acetate 9:1) to give the desired coumarins (**11** and **12**).

Biological Assays

Disk Diffusion Test: The antimicrobial activity of the compounds was assayed by the disk diffusion method, following the procedures of the Clinical and Laboratory Standards Institute (CLSI, 2006). The inoculum was prepared by making a saline suspension of colonies from a 24 h Mueller Hinton Agar (MHA) (Cultimed, Spain) plate culture of the microorganisms, containing approximately $1x10^8$ colony forming units (CFU)/mL (OD_{620} of 0,09). Colony counts on inoculum suspension were verified by the plate dilution method using MHA plates and counting the bacterial colonies produced. The entire surface of the MHA plate was inoculated by streaking with the swab containing the inoculum. Antimicrobial solution (concentration of 10000 μg/mL) was prepared by using DMSO as solvent. Sterile disks of 6 mm diameter (Liofilchem, Italy) embedded in the drug at a final concentration of 100 μg/disk were kept on agar surface. Sterile disks embedded with DMSO were used as a negative control. The plates were incubated at 37 °C for 24 h. Zones of inhibition were measured in millimeter.

Disk Minimum Inhibitory Concentrations (MICs): The MICs were evaluated using the broth microdilution method, following the procedures of the Clinical and Laboratory Standards Institute (CLSI, 2006). Broth microdilution tests were performed with 96 sterile flat-bottom microtiter plates (Becton Dickinson Labware Europe). Antimicrobials were serially diluted (512 to 1 μg/mL) in Mueller-Hinton Broth (MHB) (Cultimed, Spain) and then one hundred microliters of each dilution were deposited into each well. The inoculum was prepared by making a MHB suspension of colonies from a 24 h MHA (Cultimed, Spain) plate culture of the microorganisms, containing approximately $1x10^8$ colony forming units (CFU)/mL (OD_{620} of 0,09). The inoculum was diluted 1:100 in MHB to yield $1x10^6$ CFU/mL. Ten microliters of this suspension was inoculated into the each well. Colony counts on inoculum suspension were verified by the plate dilution method using MHA plates and counting the bacterial colonies produced. MHB with and without inoculum was used as growth-control and negative control, respectively. The plates were incubated at 37 °C for 24 h. The MIC was established comparing the amount of growth in the wells containing the antimicrobial agents with the amount of growth in the growth-control wells both with the unaided eye and by using a photometric device (OD_{620}).

CONFLICT OF INTEREST

The authors(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Thanks to the Spanish Ministry (PS09/00501), Xunta da Galicia (PGIDIT09CSA030203PR and PGIDT08MMA0112 00PR) and IN845B-2010/089 for the financial support. M.J.M. thanks to FCT for a PhD grant (SFRH/BD/61262/ 2009). S.V.R. thanks to Ministerio de Educación y Ciencia for a PhD grant (AP2008-04263). There are no competing interests.

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Received: July 13, 2011 Revised: May 06, 2012 Accepted: May 07, 2012

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Journal:	*Journal of Agricultural and Food Chemistry*
Manuscript ID:	Draft
Manuscript Type:	Article
Date Submitted by the Author:	n/a
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Synthesis and Antibacterial Evaluation of Coumarin-Chalcone Hybrids as New and Potent Agents for Tenacibaculosis

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Abstract

With the aim of finding the structural features for the antibacterial activity and selectivity of new antibacterial agents, a series of coumarin-chalcone hybrid compounds bearing amino, methoxy, ethoxy and bromo substituents at different positions were designed and synthesized. Their antibacterial activity was evaluated against three important types of human bacteria strains (*E. coli*, *S. aureus* and *P. aeruginosa)* as well as against three different strains of the *Tenacibaculum maritimum* (*T. maritimum*) bacteria, responsible for tenacibaculosis in marine fish, an important disease that causes great economical loss in the aquaculture industry. All the amino derivatives **5-12** presented high activity against *T. maritimum* and no activity against any of the three pathogenic human bacteria strains, being therefore good candidates for aquaculture treatments.

Keywords: Coumarin, chalcone, antibacterial, *Tenacibaculum maritimum*, Aquiculture

Introduction

Tenacibaculum maritimum (*T. maritimum*), formerly known as *Flexibacter maritimus* [1], is a Gram-negative and filamentous bacterium, responsible for tenacibaculosis in marine fish. The pathology of the disease caused by this marine organism has mainly been associated with characteristic gross lesions on the body surface of fish such as ulcers, necrosis, eroded mouth, frayed fins and tail rots, and sometimes necrosis on the gills and eyes [2-6].

Even though tenacibaculosis has long been recognized as a disease of fish in Japan, the infection was not considered of economical importance until serious mortalities affecting Dover sole *Solea solea* in Scotland were reported [2,7]. To date, tenacibaculosis is one of the most threatening bacterial infections limiting the culture of many species of commercial value in distinct geographical areas of the world [8].

Despite the significance of *T. maritimum* in the aquaculture industry, relatively little is known about the pathogenicity of this bacterium. *In vitro* studies on the susceptibility of *T. maritimum* to various chemotherapeutic agents indicate that bacterial strains isolated from different host species and geographical regions exhibit a similar pattern, with respect to susceptibility to nitrofurans, penicillins, erythromycin, tetracyclines, chloramphenicol, trimethoprim, potentiated sulfonamides and fluoroquinolones, and resistance to colistin, kanamicyn, neomicyn and the quinolones, oxolinic acid and flumequine [9].

Among the drugs used in the past few years in turbot and sole cultures (tetracycline, enrofloxacin, flumequine and potentiated sulfonamides), enrofloxacin proved to be the most useful compound for controlling *T. maritimum* outbreaks, although the rapid appearance of resistant strains has already been described (Figure 1) [10]. However, during the last few years, enrofloxacin, a member of the 4-quinolone group with broad

spectrum activity, has received growing attention for its potential in fish therapy against infections caused by bacterial pathogens [11,12].

Among other different heterocyclic compounds, coumarins are a large and well-known family of compounds of natural and synthetic origin with great structural variety responsible for a wide range of pharmacological activities [13,14]. Recent studies pay special attention to their anti-inflammatory [15,16], antioxidative [17,18], cardioprotective [19], antitumor [20,21], antimicrobial [22-24] and enzymatic inhibition [25,26] properties. As well as coumarins, there are important natural occurring antibiotics containing the benzopyrone moiety, such as novobiocin, chlorobiocin and coumermycin A_1 (Figure 2). Special attention can be paid to aminocoumarins, which represents a class of antibiotics that act by the inhibition of the DNA gyrase enzyme involved in the cell division in bacteria by binding tightly to its B subunit, thereby inhibiting this essential enzyme [27].

(Figure 1)

Coumarin derivatives could be considered as structurally similar quinolone drugs (benzopyridone moiety), widely used in clinic and with a broad antimicrobial spectrum, including many encountered pathogens (Figure 2) [28]. This fact makes the study of quinolone-like compounds an interesting topic on this research area.

On the other hand, chalcones are prominent secondary metabolites precursors of flavonoids and isoflavonoids in plants. Chemically, they consist of two aromatic rings linked by a three-carbon α,β-unsatured systems that are know to exhibit an impressive array of biological properties [29]. Among them, it is remarkable to mention their

antibacterial [30,31], antimalarial [32,33], antifungal [34], antiviral [35,36] and anti-inflammatory [37,38] properties.

(Figure 2)

Taking into account the economical importance of the marine fish diseases in the aquaculture industry and the increasing need of finding new drug candidates for the treatment of tenacibaculosis, we have designed and synthesized novel coumarin-chalcone hybrid compounds and tested their *in vitro* antibacterial activity (Figure 3). We have selected three human pathogenic bacteria strains (*E. coli*, *S. aureus* and *P. aeruginosa*) and three different *T. maritimum strains* (TM LC07 10.1.2, TM Chile and TM LL01 8.3.8) with the aim of finding new drug candidate, active against this marine pathogen but not active with pathogenic human bacteria in order to avoid drug resistance problems in humans. Based on previous findings, in aminocoumarin derivatives, in the quinolone-like antibacterial drugs and in our experience with 3-substituted coumarins [39-41] we have designed and synthesized coumarin-chalcone hybrid compounds and tested their antibacterial against the previous mentioned strains.

(Figure 3)

Materials and methods

Melting points (mp) are uncorrected and were determined with a Reichert Kofler thermopan or in capillary tubes in a Buchi 510 apparatus. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.4 MHz) spectra were recorder with a Bruker AMX spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) using TMS as an internal standard. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets) and m

(multiplet). Mass spectrometry was carried out with a Kratos MS-50 or a Varian MAT-711 spectrometer. Elemental analyses were performed by a Perkin-Elmer 240B microanalyzer and were within ±0.4% of calculated values in all cases. Flash Chromatography (FC) was performed on silica gel (Merck 60, 230-400 mesh); analytical TLC was performed on precoated silica gel plates (Merck 60 F254).

General procedure for the synthesis of 3-benzoylcoumarins **1-4**:

To a solution of the appropriate β-ketoester (1 mmol) and the corresponding salicylaldehyde (1 mmol) in ethanol (3 mL) was added piperidine in catalytic amount. The mixture was refluxed for 2–5 h and after completion (followed by TLC), the reaction was cooled and the precipitated was filtered and washed with cold ethanol and ether to afford the desired compound. Compounds were further recrystallized from MeOH/CH_2Cl_2.

3-Benzoyl-8-ethoxycoumarin (**3**): Yield (60%), mp 141-143 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.89 (s, 1H, H-4), 7.71 (d, J = 8.3 Hz, 2H, H-2', H-6'), 7.44 (t, J = 8.1 Hz, 1H, H-4'), 7.36-7.23 (m, 2H, H-3', H-5'), 7.14-6.91 (m, 3H, H-5, H-6, H-7), 4.05 (q, J = 7.0 Hz, 2H, CH_2), 1.35 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 192.15, 158.47, 147.00, 146.06, 145.06, 136.57, 134.16, 130.00, 128.93, 127.61, 125.18, 120.68, 119.27, 116.82, 65.50, 15.12. MS EI *m/z* (%): 295 ([M+1]$^+$, 28), 294 ([M]$^+$, 78), 266 (43), 237 (35), 105 (100), 77 (77). Anal. Calcd. for $C_{18}H_{14}O_4$: C, 73.46; H, 4.79; Found: C, 73.06; H, 4.45.

6-Bromo-3-benzoylcoumarin (**4**): Yield (58%), mp 173-175 ºC; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.01 (s, 1H, H-4), 7.91 (d, J = 7.5 Hz, 2H, H-2', H-6'), 7.81-7.72 (m, 2H, H-3', H-5'), 7.66 (t, J = 7.3 Hz, 1H, H-4'), 7.55 (d, J = 8.3 Hz, 2H, H-7, H-8), 7.33 (d, J = 9.3 Hz, 1H, H-5). ^{13}C NMR (63 MHz, $CDCl_3$) δ 191.48, 158.09, 153.91, 144.10,

136.64, 136.27, 134.43, 131.64, 129.98, 129.07, 128.51, 121.67, 120.03, 119.04, 117.91, 108.26. MS EI *m/z* (%): 331 ($[M+2]^+$, 26), 330 ($[M+1]^+$, 6), 329 ($[M]^+$, 27), 105 (100), 77 (87). Anal. Calcd. for $C_{17}H_{11}BrO_4$: C, 56.85; H, 3.09; Found: C, 56.51; H, 3.02.

General procedure for the synthesis of amino 3-benzoylcoumarins **5-12**: Following the same procedure described for the synthesis of derivatives **1-4**, the nitro 3-benzoylcoumarin derivatives were prepared and were used without further purification. To a solution of the corresponding nitro 3-benzoylcoumarin (1 mmol) in pure ethanol (5 mL) was treated with $SnCl_2.2H_2O$ and the resulting mixture was left under reflux for 3-7h. After completion of reaction monitored by TLC, water was poured over the reaction mixture (15 mL), and the solution was further neutralized with a 5% $NaHCO_3$ solution. Extraction was carried out with EtOAc (3x 20 mL), the combined organic layers were dried over anhydride Na_2SO_4 and solvent was completely removed. The crude was purified by flash chromatography (7:3 Hexane/EtOAc) to afford the desired amino derivatives.

3-(4'-aminobenzoyl)coumarin (**5**): Yield (67%), mp 201-203 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.16 (s, 1H, H-4), 7.78 (d, J = 7.5 Hz, 1H, H-5), 7.71-7.56 (m, 3H, H-7, H2', H-6'), 7.49-7.32 (m, 2H, H-6, H-8), 6.59 (d, J = 8.2 Hz, 2H, H-3', H-5'), 6.27 (bs, 2H, -NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 188.39, 158.41, 154.93, 153.83, 142.73, 132.95, 132.65, 129.39, 127.91, 124.93, 123.27, 118.51, 116.39, 112.79. MS EI *m/z* (%): 266 ($[M+1]^+$, 40), 265 ($[M]^+$, 89), 237 (18), 236 (17), 121 (31), 120 (100), 89 (16), 65 (63), 63 (18). Anal. Calcd. for $C_{16}H_{11}NO_3$ C, 72.45; H, 4.18; Found: C, 72.27; H, 4.02.

3-(4'-aminobenzoyl)-8-methoxycoumarin (**6**): Yield (82%), mp 165-167 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.16 (s, 1H, H-4), 7.62 (dd, J = 8.8, 2.3 Hz, 2H, H-2', H-6'), 7.43-7.19 (m, 3H, H-5, H-6, H-7), 6.56 (d, J = 8.8 Hz, 2H, H-3', H-5'), 6.33 (bs, 2H, NH_2), 3.92 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 188.38, 154.93, 146.55, 142.94, 132.63, 128.10, 124.84, 123.25, 120.44, 119.07, 115.07, 112.76, 56.30. MS EI *m/z* (%): 296 ($[M+1]^+$, 23), 295 ($[M]^+$, 69), 121 (15), 120 (100), 92 (28), 65 (17). Anal. Calcd. for $C_{17}H_{13}NO_4$ C, 69.15; H, 4.44; Found: C, 69.12; H, 4.43.

3-(4'-aminobenzoyl)-8-ethoxycoumarin (**7**): Yield (62%), mp 221-222 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.16 (s, 1H, H-4), 7.61 (d, J = 8.7 Hz, 2H, H-2', H-6'), 7.42-7.22 (m, 3H, H-5, H-6, H-7), 6.56 (d, J = 8.7 Hz, 2H, H-3', H-5'), 6.33 (bs, 2H, NH_2), 4.18 (q, J = 6.9 Hz, 2H, CH_2), 1.40 (t, J = 6.9 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 188.38, 158.22, 154.92, 145.78, 143.02, 132.62, 128.11, 124.84, 123.26, 120.39, 119.16, 115.92, 112.74, 64.58, 14.76. MS EI *m/z* (%): 310 ($[M+1]^+$, 24), 309 ($[M]^+$, 67), 281 (12), 280 (11), 121 (18), 120 (100), 92 (29), 65 (20). Anal. Calcd. for $C_{18}H_{15}NO_4$: C, 69.89; H, 4.89; Found: C, 69.68; H, 4.75.

3-(4'-aminobenzoyl)-6-bromocoumarin (**8**): Yield (42%), mp 243-245 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.11 (s, 1H, H-4), 8.03 (d, J = 2.5 Hz, 1H, H-5), 7.81 (dd, J = 8.9, 2.5 Hz, 1H, H-7), 7.63 (d, J = 8.6 Hz, 2H, H-2', H-6'), 7.43 (d, J = 8.9 Hz, 1H, H-8), 6.57 (d, J = 8.3 Hz, 2H, H-3', H-5'), 6.32 (bs, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 187.93, 157.92, 155.07, 152.92, 141.29, 135.13, 132.73, 131.28, 128.88, 123.03, 120.48, 118.69, 116.35, 112.76. MS EI *m/z* (%): 345 ($[M+2]^+$, 44), 343 ($[M]^+$, 44), 121 (14), 120 (100), 92 (28), 65 (20). Anal. Calcd. for $C_{16}H_{10}BrNO_3$: C, 55.84; H, 2.93; Found: C, 55.87; H, 2.88.

3-(3'-aminobenzoyl)coumarin (**9**): Yield (94%), mp 147-148 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.00 (s, 1H, H-4), 7.72-7.50 (m, 2H, H-5, H-7), 7.45-7.27 (m, 2H, H-6,

175 H-8), 7.27-7.12 (m, 3H, H-2', H-5', H-6'), 6.97-6.83 (m, 1H, H-4'), 3.85 (bs, 2H, NH_2).
176 ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 191.64, 158.23, 154.50, 146.77, 144.75, 137.05,
177 133.37, 129.32, 129.01, 126.97, 124.81, 120.35, 120.06, 117.98, 116.77, 114.97. MS EI
178 *m/z* (%): 266 ($[M+1]^+$, 30), 265 ($[M]^+$, 100), 236 (28), 172 (22), 120 (84), 89 (15), 65
179 (44). Anal. Calcd. for $C_{16}H_{11}NO_3$: C, 72.45; H, 4.18; Found: C, 72.31; H, 4.12.
180 *3-(3'-aminobenzoyl)-8-methoxycoumarin* (**10**): Yield (74%), mp 138-141 ºC; ^1H NMR
181 (300 MHz, DMSO-d_6) δ ppm 8.30 (s, 1H, H-4), 7.47-7.27 (m, 3H, 3xAr-H), 7.16 (t, J =
182 7.8 Hz, 1H, Ar-H), 7.12-6.96 (m, 2H, 2xAr-H), 6.85 (dd, J = 7.8, 2.3 Hz, 1H, Ar-H),
183 5.38 (bs, 2H, NH_2), 3.93 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 192.13,
184 157.77, 149.29, 146.57, 144.45, 143.33, 136.69, 129.40, 127.12, 125.04, 120.75,
185 119.43, 118.81, 117.22, 115.60, 114.21, 56.34. MS EI *m/z* (%): 296 ($[M+1]^+$, 31), 295
186 ($[M]^+$, 100), 266 (16), 120 (82). 92 (58), 65 (26). Anal. Calcd. for $C_{17}H_{13}NO_4$: C, 69.15;
187 H, 4.44; Found: C, 69.01; H, 4.45.
188 *3-(3'-aminobenzoyl)-8-ethoxycoumarin* (**11**): Yield (69%), mp 229-230 ºC; ^1H NMR
189 (300 MHz, DMSO-d_6) δ ppm 8.26 (s, 1H, H-4), 7.32-7.18 (m, 3H, 3xAr-H), 7.09 (t, J =
190 7.8 Hz, 1H, Ar-H), 7.01-6.84 (m, 2H, 2x Ar-H), 6.75 (dd, J = 7.8, 2.3 Hz, 1H, Ar-H),
191 5.32 (bs, 2H, NH_2), 4.05 (q, J = 7.0 Hz, 2H, CH_2), 1.35 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C
192 NMR (75 MHz, DMSO-d_6) δ ppm 192.15, 158.26, 148.52, 146.27, 144.45, 143.33,
193 136.59, 129.44, 127.77, 125.14, 120.67, 119.33, 118.81, 117.22, 115.60, 114.21, 65.50,
194 15.12. MS EI *m/z* (%): 310 ($[M+1]^+$, 20), 309 ($[M]^+$, 62), 120 (100), 92 (28), 65 (18).
195 Anal. Calcd. for $C_{18}H_{15}NO_4$: C, 69.89; H, 4.89; Found: C, 69.75; H, 4.81.
196 *3-(3'-aminobenzoyl)-6-bromoycoumarin* (**12**): Yield (61%), mp 205-207 ºC; ^1H NMR
197 (300 MHz, DMSO-d_6) δ ppm 8.26 (s, 1H, H-4), 8.07 (d, J = 2.9 Hz, 1H, H-5), 7.85 (dd,
198 J = 8.9, 2.9 Hz, 1H, H-7), 7.45 (d, J = 8.9 Hz, 1H, H-8), 7.25-6.96 (m, 3H, H-2', H-5',
199 H-6'), 6.86 (d, J = 7.3 Hz, 1H, H-4'), 5.38 (s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6)

δ ppm 191.74, 157.59, 153.11, 149.34, 142.86, 136.51, 135.73, 131.65, 129.44, 127.85, 120.19, 119.63, 118.79, 117.51, 116.54, 114.05. MS EI *m/z* (%): 345 ($[M+2]^+$, 70), 343 ($[M]^+$, 70), 120 (100), 92 (85), 65 (52). Anal. Calcd. for $C_{16}H_{10}BrNO_3$: C, 55.84; H, 2.93; Found: C, 55.77; H, 2.89.

