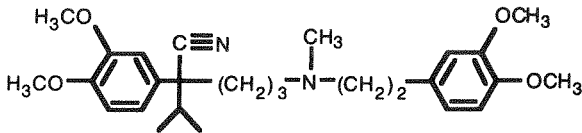


## STRUCTURE ACTIVITY RELATIONSHIPS OF CALCIUM ANTAGONISTS

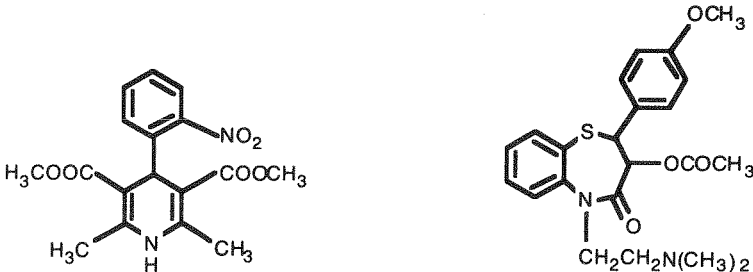
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Calcium antagonists are a structurally diverse group of compounds (figure 1) [1-3]. Although the fundamental role of calcium ions for living cells was recognized by Ringer 100 years ago, in 1963, when Verapamil [4,5] was introduced into human therapy, nobody knew its mechanism of action. Verapamil was developed as a coronary dilating agent with additional antiarrhythmic and anti-hypertensive properties. Due to the fact that it antagonized the biological effects of isoprenalin, it was considered - in these early days - to be an antiadrenergic agent. However, in contrast to classical  $\beta$ -blockers, the antagonism is functional, not competitive.



Verapamil



Nifedipine

Diltiazem

Figure 1. Structures of calcium antagonists

It was Fleckenstein in the middle and late sixties, who recognized the modulating activity of verapamil on the function of calcium channels; he created the name "calcium antagonism" for this new biological principle [6,7]. Nifedipine [8] and Diltiazem [9] followed later, and these three compounds are now not only the prototypes of highly active calcium antagonists, they are also the most important drugs in the market.

A chemically closer related class of lipophilic amines (figure 2) are Prenylamine [10], Fendiline [11], Cinnarizine [12], Flunarizine [13], Bepridil [14] and Perhexiline [15]. However, they are weak calcium antagonists, thus their structure activity relationships will not be discussed here.

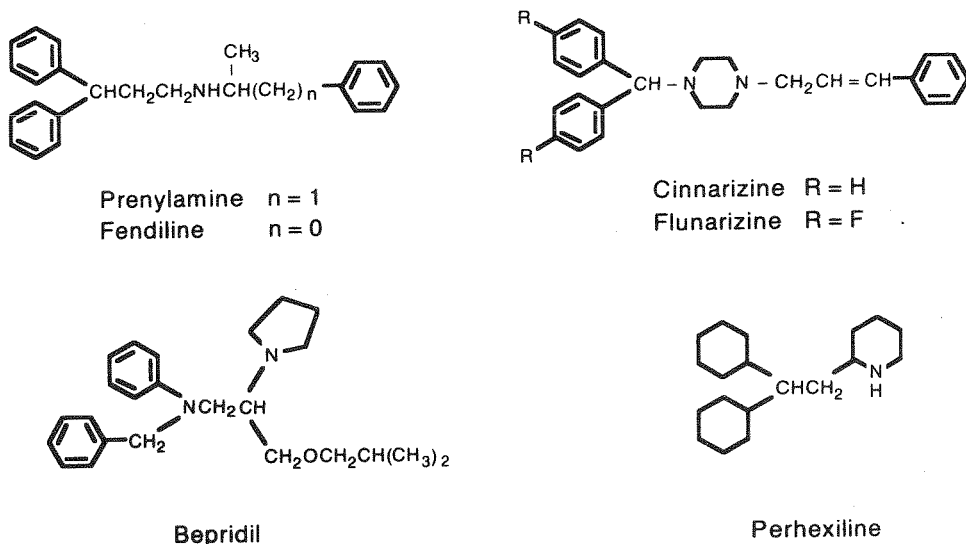


Figure 2. Structures of non-specific calcium antagonists

In a normal resting cell, the intracellular concentration of free calcium ions is rather low (figure 3), ranging from  $10^{-8}$  to  $10^{-7}$  moles per liter, which means a concentration gradient of 4 to 5 decades against the normal extracellular concentration

Ion	Extracellular concentration [mMol]	Intracellular concentration [mMol]
Na <sup>+</sup>	130 - 150	5 - 20
K <sup>+</sup>	3 - 6	120 - 140
Mg <sup>++</sup>	1 - 2	10 - 30
Ca <sup>++</sup>	2 - 5	≤ 0.0001 <sup>a)</sup>

a) resting state; 0.0001 to 0.01 during excitation

Figure 3. Extracellular and intracellular concentrations of inorganic cations

of calcium ions. This concentration gradient is effectively controlled by calcium channels and by calcium pumps located in the plasmalemma and in the internal membranes.

