Designing Irreversible Inhibitors – Worth the Effort?

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Abstract: Despite the unquestionable success of numerous irreversible drugs in clinical use, such as aspirin and penicillin, the number of approved drugs in comparison with non-covalent ones is relatively low. Over the years, the possible off-target effects of these types of compounds have been the major concern that has hampered their development. However, their remarkable advantages over non-covalent drugs and a better analysis of the risks have reduced the widespread skepticism surrounding them. The design of irreversible inhibitors is a challenge, particularly considering that in some cases their efficacy is due to complex and unexpected mechanisms of action. In this article the main advantages of irreversible inhibition are summarized and the complexity of certain covalent modification mechanisms is highlighted with selected examples.

Introduction

Many of the most widely employed and successful drugs in clinical use are irreversible drugs.[1-8] Aspirin, which was developed by Bayer in 1897 as an anti-inflammatory agent, is probably the best known example. As with many other examples, the covalent mechanism of action of Aspirin was discovered by serendipity more than 70 years after its commercialization. Aspirin causes the irreversible inhibition of cyclooxygenases 1 (COX-1) and 2 (COX-2), which are enzymes involved in the prostaglandin biosynthesis, by acylation of a serine residue that is close to the active site.[9,10] Other examples are penicillins and cephalosporins,[7] which are antibiotics that inhibit the cross-linking of bacterial cell walls catalyzed by penicillin-binding proteins (Figure 1). Fosfomycin,[11-13] which is an antibiotic that targets MurA, an enzyme involved in peptidoglycan biosynthesis, and omeprazole, which is a proton pump inhibitor for the treatment of diverse stomach diseases, are further examples.[14] Despite this success, the number of approved irreversible drugs is paradoxically relatively low in comparison with the commercially available non-covalent drugs.[1,2] The complexity in designing effective covalent inhibitors of a selected target and, more importantly, their selectivity, were the major concerns that slowed their development. The hepatotoxic properties reported in the 1970s for some compounds probably contributed greatly to the increased concern in subsequent years.[2]

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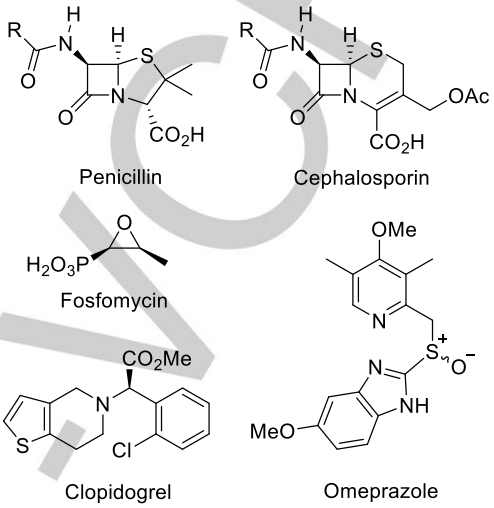


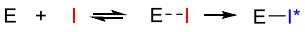
Figure 1. Examples of irreversible drugs.

Fortunately, irreversible inhibitors have started to be seen in a more favorable light in recent years. The advantages of irreversible drugs, their high economical profitability and an improved understanding of the potential risks have resulted in a clear increase in the number of compounds in clinical trials, drug approvals and scientific articles in this area.[1,2] The clinical relevance of irreversible drugs have been reviewed previously and these aspects will be briefly summarized here.[1-6] Considering the fact that the covalent mechanisms of most irreversible drugs in clinical use were found out many years after their discovery, a knowledge of the chemical modification mechanism of irreversible inhibitors and the structural basis of their efficiency could facilitate the future design of more efficient inhibitors and/or inspire the development of novel inhibitors to target unexplored enzymes.[15] This article illustrates the complex mechanism of action of some of the irreversible inhibitors. This may provide inspiration for future inhibitor design. Structural details of the modified targets to support the covalent mechanism are also provided.

Irreversible Inhibitors

Irreversible inhibitors are small molecules that initially bind at the active site of a target and then react with it by forming a stable covalent adduct. These compounds usually contain an electrophilic functional group that reacts with a nucleophilic group of a side chain residue present in the active site of the enzyme. Although examples of irreversible inhibitors that target receptors have been reported, as clopidogrel (Figure 1), which is an antiplatelet drug that inhibits the P2Y purinergic receptor,[16] most of these targets are enzymes. In the latter case, the initial

enzyme-inhibitor complex (E--I) is converted into an enzyme-adduct complex (E–I*) that does not have catalytic activity (Scheme 1). In general, irreversible inhibitors of enzymes are usually designed to react with the key residues for catalysis, which are located in the active site. However, there are also excellent examples of allosteric irreversible inhibitors, in particular, of oncology and anti-infective targets.[17-22] These compounds act by avoiding the correct geometry of the active site for catalysis, which is achieved by covalent modification of another region of the target.



Scheme 1. Irreversible inhibition.