Antibacterial activity

The antimicrobial activity of the compounds was assayed by the disk diffusion method, following the procedures of the Clinical and Laboratory Standards Institute (CLSI, 2006). The assay was carried out with the reference strains of *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923 and *Pseudomonas aeruginosa* ATCC27853 and three different strains of the Gram-negative marine bacterium *Tenacibaculum maritimum* (TM LC07 10.1.1, TM Chile and TM LL01 8.3.8). The inoculum was prepared in sterile saline from a 24 h Mueller Hinton Agar (MHA) (Cultimed, Spain) plate culture of reference strains of *E. coli*, *P. aeruginosa* y *S. aureus* and from a 48 h *Flexibacter maritimum* medium (FMM) [42] plate culture of *T. maritimum* strains, containing approximately $1x10^8$ colony forming units (CFU)/mL (OD_{620} of 0,09). Colony counts on inoculum suspension were verified by the plate dilution method using the appropriate solid medium and counting the bacterial colonies produced. Antimicrobial solution (concentration of 10000 µg/mL) was prepared by using DMSO as solvent. Sterile disks of 6 mm diameter (Liofilchem, Italy) embedded in the drug at a final concentration of 100 µg/disk were kept on agar surface. Sterile disks embedded with DMSO were used as a negative control. The plates were incubated at 37 ºC for 24 h (reference strains) and at 25 ºC for 48 h *T. maritimum* isolates. Zones of inhibition were measured in millimeter.

225

226

227 **Results and discussion**

228 **Chemistry**

229 The synthesis of the studied compounds **1-12** was performed following the synthetic
230 route outlined in Scheme 1.

231

232 (Scheme 1)

233

234 Compounds **1-4** were efficiently synthesized with 58-70% yield via Knoevenagel
235 reaction treating the appropriate salicylaldehyde and the corresponding ethyl substituted
236 benzoylacetate with piperidine in ethanol at reflux for 2–5 h. For the preparation of the
237 amino substituted derivatives **5-12**, the same strategy was employed to achieve the nitro
238 substituted 3-benzoylcoumarins precursors, which were treated without further
239 purification with $SnCl_2.2H_2O$ in ethanol at reflux for 3-7 h to achieve the final amino
240 substituted compounds **5-12** in 42-94% overall yield after purification by flash
241 chromatography in 7:3 Hexane/AcOEt.

242

243 **Antibacterial activity**

244 In order to test the antibacterial activity of the target compounds, the disk diffusion test
245 was employed. The antimicrobial drugs, oxolinic acid and enrofloxacin (quinolone
246 antibiotic, Figure 1) and ampicillin (*β*-lactamic antibiotic), were used as positive
247 controls. The antibacterial activity of compounds **1-12** was evaluated *in vitro* by
248 measuring the inhibition zone (in millimeters) for growth inhibitory effects over one
249 Gram-positive bacterium *S. aureus* and two Gram-negative bacteria (*E. coli* and *P.*

aeruginosa) and three different strains of the Gram-negative marine bacteria *T. maritimum* (TM LC07 10.1.1, TM Chile and TM LL01 8.3.8). The antibacterial activities were tested at concentrations of 100 μg/disk in DMSO. The bacterial cultures were inoculated and incubated according to the described protocol of the experimental section. The experimental results of this *in vitro* antibacterial evaluation are summarized in Table 1.

(Table 1)

The obtained results revealed that all tested compounds **1-12** were inactive against the human pathogenic bacteria strains of *E.coli*, *S. aureus* and *P. aeruginosa*. However most of the synthesized compounds (**5-7** and **9-12**) presented antibacterial activity against the three *T. maritimum* marine bacteria strains, showing inhibitions zones ranging between 11 to 35 mm. Compounds without an amino substituent (**1-4**) were inactive against any of the *T. maritimum* strains, with exception of compound **1**, which resulted to be slightly sensitive to one of the *T. maritimum* strains (TM LC07 10.1.2), presenting and inhibition zone of 12 mm. Therefore, introduction of an amino substituent on the 3-benzoyl moiety in either 3' or 4' positions, resulted to be a key substitution for the activity of these compounds over *T. maritimum* strains.

Comparing compounds of the 4'-amino substituted series (**5-8**), the 3-(4'-aminobenzoyl)coumarin **5** showed activity only against two of the three *T. maritimum* strains, TM Chile and TM LC01 8.3.8, with inhibition zones of 10 and 12 mm, respectively. When a methoxy or ethoxy substituent at position 8 of the coumarin core (compounds **6** and **7**, respectively) was introduced, the activity of this series notably increased respect to the simplest derivative of the series **5**, presenting inhibition zones

ranging from 28 to 34 mm for the three *T. maritimum* strains, reaching inhibition zone values higher than enrofloxacin, the currently used drug for the treatment of tenacibaculosis. However, when a substituent was introduced at position 6 of the coumarin (compound **8**), the activity was completely lost.

For the 3'-amino substituted series (**9-12**), no significant differences in the activity were found between the simplest compound of the series **9**, with only an amino group at position 3', and compounds in which an additional methoxy or ethoxy substituent (compounds **10** and **11**, respectively) were introduced at position 8, presented inhibition zone values higher than the referenced compounds oxolinic acid and enrofloxacin, ranging from 19 to 35 mm. In any case, the most sensitive *T. maritimum* strain was TM Chile. As for the 4'-amino substituted series, the 6-bromo substituted compound **12**, was the less active compound of the series but presenting the same activity range than the reference compound oxolinic acid.

In conclusion, a series of coumarin-chalcone hybrid compounds have been designed and synthesized and their antibacterial activity against human pathogenic bacteria (*E.coli*, *S. aureus* and *P. aeruginosa*) and three different strains of the marine pathogenic bacteria *T. maritimum* were tested. Compounds were non-active against any human pathogenic bacteria, but most of them showed high inhibition growth against the three *T. maritimum* strains, with inhibition zones in the same range or higher than the currently drug used for the treatment of tenacibaculosis, enrofloxacin, which is not selective against *T. maritimum* since it is also highly active against the tested human pathogenic bacteria. Presence of an amino group in the benzoyl moiety (compounds **5-12**) resulted to be a key substitution for the activity of compounds against *T. maritimum*. Additional substitutions on the coumarin core at position 8 or 6 also played a key role in their activity against *T. maritimum*, indicating that substitutions at position 8 (compounds **6**,

300 **7**, **10** and **11**) increases their activity, whereas substitution at position 6 (compounds **8**
301 and **12**) decreases *T. maritimum* antibacterial activity. Therefore, the high activity of
302 compounds and their selectivity only against the marine bacteria can avoid drug
303 resistance problems in humans, making these compounds good candidates for the
304 treatment of tenacibaculosis.
305
306 **Acknowledgements**
307 This Project has been partially supported by personal founds of Spanish researchers and
308 the University of Santiago de Compostela. SVR thanks to Ministerio de Educación y
309 Ciencia for her FPU PhD grant AP2008-04263 (Spain). MJM thanks Fundação para a
310 Ciência e Tecnologia for her PhD grant SFRH/BD/61262/2009 (Portugal).
311
312 **Conflict of interest**
313 The authors have declared no conflict of interest.
314
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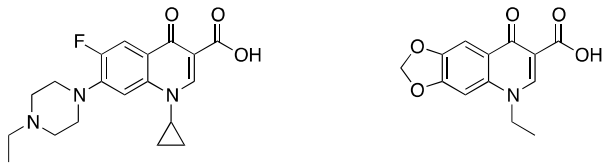

Figure 1. Quinolone derivatives as antibacterial drugs.

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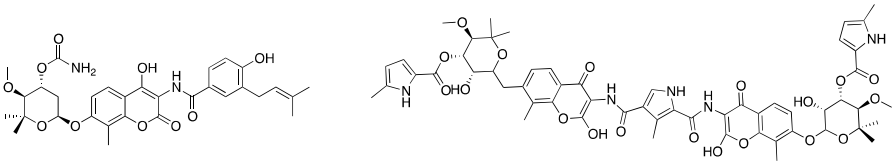


Figure 2. Benzopyrone derivatives currently used as potent antibacterial agents.

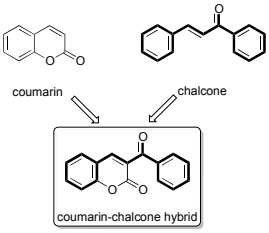
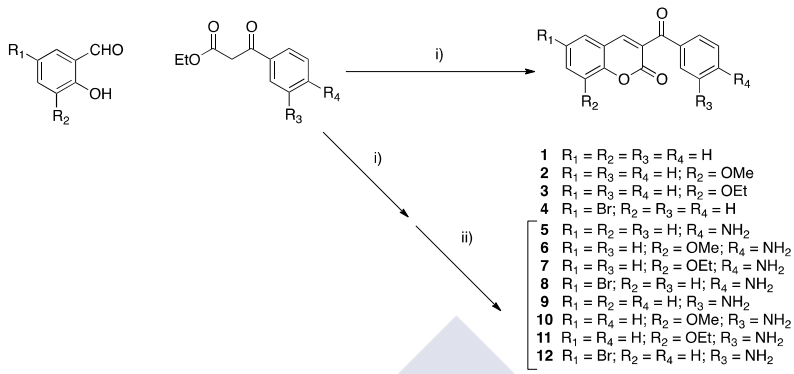


Figure 3. Rational design of the coumarin-chalcone hybrid compounds



Scheme 1. Synthesis of the target molecules **1-12**. *Reagents and conditions:* i) EtOH, piperidine, reflux, 2-5 h; ii) $SnCl_2.2H_2O$, EtOH, reflux, 3-7 h.

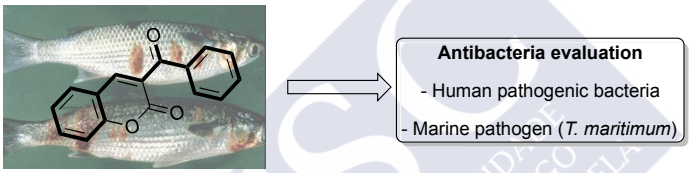
Table 1. *In vitro* antibacterial activity[a] of target compounds **1-12** expressed as inhibition zones (mm).[b]

Compound	*E. coli*	*S. aureus*	*P. aeruginosa*	TM LC07 10.1.2	TM Chile	TM LC01 8.3.8
1	NA	NA	NA	12	NA	NA
2	NA	NA	NA	NA	NA	NA
3	NA	NA	NA	NA	NA	NA
4	NA	NA	NA	NA	NA	NA
5	NA	NA	NA	NA	10	12
6	NA	NA	NA	30	33	32
7	NA	NA	NA	31	28	34
8	NA	NA	NA	NA	NA	NA
9	NA	NA	NA	28	35	33
10	NA	NA	NA	19	26	23
11	NA	NA	NA	28	35	35
12	NA	NA	NA	11	16	14
Oxolinic acid	31	25	NA	12	16	14
Enrofloxacin	42	32	18	24	29	29
Ampicillin	21	30	NA	31	30	34

[a] These results are average results of three experiments. [b] The compounds were used at the concentration of 100 µg/disk and the inhibition zones are stated in mm; NA = not active; diameter of inhibition zone ≤ 5 mm.

Table of contents

A series of coumarin-chalcone hybrid compounds has been synthesized and their antibacterial evaluation against human pathogenic bacteria and three different strains of T. maritimum has been assessed. Compounds showed high activity and selectivity against the three strains fish pathogen and presented inhibition growth zones in the same range or higher than the currently used antibacterial drug enrofloxacin, making these compounds good candidates for the treatment of tenacibaculosis.



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ARTICLE TYPE

3-Benzoylcoumarins as new selective scaffolds for modulation of the *h*A_1 and *h*A_3 binding affinity. Synthesis, biological assays and docking studies.

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

With the aim of finding new and selective adenosine receptor ligands, in the present communication we report the synthesis and the pharmacological characterization for the four subtypes of adenosine receptors of a new series of coumarin-chalcone hybrids. The synthesized compounds **1-8** were characterized in radioligand binding (A_1, A_{2A} and A_3) and adenylyl cyclase activity assays (A_{2B}) in order to determine the affinity of the compounds for the four human adenosine receptors subtypes (*h*ARs). Coumarin-chalcone hybrids resulted to be ligands with a novel structure never reported before that showed varying affinity and selectivity for the *h*A_1 or *h*A_3 subtypes depending on the methoxylate or hydroxylated nature of the substituents. Hydroxy-substituted hybrids were selective for the *h*A_1, while methoxy substituted hybrids resulted to be selective for the *h*A3. The most active compound selective for the *h*A_1 resulted to be compound **7**, whereas compound **4** was the most active selective for *h*A_3. In addition, homology models for the *h*A1 and *h*A3 were developed to analyze the structure-activity relationship through molecular docking calculations. Results showed that different residues can play an important role in ligand selectivity.

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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

1. Introduction

Adenosine is a purine nucleoside comprised of four distinct receptor subtypes designated as A_1, A_{2A}, A_{2B}, and A_3 belonging to the G protein-coupled receptor superfamily (GPCRs).[1] It acts as an endogenous modulator controlling a wide range of physiological processes due to its interaction with this four specific cell membrane GPCRs.[2,3,4] Extracellular adenosine activates receptors of the A_1 and A_{2A} subtypes, which depresses the action of the heart, brain, kidneys, and the immune system, as well as other systems.[5] Recent studies have allowed to clone the A_3 subtype of adenosine receptors, [6,7] and establish its pharmacological[8,9,10,11] and regulatory characteristics[12]. The A_{2A} and A_{2B} adenosine receptor (AR) subtypes are primarily coupled to Gs and thereby increase intracellular cAMP levels, whereas the A_1 and A_3ARs inhibit cAMP production via activation of Gi.

Because of their ubiquitous presence in cells, ARs have been seen as promising targets in the medicinal chemistry field. During the last years, the search for agonist and antagonist ligands binding to individual AR subtypes has been intensified, and therefore the role of these receptors in drug discovery is continuously expanding.[13,14,15] Most of the AR antagonists are promising new drug candidates for a number of pathological processes such as inflammation (A_3),[14] heart and renal failure (A_1),[16] or neurological disorders like Parkinson[17,18] and Alzheimer's diseases (A_{2A} and/or A_1).[19] For this reason, targeting the different ARs can open a new window for potential drug treatment of a variety of pathologies.

From the large number of compounds exerting high potency and selectivity towards ARs, the xanthine and adenine scaffolds have been used to develop the so-called classical AR antagonists.[20,21] In the search for nonclassical AR ligands, novel structures have recently been discovered. For example, flavone and isoflavone derivatives have play a remarkable role, namely genistein, which has been described to be a competitive antagonist at A_1AR in FRTL (thyroid) cells[22] or galangin, which was found to bind three subtypes of adenosine receptors presenting micromolar affinity for the A_3AR.[23] The affinity of flavonoids and other phytochemicals to adenosine receptors suggests that a wide range of natural substances in the diet may potentially block the effects of endogenous adenosine.

Coumarins (chromone isosteres) and chalcones (flavone with an opened pyrone ring) are another class of benzopyran related compounds of natural origin that present a wide range of pharmacological activities.[24,25,26] Taking into consideration that the coumarin and chalcone nucleus are structurally related flavonoids, it was found to be relevant to explore them for the design of novel AR antagonists. Our interest in this new scaffold was also motivated by the structure similarity between coumarin skeleton and chromone scaffolds previously described as AR antagonists that present a particular selectivity for the A_3 and A_{2A} AR subtypes.[27,28,29] Among them, our attention was focused on the 3-benzoylcoumarin ring system, which can be considered as hybrid compounds in which the chalcone moiety is fixed in a *trans* conformation through the double bond of the pyrone ring of the coumarin scaffold (Figure 1), which has never been proposed as a scaffold for AR ligands.

Thus, due to structural similarities between flavones, coumarins and chalcones, we decided to synthesize hybrid compounds bearing in the same molecule both scaffolds, and evaluate their activity towards the four subtypes of human AR expressed in Chinese Hamster Ovary (CHO) cells.

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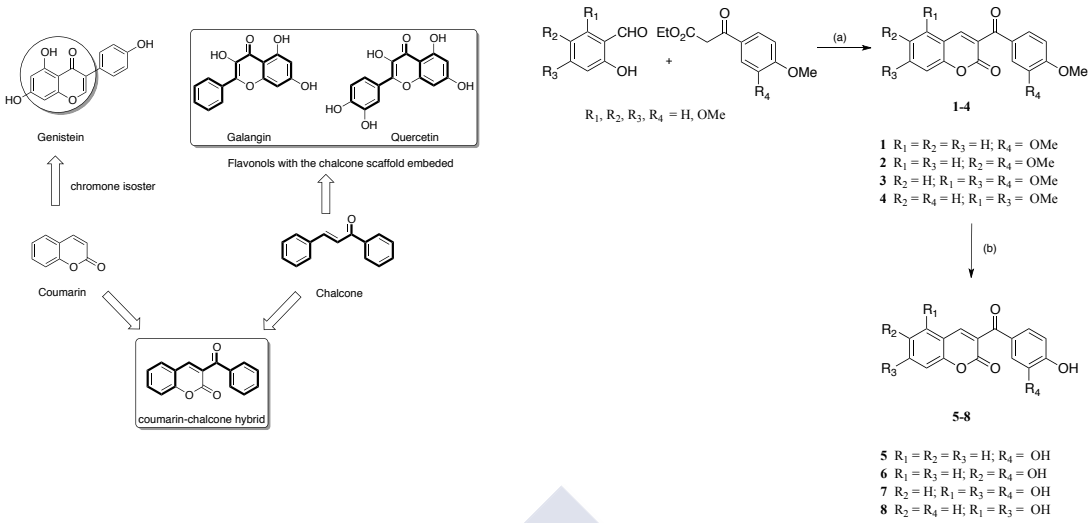

Figure 1. Rational design of coumarin-chalcone hybrid compounds based on flavonoids.

2. Chemistry

Two groups of coumarin-chalcone hybrid compounds have been synthesized: methoxy substituted hybrids (**1-4**) and hydroxyl substituted derivatives (**5-8**). In order to achieve the final products, the synthesis of the methoxy-3-benzoylcoumarins **1-4** was carried out employing a Knoevenagel reaction as the key step. Synthesis of the corresponding hydroxy-3-benzoylcoumarins **5-8** was performed via hydrolysis of the previously synthesized methoxy deivatives employing BBr_3 as deprotection reagent. The synthetic approach is highlighted in Scheme 1.

The methoxy-3-benzoylcoumarins **1-4** were efficiently synthesized with 85-97% yield by a Knoevenagel reaction treating the appropriate salicylaldehyde and the corresponding methoxylated ethyl benzoylacetate with piperidine in ethanol at reflux for 2-6 h. The resulting methoxy derivatives were treated with boron tribromide in DCM at 80 ºC in a Schlenk tube for 48 h to afford the corresponding hydroxy derivatives **5-8** with 75-94 % yield. Reaction conditions and compounds characterization are described in the experimental section.

Scheme 1. Preparation of coumarin-chalcone hybrids. *Reagents and conditions:* (a) piperidine, EtOH, reflux, 2-6 h; (b) BBr_3, DCM, 80 ºC, 48 h.

3. Pharmacology

3.1. Adenosine receptor binding affinity assays

The adenosine binding affinity of compounds **1-8** for the human AR subtypes hA$_1$, hA$_{2A}$, hA$_{2B}$ and hA$_3$, expressed in Chinese Hamster Ovary (CHO) cells, was determined in radioligand competition experiments.[30,31,32,33] In the binding affinity assay, it is measured the competition of ligands for specific binding of [^{3}H]CCPA binding at hA1ARs; specific binding of [^{3}H]NECA binding at hA$_{2A}$ ARs; and specific binding of [^{3}H]HEMADO at hA$_3$ ARs. The results were expressed as Ki (dissociation constants), which were calculated with the program SCTFIT, and given as geometric means of at least three experiments, including 95 % confidence intervals.[32] Due to the lack of a suitable radioligand for hA$_{2B}$ receptor in binding assay, the potency of antagonists at hA$_{2B}$ receptor was determined instead by inhibition of NECA-stimulated adenylyl cyclase activity with increasing concentrations of antagonist.[30,31] As a result, cAMP (cyclic adenosine monophosphate) production was inhibited in a concentration

dependent fashion, and IC50 and Ki values, respectively, were determined

The data obtained for the binding affinity assays from radioligand binding experiments for compounds **1-8** are summarized in Table 1. Data obtained from the binding affinity assays showed the that methoxylated derivative compounds showed either non binding affity for any of the *h*AR (compounds **1** and **2**) or good binding affinity and selectivity for the hA_3AR (compounds **3** and **4**). On the contrary, all hydroxylated compounds **6-8** presented moderated but selective affinity for the hA_1, except compounds **5,** which presented the same binding affinity for both hA_1 and hA_3 ARs.