In principle, these types of inhibitors can achieve full inactivation of the target when they are left for a sufficient time because the target would be permanently blocked by the inhibitor. Thus, the dissociation equilibrium observed for non-covalent inhibitors, which interact with the target through reversible forces, does not occur. This makes irreversible inhibitors more efficient and robust against pharmacokinetic liabilities (clearance, binding to serum proteins, etc.) than non-covalent ones. Moreover, as non-equilibrium binding takes place, a time-dependent kinetic is produced since the amount of active target decreases progressively as the reaction between the target and the inhibitor occurs. Considering that the rate of this reaction is relatively slow (minutes or more) whereas the association process in non-covalent inhibition is usually quite fast (seconds or less), the inhibition potency of irreversible inhibitors is not quoted as an IC_{50} value – in contrast to the non-covalent systems. The IC_{50} values would strongly depend on the pre-incubation time and the efficiency of the chemical reaction (rate). Instead, the inhibitory potency is more precisely described by rate of inactivation (k_{inact}) and inhibition constant (K_i) terms.[23]
The design of an irreversible inhibitor is a challenge in the sense that it requires a combination of two key factors in the same molecule: binding and reactivity. Thus, as for non-covalent inhibitors, a high affinity for the enzyme is required in order to achieve selectivity and an effective concentration of the enzyme-inhibitor complex. In addition to the latter, the two reactive centers (nucleophile and electrophile) must be located in close contact and in the correct geometry for the transformation to occur. In this regard, it is important to highlight that enzyme active sites are usually shielded from the solvent environment, normally by a substrate-covering loop that closes over the active site after substrate binding. In this arrangement, the reactive nucleophilic side chain residues are quite desolvated and a high effective concentration of the nucleophile or a base is achieved. This is an amazing way that Nature has found to address mechanistically challenging transformations like the formation of carbon-carbon bonds through covalent catalysis. To some extent, irreversible inhibitors are designed to imitate Nature.
The advantages of covalent drugs are:[1-6]

- Increased efficiency that favors the use of lower doses and the reduction of the side effects;
- Selectivity;
- Reduced risk of drug resistance due to active site residue changes, which is a major concern in the treatment of infectious diseases and oncology;
- Reduced sensitivity to pharmacokinetic parameters since the inhibitor is covalently attached to the target;
- Prolonged duration of the inhibition since the activity can only be recovered by synthesis of new target;

The disadvantages of covalent drugs are:[1-6]

- Low specificity leading to side effects such as hepatotoxicity, mutagenicity or carcinogenicity.
- Potential immunogenicity of the resulting target-adduct, which could cause an allergic response or drug hypersensitivity reaction.

An analysis of thirty nine irreversible drugs, which were approved by the US Food and Drug Administration (FDA), carried out by Singh *et al*.[1] in 2011 showed that irreversible drugs are mainly applied in anti-infective (~33%) and oncology therapies (~20%) (Figure 2). ~15% are used for gastrointestinal disorders, 10% for central nervous system diseases, 5% for cardiovascular disorders and 3% for inflammation diseases. The use of irreversible inhibitors for chronic diseases is limited due to the high risk of an immune response of the resulted target-adduct after continuous treatment. It is not therefore surprising that over 50% of these compounds were developed for oncology and infective diseases.

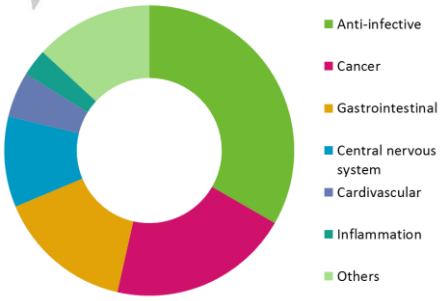


Figure 2. Distribution of approved irreversible drugs in therapeutic areas. Data from an analysis of 39 approved drugs by the US Food and Drug Administration (FDA).[1]

The most frequent electrophilic functional groups present in covalent inhibitors are α,β-unsaturated ketones, vinyl sulfones, α-substituted carbonyl derivatives, bromodihydroisoxazoles, imidazole-1-carboxamides, epoxides, β-lactams and strained lactones (Figure 3).[24,2] In a recent study on the selectivity of diverse electrophilic groups toward several relevant targets, Jöst *et al.*[19] showed that these functional groups are much less promiscuous, i.e., more selective, than initially thought. They studied the binding of six electrophiles (acrylamides, chloroacetylamides, dimethylsulfoniumacetylamides, bromodihydroisoxazoles, 2-cyanoacetamides, and imidazole-1-carboxamides) towards eleven structurally and functionally diverse enzyme targets. Specifically, seven bacterial targets

(MurA-F, bacterial methionine aminopeptidase), two viral (denge virus protease, West Nile virus protease) and two human ones (thrombin, human methionine aminopeptidase type I). Unexpectedly, chloroacetamides, which are the most electrophilic functional groups of the series, showed low off-target reactivity. Along with acryl- and 2-cyanoacetamides and imidazole-1-carboxamides proved to be good functional groups for the design of covalent inhibitors. The 3-bromo-4,5-dihydroisoxazole moiety, which is derived from the natural product acivicin and reacting by nucleophilic replacement of the bromine, proved to be a particularly selective group for antibiotic designs as showed some activity with the Mur enzymes.