Table 1. The binding affinity (K_i) for the human AR subtypes hA_1, hA_{2A} and hA_3 of compounds **1-8** was determined using radioligand competition experiments in chinese hamster ovary (CHO) cells. Highest concentrations tested were different depending on the solubility of compounds under the respective assay conditions.

Compound	hA_1 (Ki nM)	hA_3 (Ki nM)
1	>100,000	>100,000
2	>100,000	>30,000
3	>30,000	9,030 (6,280-13,000)
4	>100,000	2,490 (2,330-2,660)
5	39,500 (25,300-61,500)	34,500 (29,700-40,100)
6	54,000 (49,800-58,500)	>30,000
7	17,700 (16,000-19,500)	>30,000
8	29,100 (20,400-41-500)	>30,000

These results are geometric means of 3 experiments and given with 95% confidence intervals in parentheses.

4. Theoretical evaluation of ADME properties

To better correlate the drug-like properties of the coumarin-chalcone hybrid compounds, their theoretical ADME properties were calculated using the Molinspiration property program.[34]

Table 2. Theoretical evaluation of the ADME properties of the tested compounds[a]

Compd.	log P	TPSA (Å^2)	*n*-OH acceptors	*n*-OHNH donors	Volume (Å^3)
1	3.04	65.75	5	0	270.07
2	3.08	74.98	6	0	295.62
3	3.06	84.22	7	0	321.16
4	3.47	74.98	6	0	295.16
5	2.43	87.74	5	2	235.01
6	1.93	107.97	6	3	243.03
7	1.63	128.20	7	4	251.05
8	2.12	107.97	6	3	243.03

[a] TPSA, topological polar surface area; n-OH, number of hydrogen acceptors; n-OHNH, number of hydrogen bond donors. The data was determined with Molinspiration calculation software.

5. Molecular modeling

We successfully constructed homology models of the *h*A1 and *h*A3 (see Methods section). A selection of protein models extracted from Induce Fit calculations were tested according to their capability to discriminate between known ligands and decoys and between sub-type selective compounds. The final models selected to run docking calculations with the synthesized ligands showed excellent results in both tests. A set of 200 decoys randomly selected from ZINC database[35] were mixed up with 22 known ligands

of each adenosine sub-type receptor.[36] The database was docked to the hA_1 and hA_3 models using Glide SP precision.[37] Table 3 shows the area under the ROC curve (AUROC) for both systems. We carried out a second and more challenging test to differentiate between subtype-selective ligands. In a similar way as a previous study,[36] we docked 66 sub-type selective compounds (22 hA_1, 22 hA_{2A} and 22 hA_3 compounds) to the hA_1 model (22 true positives vs 44 false positives) and to the hA_3 (22 true positives vs 44 false positives). Results are similar to the article published by Katritch *et al.*[36] and have shown that the developed homology models can discriminate between sub-type selective compounds (see Table 3).

Table 3. Area under the ROC curve (AUROC) for both homology models. In test 1 we considered 22 hA_1 or 22 hA_3 ligands as true positives (TP) and 200 random decoys as false positives (FP). In test 2 for hA_1, we considered 22 hA_1 selective compounds as TP and 22 hA_{2A} + 22 hA_3 compounds as FP. In test 2 for hA_3, we considered 22 hA_3 compounds as TP and 22 hA_{2A} + 22 hA_1 compounds as FP.

	hA_1	hA_3
AUROC test 1	0.91	0.95
AUROC test 2	0.86	0.82

We run Glide SP molecular docking simulations[37] with our data using as the protein structure the hA_1 and hA_3 selected homology models to detect the hypothetical binding mode of the new synthesized compounds. We optimized the protein structure for each binding mode with Prime module.[38] The calculations showed great variability since all the compounds yielded different possible binding modes inside the proteins. Selection of the hypothetical binding pose was carried out considering number of similar poses extracted from the calculations and geometrical correspondence to crystallized ligands in the hA_{2A} (see Figure 2a).

6. Results and discussion

Compounds **1-8** were efficently synthesized and their *in vitro* binding affinity against human AR subtypes hA_1, hA_{2A}, hA_{2B} and hA_3, expressed in CHO cells, was tested. In the present communication, studies of the effect on the binding affinity by the presence of methoxy or hydroxy substituents in different number and positions of the 3-benzoylcoumarin scaffold, were performed.

The experimental data show that none of the coumarin-chalcone hybrid compounds have detectable binding affinity for the hA_{2A} and hA_{2B} ARs (K_i > 30,000 – 100,000 nM). Compounds **1** and **3**, presenting no substituents on the coumarin ring system or with a methoxy group at the 6 position, respectively, were the only tested compounds that showed no binding affinity for any of the four evaluated receptors (Ki > 30,000-100,000). Introduction of two methoxy groups at positions 5 and 7 of the coumarin moiety (compounds **3** and **4**) resulted in an increase of potency and selectivity of the compounds. Compound **3**, containing three methoxy groups at positions 5, 7 and 4' showed selectivity for hA_3, with Ki = 9,030 nM, whereas compound **4**, with four methoxy groups at positions 5, 7, 3' and 4', showed a slight increase in its activity comparing to compound **3**, and resulted to be selective for hA_3 too, with a Ki value of 2,490 nM. Docking results showed that the compounds orientated the oxygens in the benzopyrone ring towards the residue Asn250 and the benzoyl fragment buried in the hA_3 pocket. This hypothetical binding mode is in agreement with the conformations shown by the co-crystallized ligands in the hA_{2A} (PDB: 3EML and 3UZC)[39,40] (see Figure 2a and 2b). The pose of compound **3** yielded hydrogen bonds with the residues Gln167, Asn250 and His272.

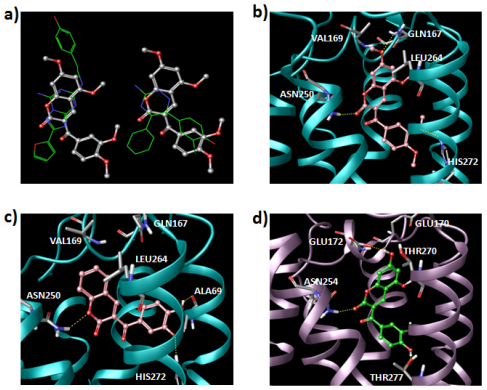

Figure 2. a) Comparison of the co-crystallized ligands (green carbons) in the hA_{2A} (3EML (left) and 3UZC (right)) with the pose of compound **3** extracted from the hA_3 docking calculations (grey carbons). Protein pockets in hA_{2A} and hA_3 were superimposed. b) Pose extracted for compound **3** inside the hA_3 after docking. Hydrogen bonds are represented in yellow color. c) Hypothetical binding mode for compound **5** (pink carbons) in the hA_3. d) Pose obtained through docking simulations for compound **7** (green carbons) in the hA_1 protein pocket.

Transformation of methoxy into hydroxy substituents resulted in a completely different selectivity, showing affinity for the hA_1 instead of affinity for the hA_3. The only compound of the hydroxylated series (**5-8**) that showed putative affinity for both receptors was compound **5** (hA_1 Ki = 39,500 and hA_3 Ki = 34,500 nM), which bears a catechol group at positions 3' and 4' and no substituents in the coumarin moiety. The hypothetical binding mode for compound **5** in the hA_3 according to docking calculations is shown in Figure 2c. The compound can establish hydrogen bonds with residues Asn250, Ala69 and His272. As it was shown in the hA_{2A} crystallized structure and previous molecular modeling studies,[41,42] the corresponding residue Asn250 seems to play an important role in ligand recognition. The pose shown by compound **5** inside the hA_1 is similar to the described pose in the hA_3. However, the pose was slightly shifted and calculations were not able to retrieve a hydrogen bond with the residue Asn250. Introduction of an additional hydroxyl group at position 6 of the coumarin (compound **6**), resulted in an increase of the binding affinity and selectivity for the hA_1AR (Ki = 54,000 nM).

As well as in the case of the methoxy derivatives, the best binding affinities were found for compounds with hydroxyl substituents at positions 5 and 7 of the coumarin. Thereby, compound **7**, with the same pattern of substitution than quercetin, that is, hydroxyl groups at positions 5, 7, 3' and 4', presented selectivity for the hA_1, and the best binding affinity, with a Ki value of 17,700 nM. Compound **8**, with the same pattern of substitution than genistein (hydroxyl substituents at positions 5, 7 and 4') presented also selectivity for the hA_1, with a Ki value of 29,100 nM. The pose obtained through docking for compound **7** in the hA_1 protein pocket showed hydrogen bonds with residues Glu172, Asn254 and Thr277 (see Figure 2d).

From these preliminary results, one can conclude that the nature and position of the substituents play a key role in both potency and selectivity for the hA_1 or hA_3 ARs. Positions 5 and 7 of the coumarin moiety resulted to be important for the selectivity and potency. In the case of the methoxylated derivatives **1-4**, only compounds presenting substituents at these positions (compounds **3** and **4**) showed not only activity, but also selectivity for the hA_3AR. For the hydroxylated series (**5-8**), compounds with higher potency and selectivity for the hA_1 were also those which bear the hydroxyl groups at positions 5 and 7 (compounds **7** and **8**). There are different residues in both hA_1 and hA_3 that can be important to explain selectivity. Hydrophobic residues in the hA_3, such as the residues Val169 and Leu264 can contribute to ligand stabilization when the compounds bear hydrophobic substituents like methoxy groups (compounds **3** and **4**) (see Figure 3). However, the corresponding residues in the *h*A1, the Glu172 and Thr270 with hydrophilic characteristics, contribute to the binding of compounds with polar substituents, such as hydroxyl groups (compounds **6-8**). Nevertheless, compound **5** does not present substitutions in the coumarin ring and could adapt to the pocket in both proteins. Figure 3 shows the molecular surface originated by the

combination of both different residues that could be responsible for protein selectivity.

Finally, analyzing the obtained theoretical results of the ADME properties, it can be observed that no violations of Lipinski's rule (molecular weight, logP, number of hydrogen donors and acceptors) were found for the described coumarins. Also, TPSA, described to be a predictive indicator of membrane penetration, was also found to be positive for these drug candidates.

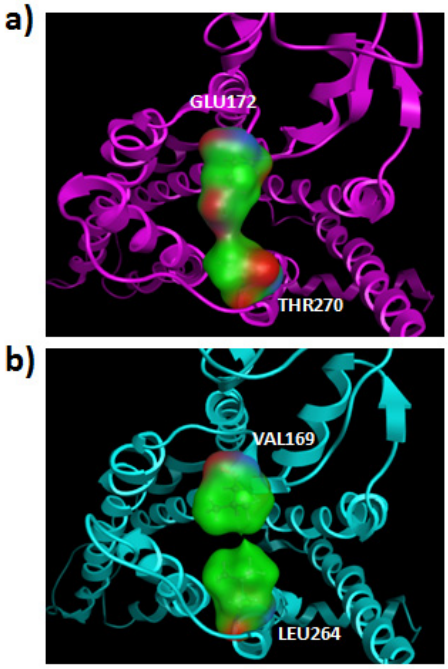

Figure 3. Molecular surface showing favoured interaction areas generated in the hA_1(a) and hA_3 (b). Red color represents hydrogen-bond areas, green color shows hydrophobic areas and blue represents mild polar. Protein structures are viewed from the extracellular side.

7. Experimental

7.1. General methods

Starting materials and reagents were obtained from commercial suppliers and were used without purification. Melting points (mp) are uncorrected and were determined with a Reichert Kofler thermopan or in capillary tubes in a Buchi 510 apparatus. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.4 MHz) spectra were recorder with a Bruker AMX spectrometer using DMSO-d_6 or $CDCl_3$ as solvent. Chemical shifts (δ) are expressed in parts per million (ppm) using TMS as an internal standard. Coupling constants *J* are expressed in hertz (Hz). Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets) and m (multiplet). Mass spectrometry was carried out with a Kratos MS-50 or a Varian MAT-711 spectrometer. Elemental analyses were performed by a Perkin-Elmer 240B microanalyzer and were within ±0.4 % of calculated values in all cases. The analytical results were ≥ 95% purity for all compounds Flash Chromatography (FC) was performed on silica gel (Merck 60, 230-400 mesh); analytical TLC was performed on precoated silica gel plates (Merck 60 F254). Organic solutions were dried over anhydrous sodium sulfate. Concentration and evaporation of the solvent after reaction or extraction was carried out on a rotary evaporator (Büchi Rotavapor) operating at reduced pressure.

7.2. Synthesis

7.2.1. General procedure for the synthesis of methoxy-3-benzoylcoumarins 1-4. To a solution of the appropriate *β*-ketoester (1 mmol) and the corresponding salicylaldehyde (1 mmol) in ethanol (5 mL) was added piperidine in catalytic amount (0.10 mL). The reaction mixture was refluxed for 2–6 h and after completion (followed by TLC), the reaction was cooled and the precipitated was filtered and washed with cold ethanol and ether. The obtained solid was recrystallized in DCM to afford the corresponding methoxy-3-benzoylcoumarin compounds.

5.2.2. General procedure for the synthesis of hydroxy-3-benzoylcoumarins 5-8. The appropriate methoxy derivative compound **1-4** (1

mmol) was dissolved in DCM (1 mL) and BBr_3 in DCM (20 mmol, 1M) was added dropwise in a Schlenk tube. Tube was sealed, and the reaction mixture was heated at 80 ºC for 48 h. The resulting crude was treated with MeOH and rotated to dryness. The obtained crude was recrystallized in MeOH or purified by flash chromatography using hexane/ethyl acetate mixtures as eluent, to afford the desired hydroxy derivatives.

3-(3',4'-Dimethoxybenzoyl)coumarin (1)

85% yield; white solid; mp 190-191 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.01 (s, 1H, H-4), 7.71 – 7.52 (m, 3H, 3xAr-H), 7.50 – 7.29 (m, 3H, 3xAr-H), 6.87 (d, *J* = 8.4 Hz, 1H, H-5'), 3.95 (s, 6H, 2xOCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 190.27, 154.83, 154.42, 149.53, 144.62, 133.55, 129.34, 129.20, 127.76, 125.71, 125.15, 118.46, 117.15, 111.24, 110.19, 56.38, 56.32; EI-MS *m/z* (%): 311 ($[M+1]^+$, 59), 310 (M^+, 100), 173 (41), 166 (25), 165 (99), 79 (22), 77 (22); Ana. Calcd. For $C_{18}H_{14}O_5$: C 69.67, H 4.55. Found: C 69.69, H 4.58.

6-Methoxy-3-(3',4'-dimethoxybenzoyl)coumarin (2)

97% yield; beige solid; mp 202-203 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.78 (s, 1H, H-4), 7.38 (d, *J* = 1.9 Hz, 1H, H-2'), 7.26 (dd, *J* = 8.4, 2.0 Hz, 1H, H-6'), 7.16 (d, *J* = 9.1 Hz, 1H, H-8), 7.04 (dd, *J* = 9.1, 2.9 Hz, 1H, H-7), 6.82 (d, *J* = 2.9 Hz, 1H, H-5), 6.70 (d, *J* = 8.4 Hz, 1H, H-5'), 3.78 (s, 6H, 2xOCH_3), 3.69 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 190.41, 156.60, 154.40, 149.51, 149.31, 144.43, 129.36, 128.02, 125.73, 121.56, 118.77, 118.18, 111.24, 110.75, 110.18, 56.39, 56.32, 56.14; EI-MS *m/z* (%): 341 ($[M+1]^+$, 58), 340 ($[M]^+$, 94), 165 (100), 77 (22); Ana. Calcd. For $C_{19}H_{16}O_6$: C 67.05, H 4.74. Found: C 67.09, H 4.75.

5,7-Dimethoxy-3-(3',4'-Dimethoxybenzoyl)coumarin (3)

91% yield; pale yellow solid; mp 210-211 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.19 (s, 1H, H-4), 7.34 (d, *J* = 1.9 Hz, 1H, H-2'), 7.26 (dd, *J* = 8.4, 2.0 Hz, 1H, H-6'), 6.70 (d, *J* = 8.4 Hz, 1H, H-5'), 6.29 (d, *J* = 1.9 Hz, 1H, H-6), 6.14 (d, *J* = 2.1 Hz, 1H, H-8), 3.77 (bs, 6H, 2x OCH_3), 3.72 (bs, 6H, 2x OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 190.91, 165.76, 159.37, 158.37, 157.97, 153.89, 149.28, 141.54, 130.03, 125.35, 121.28, 111.54, 110.11, 103.91, 95.37, 93.01, 56.32; EI-MS *m/z* (%): 371 ($[M+1]^+$, 24), 370 (M^+, 100), 339 (21), 233 (30), 165 (63); Anal. Calcd. For $C_{20}H_{18}O_7$: C 64.86, H 4.90. Found: C 64.88, H 4.93.

5,7-Dimethoxy-3-(4'-Dimethoxybenzoyl)coumarin (4)

97% yield; pale yellow solid; mp 174-175 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.21 (s, 1H, H-4), 7.69 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 6.77 (d, *J* = 8.9 Hz, 2H, H-3', H-5'), 6.29 (d, *J* = 2.1 Hz, 1H, H-6), 6.13 (d, *J* = 2.2 Hz, 1H, H-8), 3.76-3.67 (m, 9H, 3x OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 190.69, 165.61, 163.80, 159.14, 158.21, 157.81, 141.54, 132.08, 129.79, 121.16, 113.73, 103.81, 95.17, 92.81, 56.13, 56.05, 55.54; EI-MS *m/z* (%): 341 ($[M+1]^+$, 33), 340 (M^+, 88), 325 (28) 312 (30), 309 (45), 297 (20), 233 (48), 135 (100), 92 (27), 77 (38). Anal. Calcd. For $C_{19}H_{16}O_6$: C 67.05, H 4.74. Found: C 67.08, H 4.76.

5,7-Dihydroxy-3-(4'-dihydroxybenzoyl)coumarin (8)

7.3. Binding affinity assays

The binding affinity for the human AR subtypes hA_1, hA_{2A}, hA_3 of the compounds was determined using radioligand competition experiments in Chinese hamster ovary (CHO) cells that were stably transfected with the individual receptor subtypes.[30,31]

The radioligands used were 1 nM [^{3}H]CCPA for hA_1, 10 nM [^{3}H]NECA for hA_{2A}, and 1 nM [^{3}H]HEMADO for hA_3 receptors. Due to the lack of a suitable radioligand for hA_{2B} receptors the potency of antagonists at hA_{2B} receptor (expressed on CHO cells) was determined by inhibition of NECA-stimulated adenylyl cyclase activity.[30] The IC50 for inhibition of cAMP

(cyclic adenosine monophosphate) production was determined and converted to Ki values using the Cheng and Prusoff equation.[30] For all the tested compounds no measurable activity for the hA$_{2B}$ or hA$_{2A}$ (Ki > 30,000 nM) was detected.

7.4. Statistical methods

K_i values (dissociation constants) were determined in radioligand competition experiments with 7-8 different concentrations of test compound and each concentration was tested in duplicate. K_i values are given as geometric means of three independent experiments with 95% confidence intervals. For analysis of the competition curves the program SCTFIT was used.[33]

7.5. Theoretical evaluation of the ADME properties.

LogP was calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors. Topological Polar Surface Area (TPSA) was calculated based on the methodology published by Ertl *et al.* as a sum of fragment contributions.[43] Oxygen and nitrogen centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood-brain barrier penetration. Method for calculation of molecule volume developed at Molinspiration is based on group contributions. These have been obtained by fitting sum of fragment contributions to "real" 3D volume for a training set of about twelve thousand, mostly drug-like molecules. 3D molecular geometries for a training set were fully optimized by the semiempirical AM1 method.