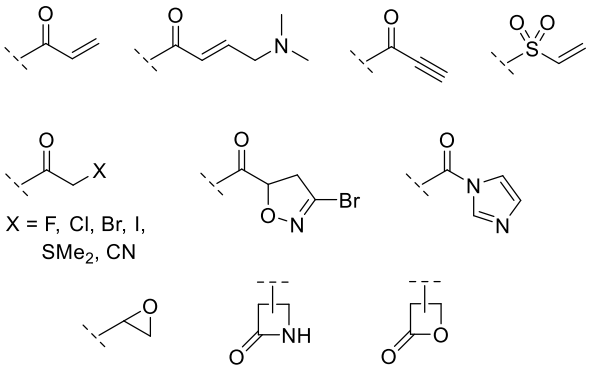


Figure 3. Examples of electrophilic functional groups typically present in irreversible inhibitors.

Chemical Modification Mechanism - Selected Examples

There are numerous relevant irreversible inhibitors in which the compound only undergoes direct attack by the nucleophilic group of a side chain residue present in the active site to the electrophilic center of the inhibitor. That is the case in several kinase enzymes in which the electrophilic group of the inhibitor is an α,β-unsaturated ketone or a vinyl sulfone,[26-29] in bacterial transcriptional activator LasR, which is inhibited by haloacetamides derived from its natural autoinducer (*N*-acyl homoserine lactones)[30] and in glyceraldehyde-3-phosphate dehydrogenase, which is covalently modified by halodihydroisoxazoles,[31] among others. From the design point of view, such inhibitors are less complex because they do not undergo further, and probably unexpected, transformations. However, in other cases the efficiency of the inhibitor is due to several chemical modifications. For instance, further reactions after covalent linkage with the enzyme take place or the inhibitor undergoes diverse intramolecular reactions to generate the reactive species that finally modifies the enzyme. Selected examples of the latter type will be discussed in this section. In particular, irreversible inhibitors that target oxacillinases (class D β-lactamases), type I dehydroquinase, and H^+/K^+ ATPase hydrogen/potassium-exchanging ATPase are discussed.

Oxacillinases

β-Lactamases (EC 3.5.2.6) are enzymes that hydrolyze the most widely used antibiotics, i.e., β-lactams, in an acylation-deacylation-based process that represents the most prevalent cause of antibiotic resistance in Gram-negative bacteria. Among the four major β-lactamase classes (A, B, C and D), class D enzymes, which are also known as 'oxacillinases' (OXA), are the most rapidly growing and diverse group of enzymes.[32-36] These enzymes hydrolyze penicillins, extended spectrum cephalosporins, and aztreonam (Figure 4A). Recently, a new type of oxacillinase that is also resistant to carbapenems, such as imipenem, has been identified and are widely dispersed in some clinically relevant species.[37-40]

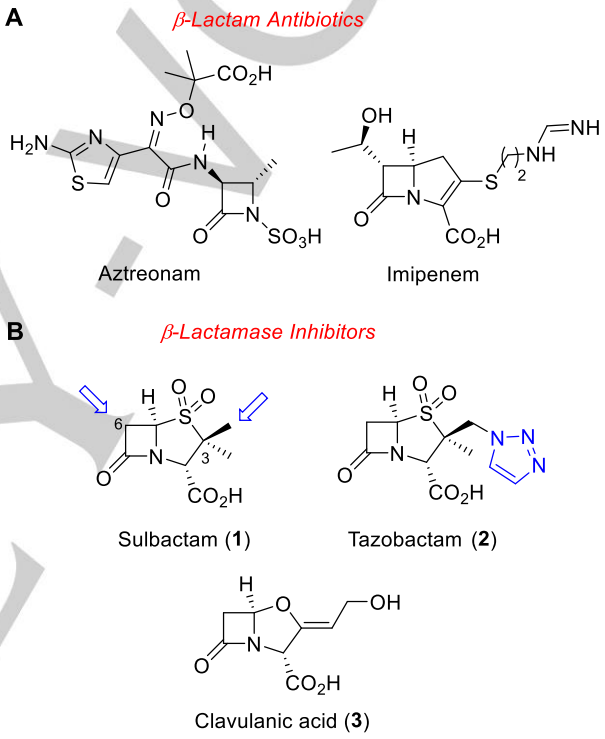
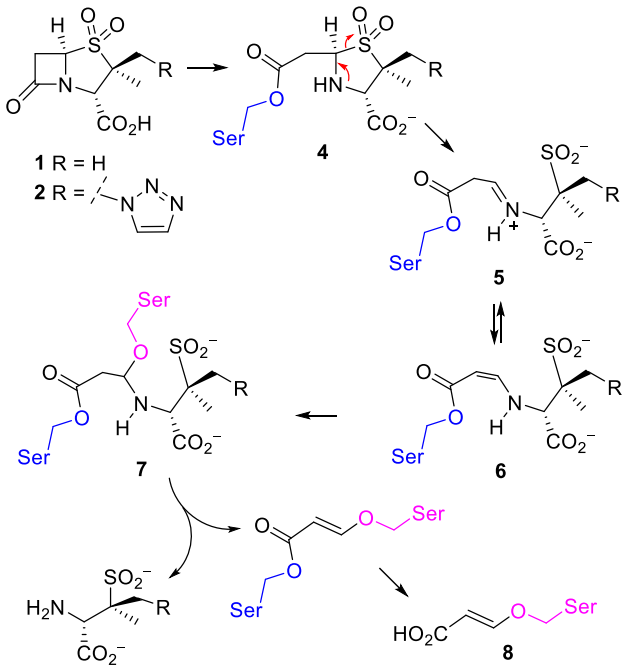


Figure 4. Most relevant β-lactam antibiotics (A) and selected β-lactamases inhibitors (B) in clinical use. The modified positions of **1** that afforded inhibitors of improved properties are highlighted with arrows.