7.6. Molecular modeling

Homology modeling was carried out using the Molecular Operating Environment (MOE) suite.[44] Molecular docking simulations were performed with the Schrodinger package.[37,38]

7.6.1. Homology models of *h*A1 and *h*A3 ARs.

We constructed homology models of the hA1 and hA$_3$ adenosine receptors. As a template we used the crystallized structure of the hA$_{2A}$ receptor (PDB: 3EML).[39] Protein sequence alignment of the 3 receptors (hA1, hA$_{2A}$ and hA3) used to generate the homology models was performed as described by Katritch *et al.*[36] The alignment was made considering the highly conserved residues in the different TM helices. Homology models were generated using the MOE software.[44] Protein geometry was evaluated for the models taking into account Phi-Psi plots, bond lengths, bond angles, dihedrals, rotamers, atom clashes and contact energies. Presence of different conserved disulfide bridges was manually checked in the models, such as the bridge between the corresponding residues Cys166 and Cys77 in the hA$_{2A}$. The final models were optimized using Induce Fit Docking Workflow in the Schrodinger package.[45] We used selective high affinity ligands (compounds coll_11 and jaco_mre3008_f20 of a previous publication)[36] to adapt the protein pocket for the hA$_1$ and hA$_3$ respectively. This procedure involved three steps: 1) Glide-based docking of the ligands using SP mode (standard-precision); 2) Protein pocket optimization using Prime and considering the residues within 5Å from the ligand poses; 3) Glide-based docking of the ligands in the refined pocket using XP mode (Extra-precision). As described previously,[36] homology models were tested for their capability to discriminate ligands from decoys and between known subtype-selective compounds. ROC curves were performed and the best models were selected for further molecular docking studies.

7.6.2. Molecular docking of *h*A1 and *h*A3 ARs.

We carried out molecular docking studies using the hA1 and hA$_3$ homology models selected in the previous step. Compounds were docked using Glide SP mode.[37] Ten poses for each ligand were collected and optimized using MM-GBSA in Prime[38] taking into account a flexible protein region defined by 5 Å from the ligand. Final binding modes were selected taking into account number of similar poses extracted from the calculations and geometrical correspondence to co-crystallized ligands in the hA$_{2A}$.

Conclusions

A series of methoxy and hydroxy-coumarin-chalcone hybrid compounds were synthesized and their binding affinity towards the four subtypes of adenosine receptors was studied. A preliminary structure-activity relationship (SAR) of the synthesized derivatives showed that in general, methoxy substituted derivatives (**1-4**) presented good binding affinity and selectivity for the hA$_3$AR, whereas hydroxy substituted derivatives (**5-8**) presented moderate but selective binding affinity for the hA$_1$AR. Positions 5 and 7 of the coumarin ring were crucial for the potency and selectivity in both series of compounds. Methoxy derivative **4** and hydroxy derivative **7** were the most active compounds, being selective for hA$_3$ (Ki = 2,490 nM) and hA$_1$ (Ki = 17,700 nM) respectively. We also performed molecular docking simulations to study the binding mode of the compounds.

In addition, from the data obtained for the theoretical evaluation of the ADME properties, one can notice that all the hybrid compounds **1-8** do not break any point of the Lipinski's rule of five, making them promising leads for drug candidates.

Therefore, according to our findings, coumarin-chalcone hybrid compounds proved to be interesting scaffolds as ARs ligands worth to deepen study.

Acknowledgements

This work was partially supported by the Ministerio de Sanidad y Consumo (PS09/00501) and Xunta de Galicia (PGIDIT09CSA030203PR and 10PXIB203303PR). SVR thanks to Ministerio de Educación y Ciencia for her PhD grant FPU (AP2008-04263).

Notes and references

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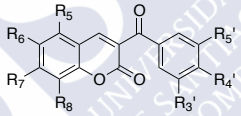
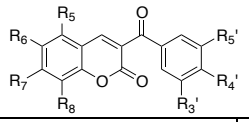
2. Compuestos preparados bajo estudio y pendientes de publicación.

Para los compuestos que no se encuentran descritos en publicaciones en el momento de la presentación de esta Memoria, pero que están bajo estudio y cuyos resultados están pendientes de ser publicados, se describirá de forma breve su caracterización estructural así como los rendimientos obtenidos en la síntesis de los mismos. Al igual que anteriormente (apartado 2.5. de Resultados y discusión), se presentan los datos de: 3-benzoilcumarinas (Tabla 7), 3-benzoil-4-hidroxicumarinas (Tabla 8), 3-cinamoilcumarinas (Tabla 9), 3-cinamoil-4-hidroxicumarinas (Tabla 10), 3-(3-aril-3-oxo-propenil)-4-clorocumarinas (Tabla 11) y 8-cinamoilcumarinas (Tabla 12).

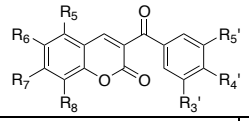
Tabla 7. 3-Benzoilcumarinas no publicadas.



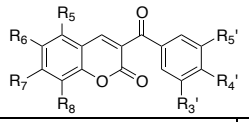
Compuesto	NMR	MS	Rdto. (%)
7	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.87 (s, 1H, H-4), 7.71 (d, *J* = 7.0 Hz, 2H, H-2', H-6'), 7.45 (t, *J* = 7.4 Hz, 1H, H-4'), 7.39-7.12 (m, 5H, H-5, H-7, H-8, H-3', H-5'), 2.27 (s, 3H, CH_3).	*m/z* (%): 265 ($[M+1]^+$, 18), 264 ($[M]^+$, 75), 235 (52), 221 (39), 105 (85), 77 (100)	82
8	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.07 (s, 1H, H-4), 7.94-7.85 (m, 2H, H-2', H-6'), 7.67-7.38 (m, 5H, H-3', H-4', H-5', H-5, H-6), 7.27 (d, *J* = 7.50 Hz, 1H, H-7), 2.51 (s, 3H, CH_3).	*m/z* (%): 265 ($[M+1]^+$, 39), 264 ($[M]^{+\cdot}$, 98), 235 (67), 221 (48), 105 (100), 77 (95).	94
9	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.35 (d, *J* = 9.3 Hz, 2H, H-7, H-8), 7.94 (s, 1H, H-4), 7.71 (d, *J* = 7.7 Hz, 2H, H-2', H-6'), 7.60-7.24 (m, 3H, H-3', H-4', H-5'), 7.09 (s, 1H, H-5).	*m/z* (%): 296 ($[M+1]^+$, 22), 295 ($[M]^{+\cdot}$, 100), 288 (31), 190 (21), 105 (55), 77 (81).	57



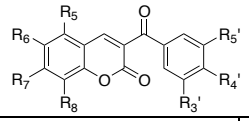
Compuesto	NMR	MS	Rdto. (%)
10	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.44 (s, 1H, H-4), 7.91-7.77 (m, 2H, Ar), 7.67-7.52 (m, 1H, Ar), 7.52-7.37 (m, 2H, aromatic0), 6.45 (d, *J* = 2.2 Hz, 1H, H-6), 6.30 (d, *J* = 2.2 Hz, 1H, H-8), 3.89 (m, 6H).	*m/z* (%): 311 ([M+1]$^{+\cdot}$, 28), 310 ([M]$^{+\cdot}$, 100), 282 (37), 281 (31), 233 (84), 105 (53), 77 (54).	88
11	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.35 (s, 1H), 7.99-7.89 (m, 3H), 7.80-7.63 (m, 2H), 7.60-7.46 (m, 3H).	*m/z* (%): 285 ([M+1]$^{+\cdot}$, 4), 284 ([M], 24), 256 (10), 255 (19), 221 (11), 105 (97), 77 (100), 69 (16).	69
12	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.08 (s, 1H), 7.84 (d, *J* = 7.8 Hz, 2H), 7.67-7.37 (m, 4H), 7.00-6.78 (m, 2H), 3.90 (s, 3H).	*m/z* (%): 281 ([M+1]$^{+\cdot}$, 18), 280 ([M]$^{+\cdot}$, 100), 252 (22), 251 (46), 221 (16), 203 (53), 119 (20), 105 (62), 77 (69).	82
20	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.80 (s, 1H, H-4), 7.71 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.34-7.05 (m, 3H, H-5, H-7, H-8), 6.77 (d, *J* = 10.2 Hz, 2H, H-3', H-5'), 3.69 (s, 3H, OCH_3), 2.24 (s, 3H, CH_3).	*m/z* (%): 295 ([M+1]$^{+\cdot}$, 18), 294 ([M]$^{+\cdot}$, 74), 250 (16), 135 (100), 77 (26).	97
21	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.83 (s, 1H, H-4), 7.71 (d, J = 9.0 Hz, 2H, H-2', H-6'), 7.13-6.92 (m, 3H, H-5, H-6, H-7), 6.77 (d, *J* = 9.0 Hz, 2H, H-3', H-5') 4.04 (q, *J* = 6.99 Hz, 2H, CH_3, (OCH_2CH_3)), 3.68 (s, 3H, OCH_3) 1.35 (t, *J* = 6.99 Hz, 3H, CH_3 (-OCH_2CH_3).	*m/z* (%): 325 ([M+1]$^{+\cdot}$, 31), 324 ([M]$^{+\cdot}$, 88), 188 (34), 135 (100), 92 (30), 77 (40).	98
22	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.81 (s, 1H, H-4), 7.72 (d, *J* = 8.78 Hz, 2H, H-2', H-6') 7.22-6.99 (m, 2H, H-3', H-5') 6.86-6.72 (m, 3H, H-5, H-6, H-7) 3.71 (s, 3H, 4'-OCH_3) 3.69 (s, 3H, 8-OCH_3).	*m/z* (%): 311 ([M+1]$^{+\cdot}$, 23), 310 ([M]$^{+\cdot}$, 87), 135 (100), 92 (27), 77 (63).	93



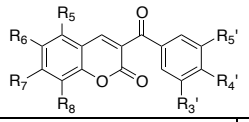
Compuesto	NMR	MS	Rdto. (%)
23	^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 8.25 (s, 1H, H-4), 8.05 (s, 1H, H-5), 7.92 (d, J = 8.5 Hz, 2H, H-2', H-6'), 7.84 (d, J = 8.8 Hz, 1H, H-7), 7.44 (d, J = 8.8 Hz, 1H, H-8), 7.05 (d, J = 8.6 Hz, 2H, H-3', H-5'), 3.85 (s, 3H, OCH_3).	*m/z* (%): 316 ([M+2]$^{+\cdot}$, 23), 314 ([M]$^{+\cdot}$, 66), 135 (100), 77 (26).	83
24	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.28 (s, 1H, H-4), 7.86 (d, J = 8.8 Hz, 2H, H-2', H-6'), 7.75 (dd, J = 8.8, 1.8 Hz, 1H, H-6), 7.14-6.94 (m, 4H), 3.88 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3).	*m/z* (%): 311 ([M+1]$^{+\cdot}$, 47), 330 ([M]$^{+\cdot}$, 80), 295 (23), 282 (29), 281 (26), 251 (35), 203 (41), 135 (100), 119 (19), 107 (28), 92 (54), 77 (74), 76 (23).	96
31	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.78 (s, 1H), 7.38 (d, J = 1.8 Hz, 1H), 7.33-7.03 (m, 4H), 6.70 (d, J = 8.4 Hz, 1H), 3.78 (d, J = 0.8 Hz, 6H), 2.26 (s, 3H).	*m/z* (%): 325 ([M+1]$^{+\cdot}$, 36), 324 ([M]$^{+\cdot}$, 99), 165 (100), 77 (20).	96
32	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.30 (s, 1H, H-4), 7.73-7.40 (m, 4H, aromático), 7.30 (t, J = 7.6 Hz, 1H, H-6'), 7.04 (d, J = 7.6 Hz, 1H, H-5'), 3.85 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3).	*m/z* (%): 325 ([M+1]$^{+\cdot}$, 16), 324 ([M]$^{+\cdot}$, 73), 187 (13), 165 (100), 77 (23).	98
33	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.80 (d, J = 2.6 Hz, 1H, H-4), 8.55-8.37 (m, 2H,), 7.70 (d, J = 9.1 Hz, 1H, H-5), 7.62 (dd, J = 8.4, 1.7 Hz, 1H, H-6'), 7.49 (d, J = 1.7 Hz, 1H, H-2'), 7.06 (d, J = 8.5 Hz, 1H, H-5'), 3.86 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3).	*m/z* (%): 356 ([M+1]$^{+\cdot}$, 31), 355 ([M]$^{+\cdot}$, 97), 218 (18), 165 (100).	99



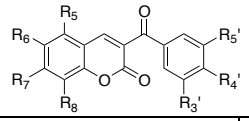
Compuesto	NMR	MS	Rdto. (%)
34	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.81 (s, 1H, H-4), 7.37 (d, *J* = 1.8 Hz, 1H, H-2'), 7.26 (dd, *J* = 8.4, 1.9 Hz, 1H, H-6'), 7.14-6.88 (m, 3H, Ar), 6.69 (d, *J* = 8.4 Hz, 1H, H-5'), 4.04 (q, *J* = 7.0 Hz, 2H, OCH_2-), 3.86 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 1.35 (t, *J* = 7.0 Hz, 3H, CH_3).	*m/z* (%): 355 ([M+1]$^{+\cdot}$, 22), 356 ([M]$^{+\cdot}$, 89), 188 (12), 165 (100), 77 (12)	91
35	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.74 (s, 1H, H-4), 7.47-7.34 (m, 3H, Ar), 7.30-7.14 (m, 2H, Ar), 6.70 (d, *J* = 8.4 Hz, 1H, H-5'), 3.79 (m, 6H, 2x OCH_3).	*m/z* (%): 420 ([M+2]$^{+\cdot}$, 95), 418 ([M]$^{+\cdot}$, 98), 165 (100), 77 (18).	73
36	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.01 (s, 1H, H-4), 7.59-7.37 (m, 3H, H-5, H-2', H-6'), 6.96-6.79 (m, 3H, H-6, H-8, H-5'), 4.02-3.93 (m, 6H, 2x OCH_3), 3.91 (s, 3H, OCH_3).	*m/z* (%): 341 ([M+1]$^{+\cdot}$, 30), 340 ([M]$^{+\cdot}$, 100), 309 (22) 165 (99), 119 (21), 79 (21), 77 (27).	60
38	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.51 (s, 1H, H-4), 8.33 (d, *J* = 8.9 Hz, 2H, H-2', H-6'), 8.14 (d, *J* = 8.9 Hz, 2H, H-3', H-5') 7.52-7.28 (m, 3H, H-5, H-7, H-8), 3.81 (s, 3H, OCH_3).	*m/z* (%): 326 ([M+1]$^{+\cdot}$, 20), 325 ([M]$^{+\cdot}$, 100), 150 (31), 77 (20).	91
39	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.50 (s, 1H, H-4), 8.33 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 8.21-8.11 (m, 3H, Ar) 7.91 (dd, *J* = 8.8, 2.39 Hz, 1H, H-7), 7.49 (d, *J* = 8.8 Hz, 1H, H-8).	*m/z* (%): 376 ([M+1]$^{+\cdot}$, 98), 375 ([M]$^{+\cdot}$, 19), 374 ([M]$^{\cdot}$, 100), 347 (32), 150 (85), 77 (48).	95
40	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.17 (s,1H, H-4), 8.12 (d, *J* = 3.9 Hz, 2H, H-2', H-6'), 7.82 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.22-7.01 (m, 3H, H-5, H-6, H-7) 3.84 (s, 3H, OCH_3).	*m/z* (%): 326 ([M+1]$^{+\cdot}$, 33), 325 ([M]$^{+\cdot}$, 100), 296 (44), 202 (35), 174 (51), 150 (62), 104 (65), 76 (60).	59



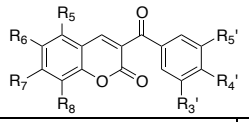
Compuesto	NMR	MS	Rdto. (%)
41	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.63 (s, 1H, H-4), 8.44 (dt, *J* = 8.0, 1.5 Hz, 1H, H-5), 8.24 (s, 1H), 8.16 (d, *J* = 7.3 Hz, 1H), 7.68 (t, *J* = 8.0 Hz, 1H, H-6), 7.41-7.21 (m, 2H, Ar), 7.05 (d, *J* = 2.8 Hz, 1H), 3.88 (s, 3H, OCH_3).	*m/z* (%): 326 ($[M+1]^{+\cdot}$, 45), 325 ($[M]^{+\cdot}$, 100), 297 (18), 296 (35), 203 (51), 175 (41), 150 (73), 119 (32), 104 (48), 76 (46).	94
42	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.48 (s, 1H, H-4), 8.33 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 8.16 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 7.65 (dd, *J* = 2.8, 1.9 Hz, 2H, H-5, H-7), 3.96 (s, 3H, OCH_3).	*m/z* (%): 388 ($[M+2]^{+\cdot}$, 97), 387 ($[M+1]^{+\cdot}$, 18), 386 ($[M]^{+\cdot}$, 100), 150 (24), 104 (19), 77 (27).	59
43	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.17 (s, 1H, H-4), 8.11 (d, *J* = 8.6 Hz, 2H, H-2', H-6'), 7.81 (d, *J* = 8.6 Hz, 2H, H-3', H-5'), 7.39-7.12 (m, 3H, H-5, H-7, H-8) 2.29 (s, 3H, CH_3).	*m/z* (%): 310 ($[M+1]^{+\cdot}$, 19), 309 ($[M]^{+\cdot}$, 100), 280 (59), 187 (38), 150 (25), 76 (19).	79
44	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.17 (s, 1H, H-4), 8.13 (d, *J* = 3.8 Hz, 2H, H-2', H-6'), 7.83 (d, *J* = 8.7 Hz, 2H, H-3', H-5'), 7.36 (dd, *J* = 12.4, 7.5 Hz, 2H, H-6, H-7), 7.14 (d, *J* = 7.5 Hz, 1H, H-5) 2.34 (s, 3H, CH_3).	*m/z* (%): 310 ($[M+1]^{+\cdot}$, 21), 309 ($[M]^{+\cdot}$, 100), 280 (64), 187 (48), 159(30), 150 (32), 76 (25).	89
45	^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 8.84 (d, *J* = 2.7 Hz, 1H, H-5), 8.67 (s, 1H, H-4), 8.51 (dd, *J* = 9.1, 2.8 Hz, 1H, H-7), 8.33 – 8.27 (m, 2H, Ar), 8.19 – 8.14 (m, 2H), 7.70 (d, *J* = 9.1 Hz, 1H, H-8).	*m/z* (%): 341 ($[M+1]^{+\cdot}$, 12), 340 ($[M]^{+\cdot}$, 100), 323 (68), 311 (36), 295 (56), 294 (25), 265 (31), 218 (46), 150 (100), 104 (78), 76 (55).	52



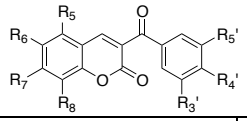
Compuesto	NMR	MS	Rdto. (%)
46	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.39 (s, 1H, H-4), 8.29 (d, J = 8.8 Hz, 2H, H-3', H-5'), 8.01 (d, J = 8.8 Hz, 2H, H-2', H-6'), 6.68 (s, 1H, H-6), 6.55 (s, 1H, H-8), 3.92 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3).	*m/z* (%): 356 ([M+1]$^{+\cdot}$, 28), 355 ([M]$^{+\cdot}$, 100), 327 (42), 326 (31), 233 (280).	61
47	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.70 (s, 1H, H-4), 8.51 (dt, J = 8.0, 1.5 Hz, 1H, H-5), 8.19 (s, 1H), 8.07 (d, J = 7.3 Hz, 1H), 7.88 (t, J = 8.0 Hz, 1H, H-6), 7.41-7.21 (m, 2H, Ar), 7.27 (d, J = 2.8 Hz, 1H), 3.88 (s, 3H, OCH_3).	*m/z* (%): 331 ([M+2]$^{+\cdot}$, 35), 329 ([M]$^{+\cdot}$, 100), 302 (15), 300 (37), 207 (38), 179 (32), 150 (99), 123 (29), 120 (24), 76 (50)	53
48	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.53 (s, 1H, H-4), 8.30 (d, J = 8.8 Hz, 2H, H-2', H-6'), 8.07 (d, J = 8.8 Hz, 2H, H-3', H-4'), 7.83 (d, J = 8.6 Hz, 1H, H-5), 7.18-6.95 (m, 2H, Ar), 3.90 (s, 3H, OCH_3).	*m/z* (%): 326 ([M+1]$^{+\cdot}$, 41), 325 ([M]$^{+\cdot}$, 100), 297 (40), 296 (58), 203 (93), 175 (33), 150 (30), 120 (24), 119 (53), 104 (52), 76 (55).	95
49	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.91 (s, 1H, H-4), 7.65 (d, J = 8.5 Hz, 2H, H-2', H-6'), 7.29 (d, J = 8.5 Hz, 2H, H-3', H-5'), 7.20-7.02 (m, 2H, Ar), 6.84 (d, J = 2.8 Hz, 1H, H-5), 3.70 (s, 3H, OCH_3).	*m/z* (%): 316 ([M+2]$^{+\cdot}$, 36), 314 ([M]$^{+\cdot}$, 90), 285 (23), 279 (15), 251 (23), 175 (22), 141 (39), 139 (100), 119 (16), 111 (52), 76 (13), 75 (15).	93
50	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.37 (s, 1H, H-4), 8.09 (s, 1H, H-5), 7.96 (d, J = 8.2 Hz, 2H, H-2', H-6'), 7.87 (dd, J = 8.9, 2.6 Hz, 1H, H-7), 7.60 (d, J = 8.2 Hz, 2H, H-3', H-5'), 7.46 (d, J = 8.9 Hz, 1H, H-8).	*m/z* (%): 365 ([M+2]$^{+\cdot}$, 6), 363 ([M]$^{+\cdot}$, 38), 361 (30), 141 (43), 139 (100), 113 (17), 111 (52).	99



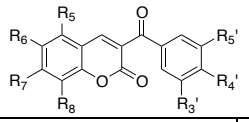
Compuesto	NMR	MS	Rdto. (%)
51	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.93 (s, 1H, H-4), 7.64 (d, *J* = 8.6 Hz, 2H, H-2', H-6'), 7.27 (d, *J* = 8.6 Hz, 2H, H-3', H-5'), 7.15-6.96 (m, 3H, Ar), 3.82 (s, 3H, OCH_3).	*m/z* (%): 316 ([M+2]$^{+\cdot}$, 27), 314 ([M]$^{+\cdot}$, 77), 285 (20), 279 (16), 251 (11), 175 (20), 141 (34), 139 (100), 111 (43), 75 (13).	95
52	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.81 (s, 1H, H-4), 7.63 (d, *J* = 8.4 Hz, 2H, H-2', H-4'), 7.28 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.14 (s, 1H, H-6), 7.10 (s, 1H, H-8), 3.82 (s, 3H, OCH_3).	*m/z* (%): 394 ([M+2]$^{+\cdot}$, 17), 392 ([M]$^{+\cdot}$, 48), 356 (62), 252 (32), 139 (100), 111 (31), 77 (18).	98
53	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.90 (s, 1H, H-4), 7.64 (d, *J* = 8.6 Hz, 2H, H-2', H-6'), 7.35-6.99 (m, 6H, Ar), 2.27 (s, 3H, CH_3).	*m/z* (%): 300 ([M+2]$^{+\cdot}$, 33), 298 ([M]$^{+\cdot}$, 81), 271 (16), 269 (38), 187 (30), 159 (29), 141 (42), 139 (100), 113 (24), 111 (66)	98
54	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.42-8.30 (m, 2H, Ar), 7.98 (s, 1H, H-4), 7.65 (d, *J* = 8.7 Hz, 2H, H-2', H-6'), 7.39 (d, *J* = 9.0 Hz, 1H, H-8), 7.31 (d, *J* = 8.7 Hz, 2H, H-3', H-5').	*m/z* (%): 331 ([M+2]$^{+\cdot}$, 12), 329 ([M]$^{+\cdot}$, 35), 141 (36), 139 (100), 113 (15), 111 (45)	41
55	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.29 (d, *J* = 10.2 Hz, 1H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.26 (d, *J* = 8.4 Hz, 2H), 6.29 (d, *J* = 2.0 Hz, 1H), 6.14 (d, *J* = 2.0 Hz, 1H), 3.73 (d, *J* = 2.5 Hz, 6H, 2x OCH_3).	*m/z* (%): 346 ([M+2]$^{+\cdot}$, 15), 342 ([M]$^{+\cdot}$, 42), 205 (31), 139 (100), 105 (27).	98
56	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.86 (s, 1H, H-4), 7.64 (d, *J* = 8.7 Hz, 2H, H-2', H-6'), 7.48-7.39 (m, 2H, Ar), 7.30 (d, *J* = 8.7 Hz, 2H, H-3', H-5'), 7.20 (d, *J* = 9.8 Hz, 1H, H-8).	*m/z* (%): 322 ([M+4]$^{+\cdot}$, 15), 320 ([M+2]$^{+\cdot}$, 82), 318 ([M]$^{+\cdot}$, 82), 285 (21), 283 (59), 257 (28), 255 (81), 141 (94), 139 (84), 113 (46), 111 (100), 75 (52).	90



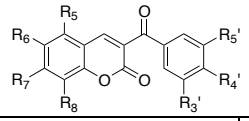
Compuesto	NMR	MS	Rdto. (%)
57	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.05 (s, 1H, H-4), 7.79 (d, *J* = 8.3 Hz, 2H, H-2', H-6'), 7.71-7.54 (m, 2H, Ar), 7.46-7.20 (m, 4H, Ar), 2.43 (s, 3H, CH_3).	*m/z* (%): 265 ([M+1]$^{+\cdot}$, 30), 264 ([M]$^{+\cdot}$, 86), 249 (27), 136 (26), 235 (24), 221 (37), 173 (21), 120 (19), 119 (100), 91 (60), 89 (26), 65 (31).	91
58	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.99 (s, 1H, H-4), 7.77 (d, *J* = 8.3 Hz, 2H, H-2', H-6'), 7.38-7.14 (m, 4H, Ar), 6.98 (d, *J* = 2.9 Hz, 1H, H-8), 3.84 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3).	*m/z* (%): 295 ([M+1]$^{+\cdot}$, 18), 194 ([M]$^{+\cdot}$, 73), 266 (17), 265 (16), 251 (31), 120 (17), 119 (100), 65 (17).	98
59	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.93 (s, 1H, H-4), 7.82-7.63 (m, 4H, Ar), 7.36-7.21 (m, 3H, Ar), 2.43 (s, 3H, CH_3).	*m/z* (%): 344 ([M+2]$^{+\cdot}$, 43), 342 ([M]$^{+\cdot}$, 43), 301 (23), 299 (23), 119 (100), 91 (57), 65 (28).	87
60	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.52 (d, *J* = 7.1 Hz, 2H, H-2', H-6'), 8.08 (s, 1H, H-4), 7.78 (d, *J* = 7.1 Hz, 2H, H-3', H-5'), 7.54 (dt, *J* = 8.6, 3.5 Hz, 1H, H-5), 7.41-7.19 (m, 2H, Ar), 2.45 (s, 3H, CH_3).	*m/z* (%): 310 ([M+1]$^{+\cdot}$, 14), 309 ([M]$^{+\cdot}$, 68), 294 (17), 119 (100), 91 (78), 65 (22).	42
61	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.41 (s, 1H, H-4), 7.75 (d, *J* = 7.5 Hz, 2H, H-2', H-6'), 7.26 (d, *J* = 7.5 Hz, 2H, H-3', H-5'), 6.45 (d, *J* = 2.7 Hz, 1H, H-6), 6.30 (d, *J* = 2.7 Hz, 1H, H-8), 3.88 (d, *J* = 3.3 Hz, 6H, 2x OCH_3), 2.42 (s, 3H, CH_3).	*m/z* (%): 341 ([M+1]$^{+\cdot}$, 33), 340 ([M]$^{+\cdot}$, 88), 325 (28) 312 (30), 309 (45), 297 (20), 233 (48), 119 (100), 92 (27), 77 (38).	90



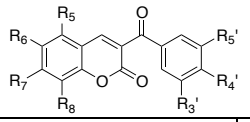
Compuesto	NMR	MS	Rdto. (%)
62	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.06 (s, 1H, H-4), 7.77 (d, *J* = 8.1 Hz, 2H, H-2', H-6'), 7.49 (d, *J* = 8.5 Hz, 1H, H-5), 7.27 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 6.97-6.80 (m, 2H, H-6, H-8'), 3.91 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3).	*m/z* (%): 295 ($[M+1]^{+\cdot}$, 30), 294 ($[M]^{+\cdot}$, 100), 279 (38), 266 (26), 265 (34), 251 (36), 203 (42), 119 (86), 91 (55), 65 (19).	63
63	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.05 (s, 1H, H-4), 7.71-7.55 (m, 2H, H-5, H-7), 7.46-7.30 (m, 2H, H-6, H-8), 7.13 (s, 2H, H-2', H-6'), 3.93 (s, 3H, OCH_3), 3.87 (s, 6H, 2x OCH_3).	*m/z* (%): 341 ($[M+1]^{+\cdot}$, 23), 340 ($[M]^{+\cdot}$, 100), 325 (42), 269 (46), 195 (27), 173 (33), 134 (18).	99
64	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.00 (s, 1H, H-4), 7.33 (d, *J* = 9.0 Hz, 1H, H-8), 7.27-7.16 (m, 1H, H-7), 7.12 (s, 2H, H-2', H-6'), 7.00 (d, *J* = 2.9 Hz, 1H, H-5), 3.93 (s, 3H, OCH_3), 3.87 (s, 6H, 2x OCH_3), 3.85 (s, 3H, OCH_3).	*m/z* (%): 371 ($[M+1]^{+\cdot}$, 23), 370 ($[M]^{+\cdot}$, 100), 355 (28), 203 (19), 195 (62), 134 (34).	95
65	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.99 (s, 1H, H-4), 7.52-7.23 (m, 3H, H-5, H-7, H-8), 7.12 (s, 2H, H-2', H-6'), 3.93 (s, 3H, OCH_3), 3.87 (s, 6H, 2x OCH_3), 2.43 (s, 3H, CH_3).	*m/z* (%): 355 ($[M+1]^{+\cdot}$, 37), 354 ($[M]^{+\cdot}$, 100), 339 (36), 283 (13), 195 (47), 187 (34).	95
66	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.04 (s, 1H, H-4), 7.27 (d, *J* = 8.4 Hz, 1H, H-5), 7.22-7.14 (m, 2H, H-6. H-7), 7.12 (s, 2H, H-2', H-6'), 3.99 (s, 3H, OCH_3), 3.92 (s, 3H, 8- OCH_3), 3.86 (s, 6H, 2x OCH_3).	*m/z* (%): 371 ($[M+1]^{+\cdot}$, 37), 370 ($[M]^{+\cdot}$, 100), 355 (31), 203 (26), 195 (65).	96



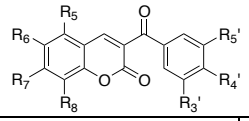
Compuesto	NMR	MS	Rdto. (%)
67	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.02 (s, 1H, H-4), 7.29-7.12 (m, 3H, H-5, H-6, H-7), 7.11 (s, 2H, H-2', H-6'), 4.19 (q, *J* = 6.9 Hz, 2H, -CH), 3.91 (s, 3H, OCH_3), 3.85 (s, 6H, 2x OCH_3), 1.47 (s, *J* = 6.9 Hz, 3H, CH_3).	*m/z* (%): 385 ([M+1]$^{+\cdot}$, 36), 384 ([M]$^{+\cdot}$, 100), 369 (31), 195 (69).	99
68	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.29 (s, 1H, H-4), 7.66 (d, *J* = 2.2 Hz, 1H, H-5), 7.58 (d, *J* = 2.2 Hz, 1H, H-7), 7.26 (s, 2H, H2', H-6'), 3.99 (s, 3H, OCH_3), 3.84 (s, 6H, 2x OCH_3), 3.80 (s, 3H, OCH_3).	*m/z* (%): 450 ([M+2]$^{+\cdot}$, 84), 448 ([M]$^{+\cdot}$, 84), 283 (11), 281 (11), 196 (15), 195 (100).	92
69	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.99 (s, 1H, H-4), 7.52-7.23 (m, 3H, H-5, H-7, H-8), 7.12 (s, 2H, H-2', H-6'), 3.93 (s, 3H, OCH_3), 3.87 (s, 6H, 2x OCH_3), 2.43 (s, 3H, CH_3).	*m/z* (%): 355 ([M+1]$^{+\cdot}$, 37), 354 ([M]$^{+\cdot}$, 100), 339 (36), 283 (13), 195 (47), 187 (34).	95
70	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.03 (s, 1H, H-4), 7.50 (d, *J* = 7.4 Hz, 1H, H-5), 7.43 (dd, *J* = 7.4, 1.5 Hz, 1H, H-7), 7.30-7.18 (m, 1H, H-6), 7.14 (s, 2H, H-2', H-6'), 3.93 (s, 3H, OCH_3), 3.87 (s, 6H, 2x OCH_3), 2.50 (s, 3H, CH_3).	*m/z* (%): 355 ([M+1]$^{+\cdot}$, 31), 354 ([M]$^{+\cdot}$, 100), 339 (42), 283 (14), 195 (39), 187 (31).	96
71	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.82 (s, 1H, H-4), 8.67-8.38 (m, 2H, H-5, H-7), 7.70 (d, *J* = 9.2 Hz, 1H, H-8), 7.27 (s, 2H, H-2', H-6'), 4.07-3.60 (m, 9H, 3x OCH_3).	*m/z* (%): 386 ([M+1]$^{+\cdot}$, 34), 385 ([M]$^{+\cdot}$, 100), 370 (31), 314 (17), 218 (30), 195 (32).	51



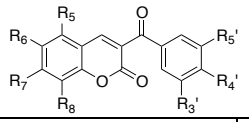
Compuesto	NMR	MS	Rdto. (%)
72	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.97 (s, 1H, H-4), 7.02 (s, 2H, H-2', H-6'), 6.31 (d, *J* = 2.7 Hz, 1H, H-6), 6.26 (d, *J* = 2.7 Hz, 1H, H-8), 3.93 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.87 (s, 6H, 2x OCH_3).	*m/z* (%): 401 ($[M+1]^{+\cdot}$, 10), 400 ($[M]^{+\cdot}$, 45), 191 (18), 135 (13), 134 (100).	95
73	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.94 (s, 1H, H-4), 7.66-7.53 (m, 2H, H-5, H-7), 7.36 (d, *J* = 7.4 Hz, 1H, H-8), 7.11 (s, 2H, H-2', H-6'), 3.93 (s, 3H, OCH_3), 3.87 (s, 6H, 2x OCH_3).	*m/z* (%): 376 ($[M+2]^{+\cdot}$, 34), 374 ($[M]^{+\cdot}$, 100), 359 (32), 207 (25), 195 (40).	94
74	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.06 (s, 1H, H-4), 7.50 (d, *J* = 8.5 Hz, 1H, H-5), 7.11 (s, 2H, H-2', H-6'), 6.95-6.83 (m, 2H, H-6, H-8), 3.93 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.87 (s, 6H, 2x OCH_3).	*m/z* (%): 371 ($[M+1]^{+\cdot}$, 22), 370 ($[M]^{+\cdot}$, 100), 355 (23), 327 (16), 203 (28), 195 (28).	97
75	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.65 (s, 1H, H-4), 8.57 (s, 1H), 8.49 (dd, *J* = 8.2, 2.2 Hz, 1H, H-2'), 8.35 (d, *J* = 6.9 Hz, 1H, H-4'), 7.95-7.69 (m, 3H, Ar), 7.56-7.38 (m, 2H, Ar).	*m/z* (%): 296 ($[M+1]^{+\cdot}$, 19), 295 ($[M]^{+\cdot}$, 100), 278 (24), 266 (47), 250 (23), 248 (25), 220 (21), 173 (74), 150 (44), 145 (36), 104 (32), 89 (25), 76 (28).	89
76	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.63 (s, 1H, H-4), 8.44 (dt, *J* = 8.0, 1.5 Hz, 1H, H-6'), 8.24 (s, 1H, H-5), 8.16 (d, *J* = 7.3 Hz, 1H, H-4'), 7.68 (t, *J* = 8.0 Hz, 1H, H-5'), 7.41-7.21 (m, 2H, Ar), 7.05 (d, *J* = 2.8 Hz, 1H, H-7), 3.88 (s, 3H, OCH_3).	*m/z* (%): 326 ($[M+1]^{+\cdot}$, 45), 325 ($[M]^{+\cdot}$, 100), 297 (18), 296 (35), 203 (51), 175 (41), 150 (73), 119 (32), 104 (48), 76 (46).	94



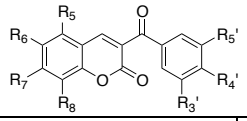
Compuesto	NMR	MS	Rdto. (%)
77	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.65 (s, 1H, H-4), 8.55-8.42 (m, 2H, H-2', H-4'), 8.35 (d, J = 7.1 Hz, 1H, H-6'), 8.14 (dd, J = 4.9, 2.5 Hz, 1H, H-5'), 7.97-7.75 (m, 2H, H-5, H-6), 7.47 (d, J = 8.8 Hz, 1H, H-8).	*m/z* (%): 375 ([M+2]$^{+\cdot}$, 99), 373 ([M]$^{+\cdot}$, 100), 346 (26), 344 (25), 253 (36), 251 (37), 225 (23), 223 (23), 150 (89), 104 (46), 76 (40)	90
78	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.64 (s, 1H, H-4), 8.58-8.39 (m, 2H, Ar), 8.34 (d, J = 7.8 Hz, 1H), 7.82 (t, J = 7.8 Hz, 1H), 7.57-7.24 (m, 3H, Ar), 3.94 (s, 3H, OCH_3).	*m/z* (%): 326 ([M+1]$^{+\cdot}$, 21), 325 ([M]$^{+\cdot}$, 100), 296 (24), 203 (27), 175 (30), 150 (43), 104 (28), 76 (26).	82
79	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.64 (td, J = 4.5, 3.9, 1.9 Hz, 1H), 8.43 (d, J = 8.1 Hz, 1H, Ar), 8.34-8.22 (m, 1H, Ar), 8.15 (d, J = 9.2 Hz, 1H, Ar), 7.68 (qd, J = 7.8, 3.9 Hz, 1H, Ar), 7.42-7.06 (m, 3H, Ar), 4.21 (q, J = 6.9 Hz, 2H, CH_2), 1.57-1.45 (t, 3H, CH_3).	*m/z* (%): 340 ([M+1]$^{+\cdot}$, 29), 339 ([M]$^{+\cdot}$, 100), 312 (21), 311 (88), 283 (23),, 282 (42), 266 (27), 264 (25), 188 (45), 160 (40), 150 (98), 120 (19), 105 (18), 104 (56), 92 (17), 76 (42).	91
80	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.70-8.56 (m, 1H, Ar), 8.56-8.39 (m, 2H, Ar), 8.34 (d, J = 7.8 Hz, 1H), 7.83 (t, J = 7.8 Hz, 1H), 7.68 (d, J = 2.0 Hz, 1H), 7.59 (d, J = 2.0 Hz, 1H), 3.97 (s, 3H, OCH_3).	*m/z* (%): 405 ([M+2]$^{+\cdot}$, 97), 403 ([M]$^{+\cdot}$, 97), 253 (23), 150 (100), 104 (50), 76 (43).	77
81	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.68-8.56 (m, 1H), 8.49 (d, J = 5.8 Hz, 2H), 8.33 (d, J = 7.7 Hz, 1H), 7.82 (t, J = 8.0 Hz, 1H), 7.67 (d, J = 2.2 Hz, 1H), 7.58 (dd, J = 8.5, 2.2 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 2.38 (s, 3H).	*m/z* (%): 310 ([M+1]$^{+\cdot}$, 27), 309 ([M]$^{+\cdot}$, 100), 281 (21), 280 (56), 264 (22), 262 (30), 234 (22), 187 (74), 159 (50), 150 (52), 104 (45), 103 (27), 77 (20), 76 (40).	88