Current β-lactamase inhibitors in clinical use include penicillin sulfones such as sulbactam (**1**), tazobactam (**2**), and clavulanic acid (**3**) (Figure 4B). As with β-lactam antibiotics, the mechanism of action of these inhibitors starts with the nucleophilic β-lactam ring opening by the catalytic serine to afford an acyl-enzyme adduct **4** (Scheme 2).[41,42] The resulting secondary amine in **4** triggers the dioxothiazolidine ring opening to give adduct **5**. The latter process does not take place with β-lactam antibiotics such as penicillin. Presumably, the driving force for this ring opening reaction is the formation of a good leaving group, a sulfinate *vs* a sulfide for the β-lactam antibiotics. The extra electrostatic interactions of the resulted sulfinate group also enhance their binding.



Scheme 2. Mechanism of action of penicillin sulfones **1** and **2**.

However, these commercially available inhibitors mainly proved to be useful for the inactivation of class A β-lactamases because the resulting adduct **5** can also undergo further transformations involving other residues in the active site that are susceptible to change by the enzyme. Thus, adduct **5** can tautomerize to give β-aminoacrylate adduct **6**, which was observed by X-ray crystallography [PDB entries 1VM1 (2.0 Å),[43] 2H5S (1.3 Å)[44]], or undergo conjugated nucleophilic addition by a second serine, which is also located in the active site, to give adduct **7**.[37] Subsequent β-elimination and hydrolysis affords adduct **8**, which was also observed experimentally by X-ray crystallography.[37]

Among the developed inhibitors against carbapenem-hydrolyzing class D β-lactamases, it is worth highlighting 1,1-dioxo-6(*Z*)-(2-pyridyl)methylenepenicillanic acid (**9a**), which was first reported by Chen *et al*.[45] from Pfizer (Figure 5). It was shown that the incorporation of a (2-pyridyl)methylene group at C6 of the sulbactam results in increased inhibitory potency against β-lactamases from *S. aureus* and *E. coli* (low micromolar range). More importantly, the authors related the increased efficiency of this inhibitor with the formation of a heterocyclic ester derivative that is resistant to hydrolysis. The inhibitory properties of **9a** were further improved by introducing a catechol ester (**9b**), a ((2-aminothiazol-4-yl)methyl)carbamate (**9c**) or a (2-aminoethyl)carbamate group (**9d**).[46,47] Derivative **9c** proved to have the lowest K_i value (500 nM), whereas inhibitor **9b** had the highest inactivation efficiency, with a k_{inact}/K_i of 0.21 μM^{-1} s^{-1}. Moreover, the catechol moiety in **9b** also facilitated entry through the outer membrane via the iron-uptake cell pathway.[47]

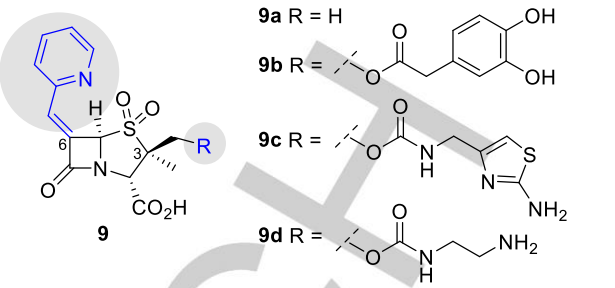


Figure 5. Selected inhibitors of carbapenem-hydrolyzing class D β-lactamases.

The crystal structure of OXA-24 from *A. baumannii* covalently modified by **9d** (PDB entry 3FZC, 2.0 Å) showed that the ligand is covalently linked to the catalytic Ser81 as an indolizine ester (Figure 6).[49] The ligand is also fixed in the active site by a series of strong interactions that further increase the stability of the complex. Specifically, the sulfinate group is anchored in the active site by a salt bridge with the guanidinium group of Arg261, two hydrogen-bonding interactions with the side chains of highly conserved Ser128 and Ser219 and an electrostatic interaction with Lys218. In addition, the carboxylate group, which as for β-lactam antibiotics proved to be essential for activity, establishes a strong hydrogen-bonding interaction with the phenol group of Tyr112. The formation of the indolizine adduct **12** would occur due to the favorable positioning of the pyridine nitrogen for nucleophilic attack on the conjugated imine adduct **11** that would result from dioxothiazolidine ring opening.[49] (2-Pyridyl)methylenecephalosporins also have a similar covalent mechanism, whereas 7-(*tert*-butoxycarbonyl)methylenecephalosporsin sulfone gives an eight-membered adduct.[50,51] The increased potency of compounds **9**, in comparison to the aforementioned penicillin sulfones **1**–**2**, against a wide range of β-lactamases seems to be due to the higher stability of the resulting heterocyclic ester as well as a set of favorable interactions that prevent hydrolysis of adduct **12**.