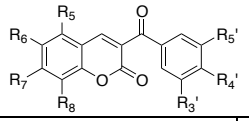
Compuesto	NMR	MS	Rdto. (%)
82	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.64 (d, *J* = 2.0 Hz, 1H, H-4), 8.44 (d, *J* = 7.7 Hz, 1H, H-4'), 8.28 (s, 1H, H-2'), 8.22-8.06 (m, 1H), 7.68 (t, *J* = 8.0 Hz, 1H), 7.61-7.39 (m, 2H), 7.40 -7.18 (m, 1H), 2.51 (s, 3H, CH_3).	*m/z* (%): 310 ($[M+1]^{+\cdot}$, 55), 309 ($[M]^{+\cdot}$, 100), 281 (36), 280 (70), 263 (26), 262 (44), 235 (28), 234 (36), 187 (85), 159 (51), 150 (64), 104 (51), 76 (50).	79
84	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.53 (t, *J* = 2.1 Hz, 1H), 8.48-8.30 (m, 2H), 8.22 (dt, *J* = 7.1, 1.2 Hz, 1H), 7.78 (t, *J* = 8.0 Hz, 1H), 6.68 (d, *J* = 2.2 Hz, 1H), 6.56 (d, *J* = 2.2 Hz, 1H), 3.92 (s, 4H), 3.90 (s, 3H).	*m/z* (%): 356 ($[M+1]^{+\cdot}$, 32), 355 ($[M]^{+\cdot}$, 100), 327 (36), 326 (25), 325 (26), 234 (20), 233 (93), 150 (21), 104 (16), 76 (17).	89
85	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.71 (s, 1H, H-4), 8.63-8.51 (m, 2H, H-2', H-4'), 8.42 (d, *J* = 7.1 Hz, 1H, H-6'), 8.14 (dd, *J* = 4.9, 2.5 Hz, 1H, H-5'), 7.98-7.77 (m, 2H, H-5, H-6), 7.57 (d, *J* = 8.8 Hz, 1H, H-8).	*m/z* (%): 331 ($[M+2]^{+\cdot}$, 50), 329 ($[M]^{+\cdot}$, 100), 302 (20), 301 (22), 300 (47), 284 (39), 282 (32), 255 (22), 254 (25), 209 (27), 207 (65), 181 (18), 151 (17), 150 (99), 123 (39), 104 (65), 76 (57), 75 (23).	80
86	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.57 (s, 1H, H-4), 8.52 (s, 1H, H-2'), 8.45 (dd, *J* = 8.0, 2.6 Hz, 1H, H-4'), 8.27 (d, *J* = 7.7 Hz, 1H, H-6'), 7.89-7.70 (m, 2H, H-5, H-5'), 7.16-6.93 (m, 2H, H-6, H-8), 3.89 (s, 3H, OCH_3).	*m/z* (%): 326 ($[M+1]^{+\cdot}$, 37), 325 ($[M]^{+\cdot}$, 100), 297 (34), 296 (36), 203 (85), 150 (23), 104 (21), 76 (24).	85
89	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 10.38 (s, 1H), 8.36 (s, 1H), 7.96-7.81 (m, 2H), 7.69 (t, *J* = 7.4 Hz, 1H), 7.61-7.47 (m, 2H), 7.33-7.09 (m, 3H).	*m/z* (%): 267 ($[M+1]^{+\cdot}$, 7), 266 ($[M]^{+\cdot}$, 42), 105 (100), 77 (60)	82



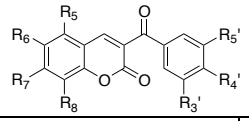
Compuesto	NMR	MS	Rdto. (%)
90	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 11.11 (s, 1H), 10.86 (s, 1H), 8.25 (s, 1H), 7.77 (d, J = 7.7 Hz, 2H), 7.71-7.33 (m, 3H), 6.28 (s, 1H), 6.24 (s, 1H).	*m/z* (%): 283 ([M+1]$^{+\cdot}$, 29), 282 ([M]$^{+\cdot}$, 100), 254 (30), 253 (69), 205 (72), 105 (88), 77 (72).	75
91	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 10.95 (s, 1H), 8.32 (s, 1H), 7.84 (d, J = 7.4 Hz, 2H), 7.75-7.58 (m, 2H), 7.50 (t, J = 7.5 Hz, 2H), 6.92-6.66 (m, 2H).	*m/z* (%): 267 ([M+1]$^{+\cdot}$, 31), 266 ([M]$^{+\cdot}$, 100), 238 (35), 237 (68), 189 (63), 161 (22), 105 (97), 77 (79).	79
98	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 10.58 (sa, 1H, OH), 8.23 (s, 1H, H-4), 7.80 (d, J = 8.7 Hz, 2H, H-2', H-6'), 7.64 – 7.46 (m, 2H, H-5, H-7), 7.38 (d, J = 8.5 Hz, 1H, H-8), 6.86 (d, J = 8.7 Hz, 2H, H-3', H-5').	*m/z* (%): 280 ([M]$^{+\cdot}$, 65), 251 (21), 237 (14), 93 (19), 76 (15), 65 (24).	89
99	^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 10.56 (sa, 1H, -OH, 4'-OH), 10.33 (sa, 1H, -OH, 8-OH), 8.22 (s, 1H, H-4), 7.79 (d, J = 8.7 Hz, 2H, H-2', H-6'), 7.29-7.06 (m, 3H, H-5, H-6, H-7), 6.86 (d, J = 8.7 Hz, 2H, H-3', H-5').	*m/z* (%): 283 ([M+1]$^{+\cdot}$, 9), 282 ([M]$^{+\cdot}$, 49), 252 (46), 120 (100).	71
100	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 10.80 (s, 1H), 10.41 (s, 1H), 8.18 (s, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.4 Hz, 1H), 6.85 (d, J = 9.0 Hz, 3H), 6.77 (s, 1H).	*m/z* (%): 283 ([M+1]$^{+\cdot}$, 22), 282 ([M]$^{+\cdot}$, 92), 254 (18), 253 (30), 237 (19), 189 (22), 121 (100), 98 (26), 93 (25), 73 (19), 71 (19), 55 (30).	68



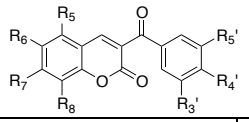
Compuesto	NMR	MS	Rdto. (%)
108	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 10.06 (s, 1H), 9.43 (s, 1H), 8.24 (d, J = 1.5 Hz, 1H), 7.71-7.49 (m, 2H), 7.42-7.19 (m, 3H), 6.82 (d, J = 8.1 Hz, 1H), 2.40 (s, 3H).	*m/z* (%): 297 ([M+1]$^{+\cdot}$, 28), 296 ([M]$^{+\cdot}$, 93), 268 (15), 267 (21), 187 (63), 137 (100), 109 (28), 81 (18), 77 (15).	77
109	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 10.85 (s, 1H), 10.00 (s, 1H), 9.42 (s, 1H), 8.16 (s, 1H), 7.63 (d, J = 8.2 Hz, 1H), 7.35-7.15 (m, 2H), 6.90-6.68 (m, 3H).	*m/z* (%): 299 ([M+1]$^{+\cdot}$, 16), 298 ([M]$^{+\cdot}$, 85), 269 (15), 189 (69), 161 (19), 137 (100), 109 (25).	74
111	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 9.85 (s, 1H, -OH), 9.29 (d, J = 19.0 Hz, 3H, 3X -OH), 8.17 (s, 1H, H-4), 7.33 (d, J = 9.6 Hz, 1H, H-8), 7.18-7.06 (m, 2H, H-5, H-7), 6.87 (s, 2H, H-2', H-6').	*m/z* (%): 315 ([M+1]$^{+\cdot}$, 31), 314 ([M]$^{+\cdot}$, 92), 189 (73), 161 (39), 153 (100), 125 (21), 79 (16).	81
112	^{1}H-NMR (250 MHz DMSO-d_6) δ ppm 9.33 (bs, 3H, 3x OH), 8.19 (s, 1H, H-4), 8.06 (s, 1H, H-5), 7.84 (d, J = 8.3 Hz, 1H, H-7), 7.45 (d, J = 8.3 Hz, 1H, H-8), 6.90 (s, 2H, H-2', H-6').	*m/z* (%): 377 ([M+2]$^{+\cdot}$, 27), 375 ([M]$^{+\cdot}$, 32), 297 (41), 251 (59), 249 (63), 153 (100), 125 (25), 79 (18).	70
113	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 9.34 (bs, 3H, 3x-OH), 8.18 (s, 1H, H-4), 7.59 (s, 1H, H-5), 7.52 (d, J = 8.4 Hz, 1H, H-7), 7.37 (d, J = 8.8 Hz, 1H, H-8), 6.88 (s, 2H, H-2', H-6'), 2.37 (s, 3H, CH_3).	*m/z* (%): 313 ([M+1]$^{+\cdot}$, 29), 312 ([M]$^{+\cdot}$, 100), 283 (15), 187 (85), 153 (94), 125 (24), 79 (15).	57
114	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 9.32 (bs, 3H, 3x OH), 8.20 (s, 1H, H-4), 7.93 (d, J = 3.1 Hz, 1H, H-5), 7.73 (d, J = 9.2, 2.9 Hz, 1H, H-7), 7.52 (d, J = 9.0 Hz, 1H, H-8), 6.90 (s, 2H, H-2', H-6').	*m/z* (%): 334 ([M+2]$^{+\cdot}$, 24), 332 ([M]$^{+\cdot}$, 70), 266 (18), 207 (48), 153 (100), 125 (21), 79 (15).	58



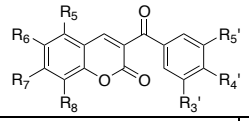
Compuesto	NMR	MS	Rdto. (%)
115	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 10.88 (s, 1H, OH), 9.29 (s, 3H, 3x OH), 8.14 (s, 1H, H-4), 7.46 (s, 1H, H-5), 7.27 (s, 1H, H-7), 6.88 (s, 2H, H-2', H-6').	*m/z* (%): 394 ([M+2]$^{+\cdot}$, 54), 392 ([M]$^{+\cdot}$, 47), 378 (13), 348 (12), 269 (12), 153 (100), 125 (18), 79 (13).	82
116	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 10.34 (s, 1H, OH), 9.31 (s, 3H, 3x OH), 8.20 (s, 1H, H-4), 7.35-7.04 (m, 3H, H-5, H-6, H-7), 6.88 (s, 2H, H-2', H-6').	*m/z* (%): 315 ([M+1]$^{+\cdot}$, 19), 314 ([M]$^{+\cdot}$, 88), 299 (21), 298 (95), 288 (42), 270 (63), 253 (21), 189 (27), 153 (100), 139 (18), 125 (20), 79 (14).	54
117	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 9.31 (bs, 3H, 2x-OH), 8.24 (s, 1H, H-4), 7.72-7.48 (m, 2H, H-5, H-6), 7.44-7.15 (m, 1H, H-7), 6.89 (s, 2H, H-2', H-6'), 2.45-2.13 (m, 3H).	*m/z* (%): 313 ([M+1]$^{+\cdot}$, 22), 312 ([M]$^{+\cdot}$, 97), 187 (92), 153 (100), 125 (27), 77 (13).	70
118	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 11.15 (s, 1H, OH), 11.01 (s, 1H, OH), 10.74 (s, 1H, OH), 9.30 (s, 2H, 2xOH), 8.05 (s, 1H, H-4), 6.80 (s, 2H, H-2', H-6'), 6.29 (d, J = 2.3 Hz, 1H, H-6), 6.24 (d, J = 2.2 Hz, 1H, H-8).	*m/z* (%): 331 ([M+1]$^{+\cdot}$, 12), 330 ([M]$^{+\cdot}$, 18), 223 (47), 211 (41), 181 (100), 180 (39), 152 (18), 115 (19), 103 (51), 91 (24), 77 (17), 69 (15).	62
120	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 9.88 (s, 1H, -OH), 8.28 (s, 1H, H-4), 7.79 (d, J = 8.2 Hz, 2H, H-2', H-6'), 7.39-7.24 (m, 3H, H-8, H-3', H-5'), 7.20-7.04 (m, 2H, H-5, H-7), 2.38 (s, 3H, CH_3).	*m/z* (%): 281 ([M+1]$^{+\cdot}$, 20), 280 ([M]$^{+\cdot}$, 80), 279 (15), 265 (17), 252 (17), 251 (18), 237 (28), 119 (100), 91 (56), 65 (16).	72



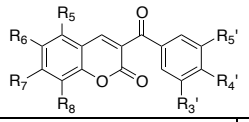
Compuesto	NMR	MS	Rdto. (%)
121	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 10.96 (s, 1H, OH), 8.29 (s, 1H, H-4), 7.74 (d, J = 8.1 Hz, 2H, H-2', H-6'), 7.68 (d, J = 8.4 Hz, 1H, H-5), 7.31 (d, J = 7.9 Hz, 2H, H-3', H-5'), 6.84 (dd, J = 8.4, 2.3 Hz, 1H, H-6), 6.77 (d, J = 2.2 Hz, 1H, H-8), 2.37 (s, 3H, CH_3).	*m/z* (%): 281 ([M+1]$^{+\cdot}$, 20), 280 ([M]$^{+\cdot}$, 100), 265 (26), 252 (28), 189 (26), 119 (99), 105 (16), 91 (61), 65 (18).	74
122	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 11.03 (s, 1H), 10.79 (s, 1H), 8.22 (s, 1H), 7.69 (d, J = 7.9 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 6.27 (dd, J = 12.6, 2.0 Hz, 2H).	*m/z* (%): 297 ([M+1]$^{+\cdot}$, 12), 296 ([M]$^{+\cdot}$, 63), 281 (22), 267 (19), 205 (25), 204 (18), 119 (100), 91 (63), 65 (18).	68
125	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.11 (s, 1H), 7.61 (d, J = 8.7 Hz, 2H), 7.44-7.18 (m, 3H), 6.57 (d, J = 8.7 Hz, 2H), 6.34 (s, 2H), 3.79 (s, 3H).	*m/z* (%): 296 ([M+1]$^{+\cdot}$, 21), 295 ([M]$^{+\cdot}$, 71), 121 (15), 120 (100), 92 (28), 65 (20).	62
126	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.10 (s, 1H), 7.69-7.53 (m, 3H), 7.49 (dd, J = 8.5, 2.2 Hz, 1H), 7.35 (d, J = 8.6 Hz, 1H), 6.57 (d, J = 8.3 Hz, 2H), 6.31 (s, 2H), 2.36 (s, 3H).	*m/z* (%): 280 ([M+1]$^{+\cdot}$, 26), 279 ([M]$^{+\cdot}$, 55), 187 (16), 120 (100), 92 (31), 65 (20).	55
127	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 7.99 (s, 1H), 7.58 (d, J = 8.8 Hz, 2H), 7.16 (d, J = 8.7 Hz, 1H), 6.91 (dd, J = 8.8, 2.7 Hz, 1H), 6.80 (d, J = 2.7 Hz, 1H), 6.56 (d, J = 8.3 Hz, 2H), 6.27 (s, 2H), 5.29 (s, 2H).	*m/z* (%): 281 ([M+1]$^{+\cdot}$, 19), 280 ([M]$^{+\cdot}$, 57), 120 (100), 92 (26), 65 (15).	92
128	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.11 (s, 1H), 7.88 (d, J = 2.6 Hz, 1H), 7.77-7.58 (m, 3H), 7.48 (d, J = 8.9 Hz, 1H), 6.58 (d, J = 8.3 Hz, 2H), 6.31 (s, 2H).	*m/z* (%): 301 ([M+2]$^{+\cdot}$, 31), 299 ([M]$^{+\cdot}$, 59), 121 (19), 120 (100), 92 (33), 65 (25).	34



Compuesto	NMR	MS	Rdto. (%)
129	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.16 (s, 1H), 7.71-7.45 (m, 4H), 7.28 (t, J = 7.6 Hz, 1H), 6.57 (d, J = 8.4 Hz, 2H), 6.31 (s, 2H), 2.39 (s, 3H).	*m/z* (%): 280 ([M+1]$^{+\cdot}$, 19), 279 ([M]$^{+\cdot}$, 79), 120 (100), 92 (26), 65 (17).	42
133	^{1}H-NMR (250 MHz, DMSO-*d6*) δ ppm 8.12 (s, 1H), 7.70 (dd, J = 8.4, 2.2 Hz, 1H), 7.60 (dd, J = 8.9, 2.4 Hz, 2H), 7.12-6.86 (m, 2H), 6.57 (d, J = 8.8 Hz, 2H), 6.24 (s, 2H), 3.87 (s, 3H).	*m/z* (%): 296 ([M+1]$^{+\cdot}$, 52), 295 ([M]$^{+\cdot}$, 87), 267 (23), 266 (24), 203 (26), 121 (27), 120 (100), 92 (56), 65 (44).	57
134	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.00 (s, 1H), 7.56 (d, J = 8.3 Hz, 2H), 6.65 (s, 1H), 6.55 (d, J = 8.6 Hz, 3H), 6.23 (s, 2H), 3.88 (s, 6H).	*m/z* (%): 326 ([M+1], 28), 325 ([M]$^{\cdot}$, 93), 310 (25), 233 (46), 120 (100), 92 (28).	71
137	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.25 (s, 1H), 7.48-7.35 (m, 2H), 7.29 (dd, J = 9.0, 3.1 Hz, 1H), 7.16 (t, J = 7.7 Hz, 1H), 7.09-6.92 (m, 2H), 6.84 (dd, J = 7.9, 2.4 Hz, 1H), 5.41 (s, 2H), 3.79 (s, 3H).	*m/z* (%): 296 ([M+1]$^{+\cdot}$, 32), 295 ([M]$^{+\cdot}$, 100), 266 (22), 251 (17), 120 (74), 92 (52), 65 (29).	65
138	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.24 (s, 1H), 7.60 (s, 1H), 7.52 (dd, J = 7.4, 2.9 Hz, 1H), 7.37 (d, J = 8.6 Hz, 1H), 7.16 (t, J = 7.7 Hz, 1H), 7.09-6.94 (m, 2H), 6.85 (dd, J = 7.7, 2.6 Hz, 1H), 5.38 (s, 2H), 2.36 (s, 3H).	*m/z* (%): 280 ([M+1]$^{+\cdot}$, 31), 279 ([M]$^{+\cdot}$, 100), 250 (26)187 (21), 120 (68), 92 (51), 65 (29).	87
139	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.27 (s, 1H), 7.94 (d, J = 2.6 Hz, 1H), 7.74 (dd, J = 8.9, 2.6 Hz, 1H), 7.52 (d, J = 8.8 Hz, 1H), 7.23-6.98 (m, 3H), 6.86 (d, J = 6.4 Hz, 1H), 5.41 (s, 2H).	*m/z* (%): 301 ([M+1]$^{+\cdot}$, 68), 299 ([M]$^{+\cdot}$, 100), 270 (21), 121 (23), 120 (99), 92 (95, 65 (68).).	59

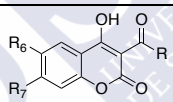


Compuesto	NMR	MS	Rdto. (%)
140	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.30 (s, 1H), 7.65 (dd, J = 7.8, 1.6 Hz, 1H), 7.58 (d, J = 7.3 Hz, 1H), 7.30 (t, J = 7.6 Hz, 1H), 7.24-6.97 (m, 3H), 6.85 (dd, J = 7.7, 2.5 Hz, 1H), 5.36 (s, 2H), 2.40 (s, 3H).	*m/z* (%): 280 ([M+1]$^{+\cdot}$, 60), 279 ([M]$^{+\cdot}$, 100), 250 (48), 223 (28), 187 (42), 120 (95), 92 (96), 65 (66).	64
143	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.23 (s, 1H), 7.58 (d, J = 15.8 Hz, 2H), 7.16 (t, J = 7.8 Hz, 1H), 7.10-6.94 (m, 2H), 6.85 (d, J = 7.9 Hz, 1H), 5.38 (s, 2H), 3.95 (s, 3H).	*m/z* (%): 375 ([M+1]$^{+\cdot}$, 77), 373 ([M]$^{+\cdot}$, 77), 121 (16), 120 (100), 92 (66), 65 (37).	61
144	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.28 (s, 1H), 7.75 (d, J = 8.6 Hz, 1H), 7.23-6.92 (m, 6H), 6.83 (d, J = 7.9 Hz, 1H), 5.39 (s, 2H), 3.88 (s, 3H).	*m/z* (%): 296 ([M+1]$^{+\cdot}$, 32), 295 ([M]$^{+\cdot}$, 100), 266 (28), 203 (55), 187 (42), 120 (75), 92 (68), 65 (39).	60
145	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.13 (s, 1H), 7.14 (t, J = 7.8 Hz, 1H), 7.00 (t, J = 2.0 Hz, 1H), 6.93 (dt, J = 7.5, 1.3 Hz, 1H), 6.81 (dd, J = 7.4, 2.3 Hz, 1H), 6.66 (d, J = 2.1 Hz, 1H), 6.54 (d, J = 2.1 Hz, 1H), 5.36 (s, 2H), 3.89 (s, 6H).	*m/z* (%): 326 ([M+1]$^{+\cdot}$, 29), 325 ([M]$^{+\cdot}$, 100), 310 (16), 297 (18), 296 (17), 233 (55), 120 (32), 92 (35), 65 (20).	59
146	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.23 (s, 1H), 7.87 (d, J = 7.2 Hz, 2H), 7.67 (t, J = 7.2 Hz, 1H), 7.61-7.45 (m, 2H), 7.19 (d, J = 8.8 Hz, 1H), 6.96 (dd, J = 8.8, 2.7 Hz, 1H), 6.85 (d, J = 2.8 Hz, 1H), 5.35 (s, 2H).	*m/z* (%): 266 ([M+1]$^{+\cdot}$, 25), 265 ([M]$^{+\cdot}$, 94), 237 (18), 236 (32), 105 (100), 77 (67).	72

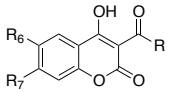


Compuesto	NMR	MS	Rdto. (%)
147	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.15 (s, 1H), 7.86 (d, J = 8.8 Hz, 2H), 7.18 (d, J = 8.7 Hz, 1H), 7.04 (d, J = 8.2 Hz, 2H), 6.94 (dd, J = 8.8, 2.6 Hz, 1H), 6.83 (d, J = 2.8 Hz, 1H), 5.33 (s, 2H), 3.84 (s, 3H).	*m/z* (%): 296 ([M+1]$^{+\cdot}$, 34), 295 ([M]$^{+\cdot}$, 100), 136 (26), 135 (79), 107 (19), 92 (25), 77 (35).	92
148	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.12 (s, 1H), 7.55-7.38 (m, 2H), 7.17 (d, J = 8.7 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 6.94 (d, J = 8.9 Hz, 1H), 6.83 (s, 1H), 5.30 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H).	*m/z* (%): 326 ([M+1]$^{+\cdot}$, 27), 325 ([M]$^{+\cdot}$, 83), 166 (19), 165 (100), 77 (18).	81

Tabla 8. 3-Benzoil-4-hidroxicumarinas no publicadas.

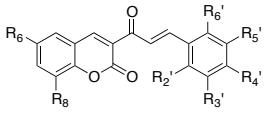
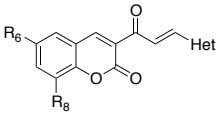


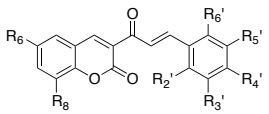
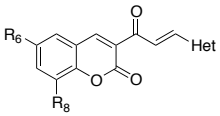
Compuesto	NMR	MS	Rdto. (%)
149	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm (dd, J = 7.8, 1.6 Hz, 1H, H-5), 7.87 (dd, J = 8.1, 1.4 Hz, 2H, H-2', H-6'), 7.74 (ddd, J = 8.5, 7.1, 1.7 Hz, 1H, H-7), 7.64 (t, J = 7.3 Hz, 1H, H-4'), 7.56-7.37 (m, 4H, H-6, H-8, H-3', H-5').	*m/z* (%): 267 ([M+1]$^{+\cdot}$, 55), 266 ([M]$^{+\cdot}$, 94), 120 (26), 105 (100), 77 (99).	87
152	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.31 (dd, J = 8.8, 2.3 Hz, 2H, H-2', H-6'), 8.15 (dd, J = 9.7, 2.2 Hz, 1H, H-5), 7.89-7.64 (m, 3H, H-7, H-3', H-5'), 7.49-7.20 (m, 2H, H-6, H-8).	*m/z* (%): 311 ([M+1]$^{+\cdot}$, 54), 310 ([M]$^{+\cdot}$, 100), 294 (41), 264 (30), 150 (77), 120 (42), 105 (34), 76 (32).	69

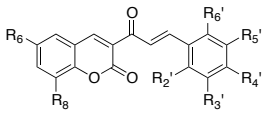
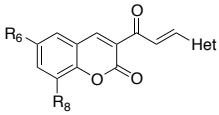


Compuesto	NMR	MS	Rdto. (%)
153	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.10 (dd, *J* = 7.8, 1.6 Hz, 1H, H-5), 7.71 (ddt, *J* = 8.8, 7.4, 1.8 Hz, 1H, H-7), 7.64 (d, *J* = 8.2 Hz, 2H, H-2', H-6'), 7.41-7.21 (m, 4H, H-6, H-8, H-3', H-5'), 2.43 (d, *J* = 1.6 Hz, 3H, -CH_3).	*m/z* (%): 281 ([M+1]$^{+\cdot}$, 75), 280 ([M]$^{+\cdot}$, 100), 265 (17), 119 (75), 91 (34).	81
154	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.12 (dd, *J* = 7.9, 1.7 Hz, 1H, H-5), 7.79-7.70 (m, 2H, 2xAr-H), 7.55-7-48 (m, 2H, 2x Ar-H), 7.43-7.30 (m, 2H, 2x Ar-H).	*m/z* (%): 337 ([M+4]$^{+\cdot}$, 16), 335 ([M+2]$^{+\cdot}$, 74), 333 ([M]$^{+\cdot}$, 100), 175 (49), 173 (77), 147 (18), 145 (28), 92 (17), 69 (21).	71
156	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.15-8.02 (m, 2H, H-3', H-5'), 7.81-7.63 (m, 2H, H-5, H-7), 7.41-7.23 (m, 2H, H-6, H-8), 6.64 (dd, *J* = 3.7, 1.7 Hz, 1H, H-4').	*m/z* (%): 257 ([M+1]$^{+\cdot}$, 21), 256 ([M]$^{+\cdot}$, 76), 229 (25), 228 (92), 121 (100), 120 (57), 95 (70), 65 (21).	52
159	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 7.82 (s, 1H, H-5), 7.74-7.63 (m, 2H, H-2', H-6'), 7.62-7.51 (m, 1H, H-4'), 7.51-7.38 (m, 2H, H3', H-5'), 7.10 (s, 1H, H-8), 2.39 (s, 3H, 7-CH_3), 2.35 (s, 3H, 6-CH_3).	*m/z* (%): 294 ([M+1]$^{+\cdot}$, 70), 293 ([M]$^{+\cdot}$, 100), 149 (25), 148 (30), 105 (99), 91 (23), 77 (65).	68

Tabla 9. 3-Cinamoilcumarinas no publicadas.

			
Compuesto	NMR	MS	Rdto. (%)
163	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.57 (s, 1H), 7.88 (d, *J* = 15.5 Hz 1H), 7.87 (s, *J* = 15.5 Hz, 1H), 7.70-7.61 (m, 2H), 7.57 (d, *J* = 8.1 Hz, 2H), 7.43-7.33 (m, 2H), 7.21 (d, *J* = 7.8 Hz, 2H), 2.39 (s, 3H).	*m/z (%):* 291 ([M+1]$^{+\cdot}$, 20), 290 ([M]$^{+\cdot}$, 100), 289 (31), 276 (18), 275 (92), 262 (33), 261 (28), 145 (68), 117 (47), 116 (20), 115 (62), 91 (40), 77 (45), 89 (27).	72
164	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.37 (s, 1H), 8.00 (d, *J* = 15.8 Hz, 1H), 7.72 (d, *J* = 15.7 Hz, 1H), 7.55-7.39 (m, 3H), 7.27-7.11 (m, 2H), 6.36 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.29 (d, *J* = 2.4 Hz, 1H), 3.73 (s, 3H), 3.69 (s, 3H).	*m/z (%):* 337 ([M+1]$^{+\cdot}$, 24), 336 ([M]$^{+\cdot}$, 100), 305 (42), 191 (53), 145 (41), 137 (29), 92 (18), 77 (15).	94
165	^{1}H-NMR (250 MHz,$CDCl_3$) δ ppm 8.38 (s, 1H), 8.05 (d, *J* = 15.8 Hz, 1H), 7.65 (d, *J* = 15.7 Hz, 1H), 7.56-7.39 (m, 2H), 7.27-7.12 (m, 2H), 7.01 (s, 1H), 6.33 (s, 1H), 3.78 (s, 3H), 3.74 (d, *J* = 1.7 Hz, 6H).	*m/z (%):* 337 ([M+1]$^{+\cdot}$, 28), 336 ([M]$^{+\cdot}$, 100), 308 (32), 293 (42), 161 (91), 133 (19), 89 (17).	96
166	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.48 (s, 1H), 8.33 (d, *J* = 16.1 Hz, 1H), 8.15 (d, *J* = 15.9 Hz, 1H), 7.72-7.53 (m, 2H), 7.43-7.22 (m, 2H), 6.09 (s, 2H), 3.89 (s, 6H), 3.84 (s, 3H).	*m/z (%):* 367 ([M+1]$^{+\cdot}$, 14), 366 ([M]$^{+\cdot}$, 46), 336 (34), 335 (100), 221 (18), 178 (21), 168 (18).	98
169	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.57 (s, 1H), 7.89 (d, *J* = 15.7 Hz, 1H), 7.77 (d, *J* = 15.7 Hz, 1H), 7.72-7.58 (m, 2H), 7.44-7.30 (m, 2H), 6.80 (d, *J* = 2.3 Hz, 2H), 6.52 (t, *J* = 2.3 Hz, 1H), 3.83 (s, 6H).	*m/z (%):* 336 ([M]$^{+\cdot}$, 81), 307 (42), 292 (53), 291 (100), 148 (17), 89 (30), 69 (18).	91

			
Compuesto	NMR	MS	Rdto. (%)
170	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.65 (s, 1H), 8.03-7.82 (m, 2H), 7.82-7.63 (m, 2H), 7.56-7.24 (m, 3H), 7.24-7.08 (m, 2H), 3.81 (s, 3H), 3.76 (s, 3H).	*m/z (%):* 336 ([M+1]$^{+\cdot}$, 81), 337 ([M]$^{+\cdot}$, 42), 292 (53), 291 (100), 148 (17), 89 (30), 69 (18).	87
171	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.54 (s, 1H), 8.18 (d, *J* = 15.8 Hz, 1H), 7.93 (d, *J* = 15.8 Hz, 1H), 7.73-7.57 (m, 2H), 7.44-7.28 (m, 2H), 7.21 (d, *J* = 3.0 Hz, 1H), 7.00-6.79 (m, 2H), 3.87 (s, 3H), 3.81 (s, 3H).	*m/z (%):* 337 ([M+1]$^{+\cdot}$, 10), 336 ([M]$^{+\cdot}$, 29), 306 (34), 305 (100), 173 (23), 89 (26).	83
173	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.47 (s, 1H), 7.85 (s, 2H), 7.70-7.46 (m, 4H), 7.34 (d, *J* = 8.9 Hz, 1H), 7.22 (d, *J* = 7.9 Hz, 2H), 2.39 (s, 3H).	*m/z (%):* 326 ([M+2]$^{+\cdot}$, 40), 324 ([M]$^{+\cdot}$, 83), 311 (49), 310 (29), 309 (91), 145 (100), 117 (66), 116 (27), 115 (75), 91 (50).	53
174	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.43 (s, 1H), 8.16 (d, *J* = 15.8 Hz, 1H), 7.83 (d, *J* = 15.8 Hz, 1H), 7.69-7.50 (m, 3H), 7.34 (s, 1H), 6.52 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.45 (d, *J* = 2.4 Hz, 1H), 3.89 (s, 3H), 3.85 (s, 3H).	*m/z (%):* 372 ([M+2]$^{+\cdot}$, 35), 370 ([M]$^{+\cdot}$, 81), 341 (48), 430 (28), 339 (100), 191 (60), 148 (39), 133 (26).	56
175	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.43 (s, 1H), 8.20 (d, *J* = 15.7 Hz, 1H), 7.75 (d, *J* = 15.9 Hz, 1H), 7.66-7.48 (m, 2H), 7.34 (s, 1H), 7.15 (s, 1H), 6.49 (s, 1H), 3.94 (s, 3H), 3.89 (d, *J* = 2.4 Hz, 6H).	*m/z (%):* 402 ([M+2]$^{+\cdot}$, 40), 400 ([M]$^{+\cdot}$, 81), 371 (69), 370 (49), 369 (100), 207 (52).	42

			
Compuesto	NMR	MS	Rdto. (%)
177	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.45 (s, 1H), 8.19 (d, *J* = 15.9 Hz, 1H), 7.88 (d, *J* = 15.8 Hz, 1H), 7.72-7.52 (m, 2H), 7.34 (d, *J* = 8.8 Hz, 1H), 7.20 (d, *J* = 3.0 Hz, 1H), 6.96 (dd, *J* = 9.0, 3.1 Hz, 1H), 6.86 (d, *J* = 9.0 Hz, 1H), 3.87 (s, 3H), 3.81 (s, 3H).	*m/z (%):* 372 ([M+2]$^{+\cdot}$, 18), 370 ([M]$^{+\cdot}$, 45), 341 (68), 339 (100), 207 (37), 176 (28), 148 (18), 123 (14).	41
178	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.54 (s, 1H), 8.03 (d, *J* = 2.5 Hz, 1H), 7.83-7.66 (m, 1H), 7.64 (s, 1H), 7.58-7.45 (m, 2H), 6.91 (d, *J* = 2.3 Hz, 2H), 6.59 (t, *J* = 2.2 Hz, 1H), 3.77 (s, 6H).	*m/z (%):* 372 ([M+2]$^{+\cdot}$, 34), 370 ([M]$^{+\cdot}$, 84), 342 (31), 341 (62), 327 (61), 326 (63), 325 (100), 230 (33), 191 (63), 148 (60).	40
179	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.51 (s, 1H, H-4), 7.80 (s, 2H, Hα, Hβ), 7.60 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.31-7.04 (m, 3H, H-5, H-6, H-7), 6.89 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 3.96 (s, 3H, 8-OCH_3), 3.82 (s, 3H, 4'-OCH_3).	*m/z (%):* 337 ([M+1]$^{+\cdot}$, 37), 336 ([M]$^{+\cdot}$, 100), 308 (26), 293 (19), 161 (87), 133 (47), 89 (21).	37
180	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.47 (s, 1H, H-4), 8.12 (d, *J* = 15.8 Hz, 1H, Hβ), 7.85 (d, *J* = 15.8 Hz, 1H, Hα), 7.60 (d, *J* = 8.6 Hz, 1H, H-6'), 7.27-7.06 (m, 2H, H-5, H-6, H-7), 6.49 (dd, *J* = 8.6, 2.4 Hz, 1H, H.5'), 6.41 (d, *J* = 2.3 Hz, 1H, H-3'), 3.95 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3).	*m/z (%):* 367 ([M+1]$^{+\cdot}$, 33), 366 ([M]$^{+\cdot}$, 95), 336 (35), 335 (100), 203 (27), 191 (75), 148 (40), 133 (26), 89 (19), 77 (17).	68
181	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.46 (s, 1H, H-4), 8.16 (d, *J* = 15.8 Hz, 1H, Hβ), 7.78 (d, *J* = 15.8 Hz, 1H, Hα), 7.29-7.04 (m, 4H, H-5, H-6, H-7, H-6'), 6.46 (s, 1H, H-3'), 3.95 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.86 (s, 6H, 2x OCH_3).	*m/z (%):* 397 ([M+1]$^{+\cdot}$, 26), 396 ([M]$^{+\cdot}$, 71), 366 (45), 365 (100), 221 (20), 203 (33).	71

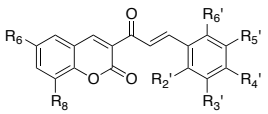
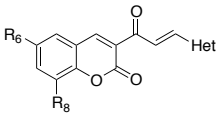
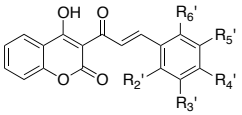
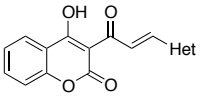
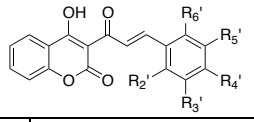
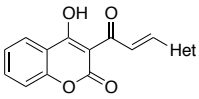
			
Compuesto	NMR	MS	Rdto. (%)
182	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.51 (s, 1H, H-4), 8.18 (d, *J* = 15.8 Hz, 1H, Hβ), 7.93 (d, *J* = 15.9 Hz, 1H, Hα), 7.28-7.14 (m, 4H, H-5, H-6, H-7, H-6'), 6.95 (dd, *J* = 9.0, 2.9 Hz, 1H, H-4'), 6.86 (d, *J* = 9.0 Hz, 1H, H-3'), 3.99 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3).	*m/z (%):* 397 ([M+1]$^{+\cdot}$, 16), 396 ([M]$^{+\cdot}$, 55), 336 (57), 335 (100), 176 (39), 161 (18), 133 (20).	59
183	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.53 (s, 1H, H-4), 7.88 (d, *J* = 15.7 Hz, 1H, Hβ), 7.75 (d, *J* = 15.7 Hz, 1H, Hα), 7.33-7.09 (m, 3H, H-5, H-6, H-7), 6.79 (d, *J* = 2.3 Hz, 2H, H-2', H-6'), 6.51 (t, *J* = 2.3 Hz, 1H, H-4'), 3.98 (s, 3H, 8-OCH_3), 3.82 (s, 6H, 2x OCH_3).	*m/z (%):* 367 ([M+1]$^{+\cdot}$, 19), 366 ([M]$^{+\cdot}$, 83), 338 (29), 337 (60), 323 (29), 322 (65), 321 (100), 203 (30), 148 (53), 89 (23), 77 (18).	37
185	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.71 (s, 1H), 8.68 (dd, *J* = 4.9, 1.7 Hz, 1H), 8.06 (d, *J* = 15.7 Hz, 1H), 7.96 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.88 (td, *J* = 7.5, 1.7 Hz, 1H), 7.81-7.68 (m, 3H), 7.53-7.38 (m, 3H).	*m/z (%):* 278 ([M+1]$^{+\cdot}$, 26), 277 ([M]$^{+\cdot}$, 77), 249 (21), 248 (48), 221 (54), 220 (100), 173 (29), 132 (65), 104 (53), 89 (30), 78 (50), 63 (21).	72
186	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.52 (s, 1H), 8.13-7.93 (m, 2H), 7.85-7.57 (m, 3H), 7.50 (t, *J* = 6.3 Hz, 2H), 7.36 (d, *J* = 15.8 Hz, 1H).	*m/z (%):* 318 ([M+2]$^{+\cdot}$, 16), 316 ([M]$^{+\cdot}$, 36), 288 (79), 287 (52), 260 (67), 137 (100), 109 (82), 65 (42).	65

Tabla 10. Caracterización de 3-cinamoil-4-hidroxicumarinas no publicadas.