Type I Dehydroquinase

The increasing and widespread development of resistance to antibiotics in both community and clinical settings, along with the evident decline in antibiotic research by the major pharmaceutical companies during the last 50 years, has recently triggered the search for new antibiotics and alternative therapies. In particular, a great deal of effort has been devoted to the development of compounds that target bacterial virulence. The inhibition of virulence factors will lead to a loss of the ability to cause infection in the host and, as a consequence, they should be more easily eliminated by the immune system.[52-55] Anti-virulence drugs will disarm bacteria and create an *in vivo* scenario similar to that achieved by vaccination with a live attenuated strain. In comparison with antibiotic therapies, which target bacterial survival, the anti-virulence strategy would not

cause substantial stress to the bacterium, which is one of the causes of the growing emergence of antibiotic-resistant strains.

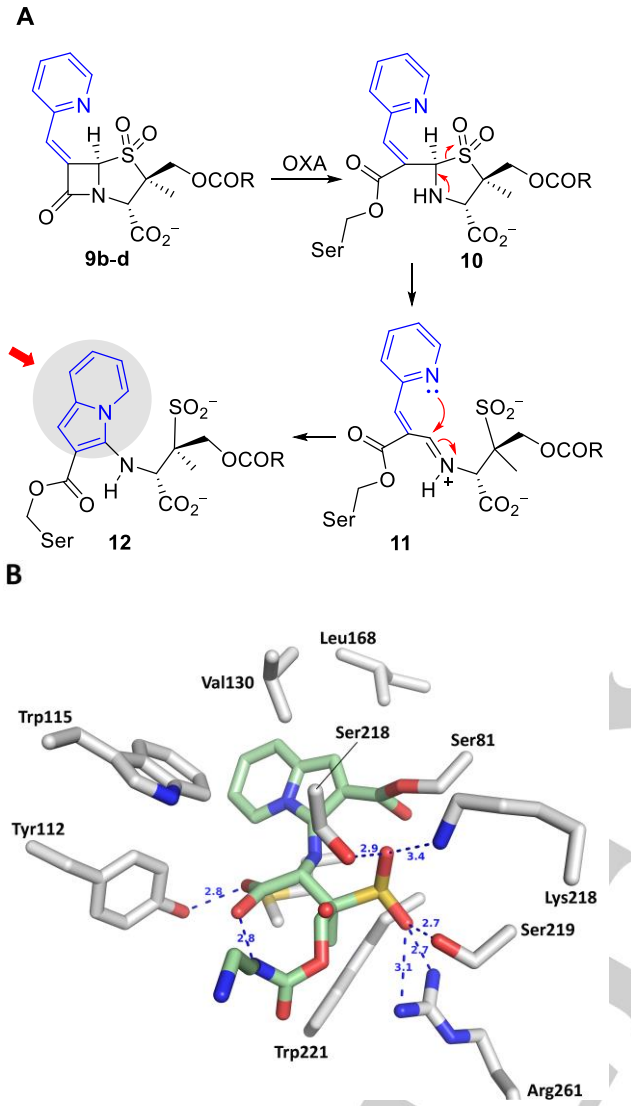


Figure 6. (A) Proposed mechanism for the covalent modification of OXA-24 caused by inhibitors **9b**–**d** and (B) detail view of the active site of OXA-24 from *A. baumannii* covalently modified by irreversible inhibitor **9d** (PDB entry 3FZC, 2.0 Å)[49]. Hydrogen bonding and electrostatic interactions between the ligand and the OXA-24 enzyme are shown. Relevant side chain residues are shown and labeled.

A promising target for the development of new anti-virulence agents is the type I dehydroquinase enzyme (3-dehydroquinate dehydratase, EC 4.2.1.10, DHQ1) because it seems to act as a virulence factor *in vivo*.[56-59] DHQ1 catalyzes the reversible dehydration of 3-dehydroquinic acid (**13**) to afford 3-dehydroshikimic acid (**14**) involving the formation of several Schiff base species' with an essential lysine (Lys170 in *S. typhi*) (Figure 7). The final dehydratation step is mediated by an essential histidine (His143 in *S. typhi*) acting as a proton donor.[60-63] Computational studies carried out on the Michaelis complex of DHQ1 from *S. typhi* (*St*-DHQ1) suggest that the formation of the substrate-Schiff base occurs by activation of the oxygen atom of the ketone group in **13** by the essential histidine, followed by nucleophilic attack from the ketone *Si* face by the essential lysine.[64,65] Based on the mechanism of action, a mimetic of the natural substrate that does not bear such reactive functional groups, namely ammonium derivative **15**, was recently developed (Figure 7).[66] This compound proved to be a time-dependent irreversible inhibitor of DHQ1 from *S. aureus* (*Sa*-DHQ1) and *St*-DHQ1, but with some differences in the formation of the resulting adducts.

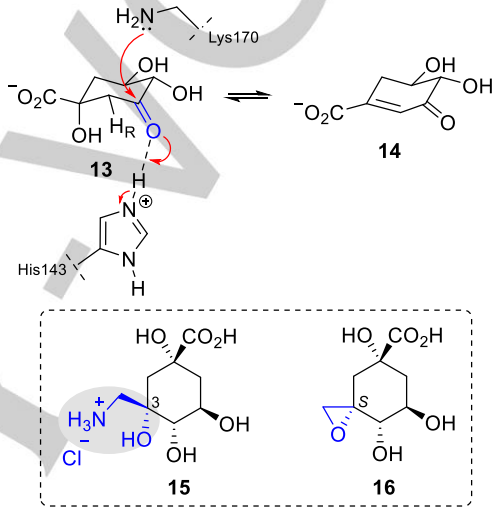


Figure 7. Reaction catalyzed by DHQ1 and irreversible inhibitors **15–16**.

In the case of *St*-DHQ1, the crystal structure of the *St*-DHQ1/**15** adduct (PDB entry 4UIO, 1.35 Å),[66] which was obtained by soaking apo-*St*-DHQ1 crystals, showed that the ligand is covalently linked to Lys170 as an amine (Figure 8A). This chemical modification would occur by activation of the essential histidine and subsequent nucleophilic attack by the essential lysine with the release of ammonia (Scheme 3). Further chemical modifications of adduct **17**, which involved dehydration/aromatization reactions and the formation of Schiff base species', were detected by MALDI-MS. The crystal structure of the *St*-DHQ1 obtained after several weeks by co-crystallization with epoxide **16** showed that the enzyme is covalently modified as a stable Schiff base (PDB entry 4CLM, 1.4 Å, Figure 8B)[65] and this suggests that ammonium derivative **15** might also be a prodrug of epoxide **16**. The results of computational studies suggest that the conserved Asp114 residue would be the base that generates a hydroxide group, which triggers the elimination reaction by deprotonation of a conserved water molecule. In contrast, only adduct **17** was obtained in the case of *Sa*-DHQ1 enzyme. The replacement of Phe225 in *St*-DHQ1 by Tyr214 in *Sa*-DHQ1, along with its hydrogen-bonding interaction with a conserved water molecule, seems to be responsible for preventing the initial adduct **17** from undergoing further transformations. Ammonium derivative **15** seems to be a good scaffold for development as it was able to

reduce the ability of *Salmonella Enteritidis* to kill A549 respiratory cells *in vitro*.[66]

Hydrogen/potassium-exchanging ATPase (H^+/K^+ ATPase)

High levels of gastric acid cause a wide range of stomach diseases, including gastroesophageal reflux disease, which can irritate the esophagus and cause heartburn and peptic ulcers. This is a common chronic disorder that affects millions of people in Western countries. Until the late 1970s, this disorder was difficult to treat and it clearly decreases the quality of life of those affected. Fortunately, the discovery in the late 1970s of cimetidine, an antagonist of the histamine 2 (H2) receptor that is involved in gastric acid secretion, and the subsequent investigations by Astra Pharmaceuticals led to the discovery of omeprazole (Scheme 4).[16,67-68]