			
Compuesto	NMR	MS	Rdto. (%)
187	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.23 (d, J = 14.5 Hz, 1H), 8.07-7.91 (m, 2H), 7.85-7.66 (m, 3H), 7.53-7.33 (m, 5H).	*m/z (%):* 293 ([M+1]$^{+\cdot}$, 20), 292 ([M]$^{+\cdot}$, 89), 291 (72), 290 (21), 289 (34), 272 (20), 247 (30), 215 (100), 171 (42), 144 (32), 121 (55), 103 (67), 77 (43).	75
189	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.41 (d, J = 15.8 Hz, 1H), 8.09 (dd, J = 7.9, 1.7 Hz, 1H), 7.98 (d, J = 15.7 Hz, 1H), 7.74-7.58 (m, 3H), 7.44-7.24 (m, 4H).	*m/z (%):* 328 ([M+2]$^{+\cdot}$, 53), 326 ([M]$^{+\cdot}$, 97), 281 (20), 216 (100), 205 (36), 178 (30), 167 (34), 165 (92), 139 (21), 138 (21), 137 (50), 121 (93), 102 (42), 101 (46), 92 (22), 57 (25).	83
190	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.41 (d, J = 15.7 Hz, 1H), 8.15-7.96 (m, 2H), 7.75-7.54 (m, 3H), 7.40-7.16 (m, 4H), 2.40 (s, 3H).	*m/z (%):* 307 ([M+1]$^{+\cdot}$, 22), 306 ([M]$^{+\cdot}$, 100), 305 (50), 215 (43), 189 (20), 185 (37), 158 (31), 145 (43), 121 (34), 117 (26), 115 (23).	79
191	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.43 (s, 2H), 8.09 (dd, J = 7.7, 1.7 Hz, 1H), 7.80-7.59 (m, 2H), 7.39-7.21 (m, 3H), 6.56 (dd, J = 8.7, 2.3 Hz, 1H), 6.45 (d, J = 2.3 Hz, 1H), 3.93 (s, 3H), 3.87 (s, 3H).	*m/z (%):* 353 ([M+1]$^{+\cdot}$, 20), 352 ([M]$^{+\cdot}$, 100), 321 (31), 191 (33), 149 (41), 121 (50), 92 (21), 77 (16).	82
192	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.49 (d, J = 15.7 Hz, 1H), 8.34 (d, J = 15.7 Hz, 1H), 8.08 (dd, J = 7.9, 1.7 Hz, 1H), 7.66 (ddd, J = 8.8, 7.4, 1.7 Hz, 1H), 7.38-7.14 (m, 3H), 6.48 (s, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 3.92 (s, 3H).	*m/z (%):* 382 ([M]$^{+\cdot}$, 34), 351 (16), 61 (100).	87

			
Compuesto	NMR	MS	Rdto. (%)
193	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.75 (d, *J* = 15.8 Hz, 1H), 8.59 (d, *J* = 15.9 Hz, 1H), 8.07 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.62 (ddd, *J* = 8.7, 7.3, 1.7 Hz, 1H), 7.35-7.19 (m, 2H), 6.09 (s, 2H), 3.95 (s, 6H), 3.84 (s, 3H).	*m/z (%):* 383 ([M]$^{+\cdot}$, 12), 382 ([M]$^{+\cdot}$, 57), 380 (22), 351 (48), 350 (69), 349 (100), 192 (21), 179 (63), 178 (34), 121 (31), 92 (21), 91 (29), 69 (27).	84
196	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.07 (d, *J* = 8.6 Hz, 1H), 7.66 (d, *J* = 15.5 Hz, 1H), 7.49 (d, *J* = 6.7 Hz, 1H), 7.35-7.11 (m, 3H), 7.03 (d, *J* = 3.1 Hz, 1H), 6.99-6.74 (m, 2H), 3.77 (s, 6H).	*m/z (%):* 352 ([M]$^{+\cdot}$, 39), 321 (50), 268 (21), 267 (26), 239 (30), 134 (30), 121 (26), 112 (25), 111 (21), 99 (21), 98 (58), 97 (26), 85 (54), 84 (57), 74 (33), 71 (65), 69 (33), 57 (100), 56 (25), 55 (55).	70
197	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.44 (d, *J* = 15.7 Hz, 1H), 8.42 (d, *J* = 15.7 Hz, 1H), 8.08 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.67 (ddd, *J* = 8.7, 7.4, 1.7 Hz, 1H), 7.41 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.36-7.23 (m, 2H), 7.11 (t, *J* = 8.0 Hz, 1H), 6.99 (dd, *J* = 8.1, 1.5 Hz, 1H), 3.93 (d, *J* = 1.2 Hz, 3H), 3.89 (s, 3H).	*m/z (%):* 352 ([M]$^{+\cdot}$, 24), 322 (28), 321 (100), 319 (23), 121 (24).	68
199	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.52 (d, *J* = 15.9 Hz, 1H), 8.44 (d, *J* = 15.9 Hz, 1H), 8.09 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.76 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.67 (ddd, *J* = 8.3, 7.4, 1.7 Hz, 1H), 7.45-7.23 (m, 3H), 7.07-6.89 (m, 2H), 3.94 (s, 3H).	*m/z (%):* 323 ([M+1]$^{+\cdot}$, 15), 322 ([M]$^{+\cdot}$, 100), 291 (75), 215 (33), 204 (19), 189 (26), 161 (49), 121 (50), 105 (24), 96 (28), 95 (24), 77 (30), 69 (52).	64

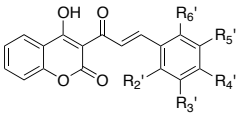
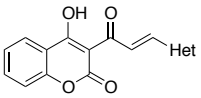
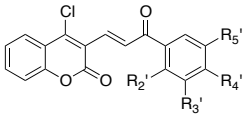
			
Compuesto	NMR	MS	Rdto. (%)
200	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.43 (d, *J* = 16.3 Hz, 1H), 8.10 (d, *J* = 7.9 Hz, 1H), 8.03 (d, *J* = 16.1 Hz, 1H), 7.69 (t, *J* = 8.0 Hz, 1H), 7.43-7.18 (m, 5H), 7.05 -6.94 (m, 1H), 3.87 (s, 3H).	*m/z (%):* 323 ([M+1]$^{+\cdot}$, 19), 322 ([M]$^{+\cdot}$, 97), 321 (40), 304 (26), 278 (49), 277 (82), 215 (80), 204 (25), 202 (70), 201 (94), 200 (33), 189 (29), 174 (77), 162 (22), 161 (100), 133 (43), 121 (93), 120 (30), 118 (41), 92 (38), 90 (26), 89 (20), 77 (40), 65 (22).	55
202	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.39 (d, *J* = 15.6 Hz, 1H), 8.09 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.97 (d, *J* = 15.7 Hz, 1H), 7.69 (td, *J* = 8.5, 7.9, 1.7 Hz, 1H), 7.39-7.27 (m, 2H), 6.84 (d, *J* = 2.3 Hz, 2H), 6.55 (t, *J* = 2.2 Hz, 1H), 3.84 (s, 6H).	*m/z (%):* 353 ([M+1]$^{+\cdot}$, 19), 352 ([M]$^{+\cdot}$, 92), 308 (43), 307 (78), 232 (44), 231 (62), 230 (21), 215 (32), 205 (100), 191 (34), 189 (44), 176 (25), 165 (27), 148 (34), 121 (79), 120 (39), 77 (22).	79
204	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.81-8.66 (m, 2H), 8.10 (dd, *J* = 8.1, 1.7 Hz, 1H), 8.03 (d, *J* = 15.6 Hz, 1H), 7.83-7.62 (m, 3H), 7.41-7.21 (m, 3H).	*m/z (%):* 294 ([M+1]$^{+\cdot}$, 18), 293 ([M]$^{+\cdot}$, 100), 291 (38), 264 (27), 215 (33), 145 (55), 132 (39), 121 (26), 120 (37), 117 (26), 104 (42), 92 (31), 78 (34).	63
206	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.31 (s, 1H), 8.08-7.93 (m, 3H), 7.88-7.73 (m, 2H), 7.48-7.35 (m, 2H), 6.88 (d, *J* = 2.0 Hz, 1H).	*m/z (%):* 283 ([M+1]$^{+\cdot}$, 13) 282 ([M]$^{+\cdot}$, 48), 254 (65), 239 (27), 134 (23), 121 (100), 120 (20), 97 (27), 93 (33), 92 (21), 85 (33), 71 (42), 65 (31).	56

Tabla 11. Caracterización de 3-(3-aril-3-oxo-propenil)-4-clorocumarinas no publicadas.



Compuesto	NMR	MS	Rendimiento (%)
207	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.60 (d, *J* = 15.5 Hz, 1H), 8.20-7.97 (m, 4H), 7.72-7.23 (m, 7H).	*m/z (%):* 312 ([M+2]$^{+\cdot}$, 12) 310 ([M]$^{+\cdot}$, 30), 207 (34), 205 (100), 187 (15), 105 (41), 77 (30).	62
208	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.59 (d, *J* = 15.4 Hz, 1H), 8.17-8.04 (m, 3H), 8.00 (d, *J* = 9.5 Hz, 1H), 7.65 (ddd, *J* = 8.9, 7.3, 1.6 Hz, 1H), 7.40 (td, *J* = 7.6, 1.1 Hz, 2H), 6.98 (d, *J* = 9.0 Hz, 2H), 3.89 (s, 3H).	*m/z (%):* 326 ([M+2]$^{+\cdot}$, 10) 324 ([M]$^{+\cdot}$, 29), 289 (51), 207 (41), 205 (100), 119 (28), 91 (26).	68
209	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.58 (d, *J* = 15.4 Hz, 1H), 8.10 (d, *J* = 15.4 Hz, 1H), 8.00 (d, *J* = 8.3 Hz, 3H), 7.65 (ddd, *J* = 8.8, 7.3, 1.5 Hz, 1H), 7.45-7.24 (m, 4H), 2.43 (s, 3H).	*m/z (%):* 342 ([M+2]$^{+\cdot}$, 7) 340 ([M]$^{+\cdot}$, 18), 305 (57), 207 (36), 205 (100), 135 (74), 77 (14).	71
210	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.59 (dd, *J* = 15.7, 1.8 Hz, 1H), 8.37 (dd, *J* = 8.8, 2.0 Hz, 2H), 8.29-8.11 (m, 3H), 8.04 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.70 (td, *J* = 7.8, 1.6 Hz, 1H), 7.43 (t, *J* = 8.3 Hz, 2H).	*m/z (%):* 357 ([M+2]$^{+\cdot}$, 9) 355 ([M]$^{+\cdot}$, 23), 321 (25), 320 (93), 274 (28), 207 (96), 205 (100), 149 (27), 76 (22).	54
211	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.57 (d, *J* = 15.4 Hz, 1H, Hβ), 8.12 (d, *J* = 15.4 Hz, 1Hα), 8.01 (d, *J* = 7.2 Hz, 1H, H-5), 7.77-7.54 (m, 3H), 7.48-7.33 (m, 3H), 7.15 (dd, *J* = 8.2, 2.7 Hz, 1H), 3.89 (s, 3H, OCH_3).	*m/z* (%): 342 ([M+2]$^{+\cdot}$, 5), 340 ([M]$^{+\cdot}$, 15), 306 (20), 305 (56), 207 (47), 205 (100), 135 (42), 77 (17)	47

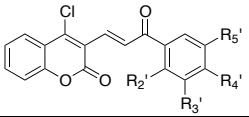
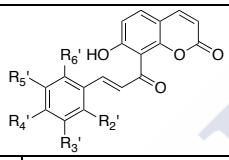
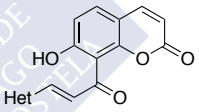
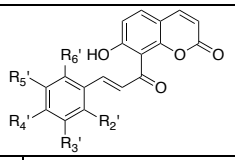
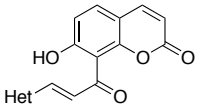
			
Compuesto	NMR	MS	Rendimiento (%)
213	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 8.44 (d, *J* = 15.5 Hz, 1H), 8.09-7.90 (m, 2H), 7.63 (ddd, *J* = 7.2, 5.5, 3.3 Hz, 1H), 7.49-7.31 (m, 2H), 7.31-7.19 (m, 1H), 7.13-7.00 (m, 1H), 7.00-6.88 (m, 1H), 3.91 (s, 3H), 3.82 (s, 3H).	*m/z (%):* 372 ([M+2]$^{+\cdot}$, 9) 375 ([M]$^{+\cdot}$, 23), 335 (21), 207 (16), 205 (48), 165 (100).	62

Tabla 12. Caracterización de 8-cinamoilcumarinas no publicadas.

			
Compuesto	NMR	MS	Rdto.(%)
215	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 14.30 (s, 1H), 8.25 (d, *J* = 15.5 Hz, 1H), 8.08 (d, *J* = 15.5 Hz, 1H), 7.58 -7.39 (m, 2H), 7.32 (d, *J* = 8.7 Hz, 1H), 6.75 (d, *J* = 8.7 Hz, 1H), 6.40 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.32 (d, *J* = 2.4 Hz, 1H), 6.11 (d, *J* = 9.5 Hz, 1H), 3.82 (s, 3H), 3.70 (s, 3H).	*m/z (%):* 353 ([M+1]$^{+\cdot}$, 14), 352 ([M]$^{+\cdot}$, 100), 321 (45), 191 (52), 149 (29), 121 (63), 92 (19), 77 (12).	95
217	^{1}H-NMR (250 MHz, DMSO-d_6) *δ* ppm 11.54 (s, 1H), 7.98 (d, *J* = 9.6 Hz, 1H), 7.81 (d, *J* = 16.1 Hz, 1H), 7.63 (d, *J* = 8.6 Hz, 1H), 7.43 (d, *J* = 16.0 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 1H), 6.36-6.16 (m, 3H), 3.81 (s, 9H).	*m/z (%):* 383 ([M+1]$^{+\cdot}$, 24), 382 ([M+1]$^{+\cdot}$, 84), 352 (30), 351 (100), 349 (39), 194 (31), 191 (15), 189 (15), 181 (27), 179 (56), 168 (16).	93

			
Compuesto	NMR	MS	Rdto.(%)
218	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 14.30 (s, 1H, OH), 8.29 (d, *J* = 15.3 Hz, 1H, Hβ), 7.92 (d, *J* = 15.3 Hz, 1H, Hα), 7.65 (d, *J* = 9.5 Hz, 1H, H-4), 7.50 (d, *J* = 8.7 Hz, 1H, H-5), 7.38 (d, *J* = 2.0 Hz, 1H, H-6'), 7.22 (d, *J* = 2.0 Hz, 1H, H-2), 6.90 (m, 2H, H-5', H-6), 6.26 (d, *J* = 9.6 Hz, 1H, H-3), 4.00 (s, 3H, 4'-OCH_3), 3.93 (s, 3H, 3'-OCH_3).	*m/z (%):* 353 ($[M+1]^{+\cdot}$, 81), 352 ($[M]^{+\cdot}$, 100), 292 (53), 291 (100), 191 (27), 161 (32), 148 (17), 89 (30), 69 (18).	81
221	^{1}H-NMR (250 MHz, DMSO-d_6) δ ppm 8.53 (s, 1H), 8.30-8.14 (m, 2H), 7.97 (d, *J* = 9.3 Hz, 1H), 7.78-7.49 (m, 3H), 7.35 (d, *J* = 16.4 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.22 (d, *J* = 9.7 Hz, 1H).	*m/z (%):* 338 ($[M+1]^{+\cdot}$, 17), 337 ($[M]^{+\cdot}$, 49), 291 (100), 274 (62), 176 (34) 161 (23).	63
222	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 13.94 (s, 1H), 8.34 (d, *J* = 15.8 Hz, 1H), 8.25 (d, *J* = 15.7 Hz, 1H), 7.65 (dd, *J* = 9.6, 2.6 Hz, 1H), 7.56-7.36 (m, 2H), 7.22-7.05 (m, 1H), 7.05-6.83 (m, 2H), 6.28 (dd, *J* = 9.5, 2.6 Hz, 1H), 3.93 (s, 3H), 3.89 (s, 3H).	*m/z (%):* 352 ($[M+1]^{+\cdot}$, 18), 322 ($[M]^{+\cdot}$, 100), 321 (35), 319 (21), 121 (42) 92 (25), 77 (18).	85
223	^{1}H-NMR (250 MHz, $CDCl_3$) δ ppm 14.04 (s, 1H), 8.32 (d, *J* = 15.3 Hz, 1H), 7.84 (d, *J* = 15.4 Hz, 1H), 7.65 (d, *J* = 9.5 Hz, 1H), 7.51 (d, *J* = 8.7 Hz, 1H), 6.99-6.74 (m, 3H), 6.52 (t, *J* = 2.2 Hz, 1H), 6.27 (d, *J* = 9.6 Hz, 1H), 3.86 (s, 6H).	*m/z (%):* 353 ($[M+1]^{+\cdot}$, 27), 352 ($[M]^{+\cdot}$, 100), 351 (33), 350 (16), 335 (19), 334 (51), 321 (33), 319 (24), 164 (53), 98 (17).	90

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Conclusiones

• Se han desarrollado y puesto a punto múltiples metodologías sintéticas para la preparación de forma sencilla, rápida y versátil de las series de compuestos híbridos seleccionadas. Todos los compuestos híbridos contienen como núcleo principal el esqueleto de cumarina, al que se le incorporan los farmacóforos derivados de estilbenos (3-arilcumarinas) o de chalconas ("endo" cumarina-chalcona y "exo" cumarina-chalconas), obteniéndose una amplia quimioteca que ha sido completamente caracterizada.

• Se han obtenido series de compuestos con sustituyentes de distinta naturaleza, en diferente número y en distintas posiciones de los esqueletos que nos han permitido realizar estudios de la relación estructura-actividad sobre aquellas dianas estudiadas.

• Las 3-arilcumarinas estudiadas resultaron ser excelentes inhibidores selectivos de la MAO-B (en concreto, 6-bromo-8-metoxi-3-(4'-metoxifenil)cumarina, IC_{50} = 1.35 nM), mientras que las 3-benzoilcumarinas presentaban una moderada pero selectiva actividad frente a MAO-A (en concreto, 6-bromo-8-hidroxi-3-(4'-hidroxibenzoil)cumarina, IC_{50} =19.17 μM).

• Los compuestos híbridos hidroxilados mostraron una muy buena actividad antioxidante, con excelentes valores de ORAC, buena capacidad para atrapar radicales, bajos potenciales de oxidación, baja citotoxicidad y elevados valores de citoprotección. En particular, la 6-hidroxi-3-(4'-hidroxibenzoil)cumarina resultó ser el compuesto más prometedor de la serie estudiada con un ORAC de 14.1 y un 100% de viabilidad celular en BAEC.

• Los híbridos "exo" cumarina-chalcona presentaron buena afinidad como ligandos de uno o varios receptores de adenosina. En concreto, el compuesto (*E*)-3-(3-(tiofen-3-il)acriloil)cumarina presentó la mejor actividad y selectividad (hA_3 = 5.16 μM) siendo clave la sustitución del anillo B de la chalcona por un grupo tiofeno.

• Los compuestos estudiados presentaron moderadas actividades antibacterianas frente a un gran número de patógenos humanos y excelentes

frente a otros de origen marino. En particular, los derivados aminados de las 3-benzoilcumarinas resultaron ser tan activos frente al patógeno marino *Tenacibaculum maritimum* como los actuales fármacos empleados, sin afectar a las cepas bacterianas humanas estudiadas.

- Se ha desarrollado una nueva metodología sintética que permite llevar a cabo la reacción de ciclación de Nazarov de dienonas completamente sustituidas de forma diastereoespecífica, generando estereocentros vecinales con todos los átomos de carbono cuaternarios. Dicha metodología sintética presenta una gran aplicación para la síntesis total de compuestos derivados de productos naturales de estructura oxacíclica.

- El carácter multidisciplinar de este trabajo deja patente la importancia y la necesidad de la colaboración entre grupos de distintas áreas. Sin estas colaboraciones, los resultados y conclusiones de este trabajo no hubiesen sido posibles.

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Conclusions

- Multiple synthetic methodologies have been developed and tuned up in order to prepare hybrid compounds series in a simple, fast and versatile way. All the hybrid compounds contain the coumarin scaffold as the main nucleus. Stilbene (3-arylcoumarin) and chalcone ("endo" and "exo" coumarin-chalcones) pharmacophores have been incorporated into the coumarin core, obtaining a broad compound library fully characterized.

- Series of compounds with a wide variety of substituents of different nature number and in various positions of the scaffolds have been obtained. This allowed us to conduct a study of the structure-activity relationship on the considered targets.

- The studied 3-arylcoumarins were found to be excellent selective MAO-B inhibitors (in particular, 6-bromo-8-methoxy-3-(4'-methoxyphenyl)coumarin, IC_{50} = 1.35 nM), while the 3-benzoulcoumarins showed a moderated but selective MAO-A inhibitory activity (in particular, 6-bromo-8-hydroxy-3-(4'-hydroxybenzoyl)coumarin, IC_{50} =19.17 μM).

- The hydroxylated hybrid compounds showed very good antioxidant activity, presenting excellent ORAC values, good scavenging capacity, low oxidation potential, low cytotoxicity and high citoprotective properties. In particular, 6-hydroxy-3-(4'-hydroxybenzoyl)coumarin was the most promising compound, with an ORAC value of 14.1 and 100% of cell viability in BAEC.

- The "exo" coumarin-chalcone hybrids showed good affinity as ligands for one or more adenosine receptors. Specifically, compound (*E*)-3-(3-(thiophen-3-yl)acryloyl)coumarin presented the best activity and selectivity (hA_3 = 5.16 mM) being essential the substitution of the chalcone ring B by a thiophene.

- Some of the studied compounds showed moderated antibacterial activities against human pathogens pathogens and some others excellent activities against marine origin pathogens. In particular, amino-3-benzoylcoumarin derivatives proved to be as active as the current drugs used against the marine pathogen *Tenacibaculum maritimum*, with no effect on human bacteria strains.

- A new synthetic methodology to carry out the diastereospecific Nazarov cyclization of fully substituted dienones has been developed, generating vicinal all-carbon-atom quaternary stereocentres. This synthetic methodology has a broad application for the total synthesis of oxacyclic natural products derivatives.

- The multidisciplinary nature of this work makes clear the importance and necessity of collaboration between groups from different areas. Without these contributions, the results and conclusions of this work would not have been possible.

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