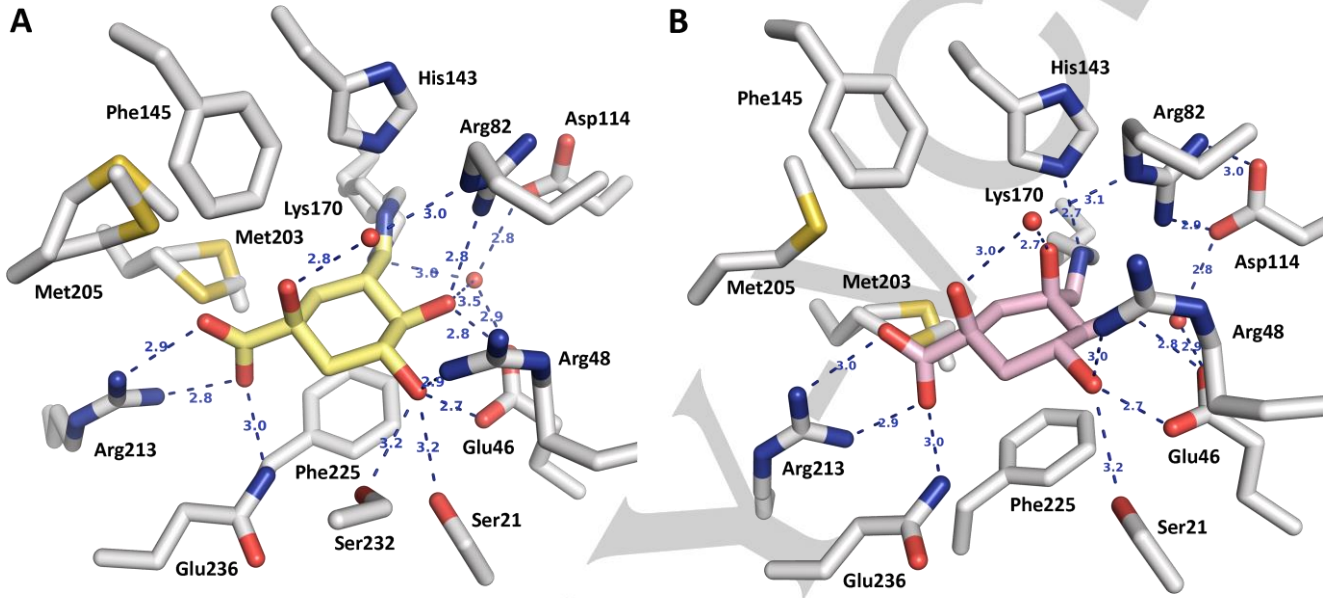
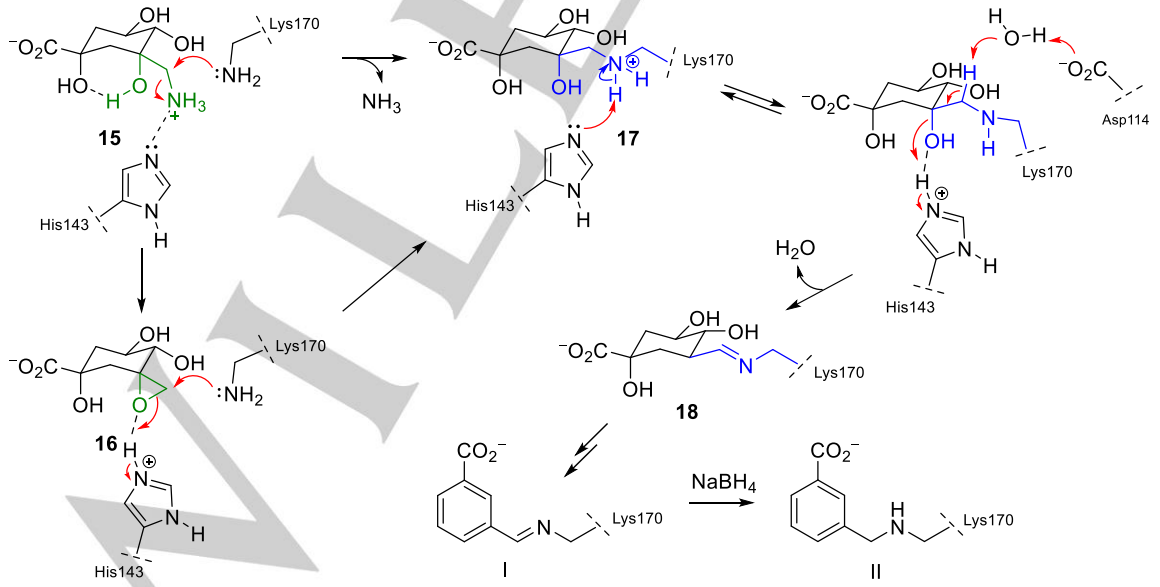
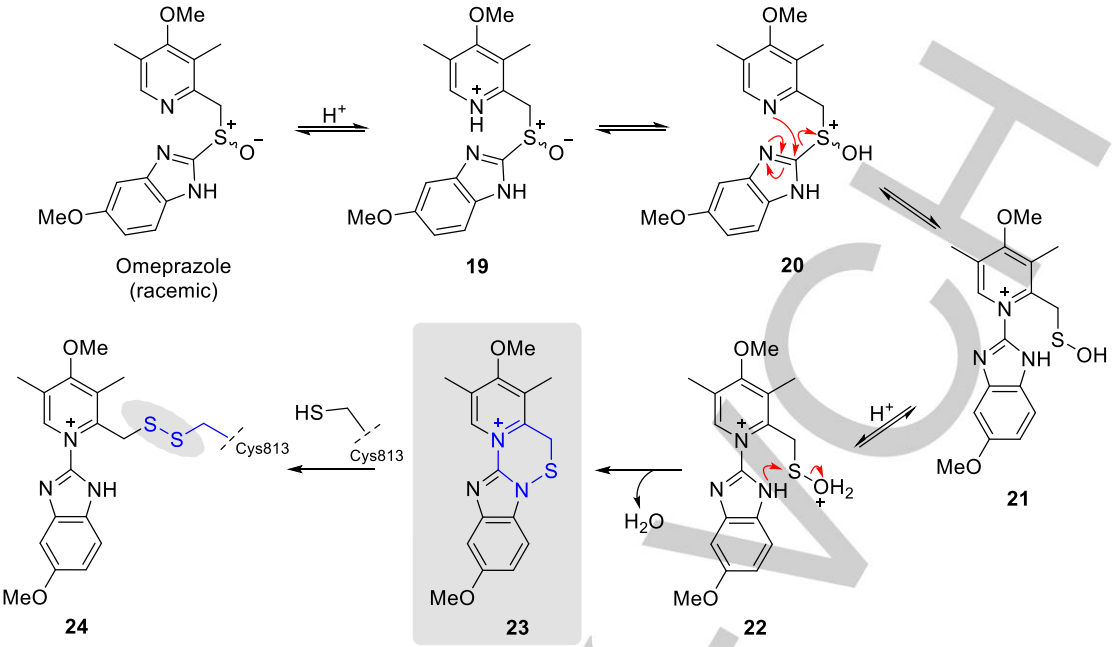


Figure 8. Crystal structures of *St*-DHQ1 covalently modified by ammonium derivative **15** (PDB entries 4UIO, 1.35 Å)[65] and epoxide **16** (PDB entries 4CLM, 1.4 Å).[64] Interactions of the modified inhibitors **15** (A) and **16** (B) with *St*-DHQ1. Hydrogen bonding and electrostatic interactions (blue) between the ligand and the *St*-DHQ1 are shown. Relevant residues are shown and labeled.



Scheme 3. Proposed covalent modification mechanism of *St*-DHQ1 caused by irreversible inhibitors **15** and **16**. Covalent adducts **17** and **18** of *St*-DHQ1 have been identified by X-ray crystallography (PDB entries 4UIO[65] and 4CLM[64], respectively). For *Sa*-DHQ1, adduct **17** is the major species detected by MALDI-MS. For *St*-DHQ1, adduct **18** undergoes elimination/aromatization reactions and adduct I and its reduced form, II, have been detected by MALDI-MS.



Scheme 4. Mechanism of action of omeprazole, the prodrug form of active metabolite **23**. This reactive compound is generated by an intramolecular rearrangement followed by dehydration under the acidic conditions in the stomach.

It was found that omeprazole modifies irreversibly an integral membrane enzyme responsible for the secretion of acid into the stomach, namely hydrogen/potassium-exchanging ATPase (EC 3.6.3.10), which is also known as H^+/K^+ ATPase.[69] This enzyme catalyzes the exchange of extracytoplasmic K^+ for cytosolic H_3O^+ in a process that occurs through phosphorylation of the enzyme by ATP, which in turn triggers a conformational change in the protein that helps to drive ion transport.

At neutral pH, omeprazole is stable and does not inhibit the enzyme. However, in the acidic medium of the stomach omeprazole undergoes an acid-promoted rearrangement that leads to the formation of the highly reactive cyclic sulfenamide **23**, which inhibits H^+/K^+ ATPase (Scheme 4).[16,67-69] The half-life of omeprazole at pH 1 is about 2 minutes whereas it is stable for around 20 hours at pH 7.4.[67]

The proposed mechanism of action involves the initial formation of sulfenic acid **20** under the acidic conditions in the stomach. This compound subsequently undergoes an intramolecular cyclization initiated by nucleophilic attack of the pyridine nitrogen atom in **20** on the C2 carbon of the benzimidazole moiety. The resulting compound **21** undergoes intramolecular nucleophilic attack of one of the nitrogen atoms of the benzimidazole moiety with elimination of water to give the highly reactive cyclic sulfenamide **23**, which is the active inhibitor formed *in vivo* from omeprazole. Finally, compound **23** undergoes nucleophilic attack by the Cys813 side chain to afford a stable disulfide adduct that causes inhibition of the enzyme.[70] Although, the two enantiomers of omeprazole has similar inhibitory potency, the *S* enantiomer (esomeprazole), proved to have better bioavailability and oral potency. This compound is commercialized as Nexium®.[71,72]

Conclusions and Outlook

For many years, irreversible inhibitors have not been a high priority in the pharmaceutical industry. This general skepticism was triggered by the discovery of the unexpected covalent mechanisms of successful drugs after many years of use and, more importantly, after hepatotoxic properties were reported in the 1970s for some compounds. These two factors retarded the development of irreversible systems over the subsequent years. The efficacy and selectivity shown by widely used and successful drugs in clinic use over the years, e.g., penicillin, aspirin and omeprazole, suggests that there was a kind of 'blindspot' when assessing the real risks of this type of compound. Fortunately, the attitude towards irreversible inhibitors has changed. Probably, we all agree that the potential toxicity of irreversible inhibitors *vs* the non-covalent ones is much higher. Mainly due to the higher risks in: 1) the possible formation of non-selective or non-specific covalent adducts with off-target proteins; and 2) the potential immune reactions. However, their potential in non-chronic therapies, such as oncology and infectious diseases, is evident considering the numerous successful examples in clinical use over the years. Moreover, a better analysis of the risks and the recognized advantages of irreversible inhibitors over the non-covalent systems has resulted in a clear increase in the number of approved irreversible drugs, of compounds in clinical trials and in the number of scientific articles in this area.

The design of a covalent inhibitor is challenging because, in addition to a high affinity for the enzyme, it requires an efficient reaction, which implies a suitable arrangement of the reactive centers in both the inhibitor and the enzyme. From the synthetic point of view, if we want to perform such reaction in the laboratory, it would probably be impossible. Specially, if we consider that the ligand might have other groups such as hydroxyl or carboxylate groups to achieve good solubility in aqueous media, which also react with the selected electrophilic reagent. Also, it would normally require the use of highly reactive electrophilic groups that would be too reactive to be even considered in any medicinal chemistry program. However, by designing a ligand with high affinity for the selected therapeutic target, less reactive electrophilic groups, which under standard conditions would be unreactive, become efficient. A good example is the herein highlighted ammonium derivative **15** that reacts with an essential lysine by forming an amino adduct with the release of ammonia. Such less reactive and safer functional groups would enhance inhibitor specificity and reduce potential off-target effects. Therefore, for an irreversible approach, by achieving an exquisite anchoring of the ligand to the active site, i.e. “geometric perfection”, is possible to use less reactive electrophilic groups and thereby to reduce inhibitor toxicity. More work must be done in exploring alternative less reactive groups to be incorporated into ligand design that would be a step forward in this area. Furthermore, in many cases the effectiveness of inhibitors is due to additional reactions, which are sometimes difficult to predict – as discussed in this review with selected examples. However, the increased selectivity and efficiency of covalent inhibitors, their prolonged effects and the reduced risk of drug resistance are unique benefits that make their development worthwhile, specially in oncology and anti-infectives. The knowledge in atomic detail of the covalent mechanisms of these inhibitors, the mechanism of action of the enzymes targeted by them, and further studies of the potential toxicity of the typically used electrophilic functional groups present in covalent inhibitors should make a positive contribution to future designs. Moreover, the development of proactive approaches could also contribute to reduce the potential toxicity of these compounds.

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Keywords: irreversible inhibition • covalent catalysis • selectivity• efficiency • toxicity

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