External stimuli are able to trigger the opening of calcium channels, whereby the intracellular calcium concentration rises to about  $10^{-5}$  moles per liter (figure 4) and binding of calcium

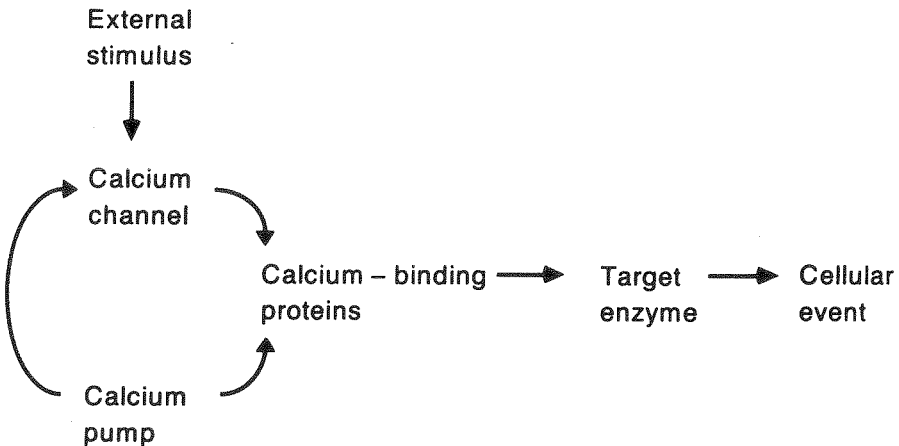


Figure 4. Scheme of calcium regulation in cells [1,3]

ions to intracellular calcium-binding proteins is enabled. The activated binding proteins can now interact with target proteins, stimulate their enzymatic activities, and trigger various cellular events. Afterwards the channels close and calcium pumps restore the initial low calcium concentration. The ubiquitous role of calcium as a second messenger in numerous cellular events ranges from contraction of smooth and striated muscle cells to glycogen metabolism and to the synthesis and release of neurotransmitters.

The therapeutic use of calcium antagonists (figure 5) [16] results from their ability to inhibit the slow inward current of calcium ions through the calcium channels. In this sense, the calcium antagonists are no antagonists according to classical definitions. They bind to the channel proteins and modify the properties of the calcium channel, according to the definition of an agonist.

### Therapeutical Use of Ca<sup>++</sup> Antagonists (Godfraind, 1986)

Clinical condition	Type
<b>Cardiovascular</b>	
Exertional angina	I, II, III
Angina at rest	I, II, III
Supraventricular tachyarrhythmias	I, III
Atrial fibrillation and flutter	I, III
Hypertension	I, II, III
Hypertrophic cardiomyopathy	I
Raynaud's phenomenon	I, II, III, IV
Cerebral vasospasms (subarachnoid hemorrhage)	II
<b>Neurological</b>	
Prophylaxis of migraine	II, IV
Vertigo	IV
<b>Under clinical examination</b>	
Protection against myocardial ischemia	I, II, III
Primary pulmonary hypertension	II
Leg ischemia	IV
Protection against cerebral ischemia	I, II, IV

Figure 5. Therapeutical use of calcium antagonists [16].  
 Type I, verapamil like; type II, nifedipine like;  
 type III, diltiazem like; type IV, flunarizine like

Calcium antagonists diminish the oxygen consumption of the heart muscle and increase its economy, they lead to a higher oxygen supply by a dilation of the coronary vessels, they lower the blood pressure by a dilation of the peripheral vessels and - with the exception of nifedipine - they lower the heart rate and prevent arrhythmias by a retardation of the transmission of electric signals in the conductive system of the heart.

One of the very first quantitative structure activity relationships for calcium antagonists showed a negative dependence of biological activity on the lipophilicity, expressed by the chromatographic parameter  $R_M$ , as opposed to a positive dependence on lipophilicity for a relatively large group of non-specifically acting cardiodepressive agents (figure 6) [1,3]. However, from a today's view this relationship must be seen as a chance correlation because we now know lipophilic nifedipine analogs with higher biological activity as well as polar verapamil analogs with lower biological activities. It is obvious that for drugs

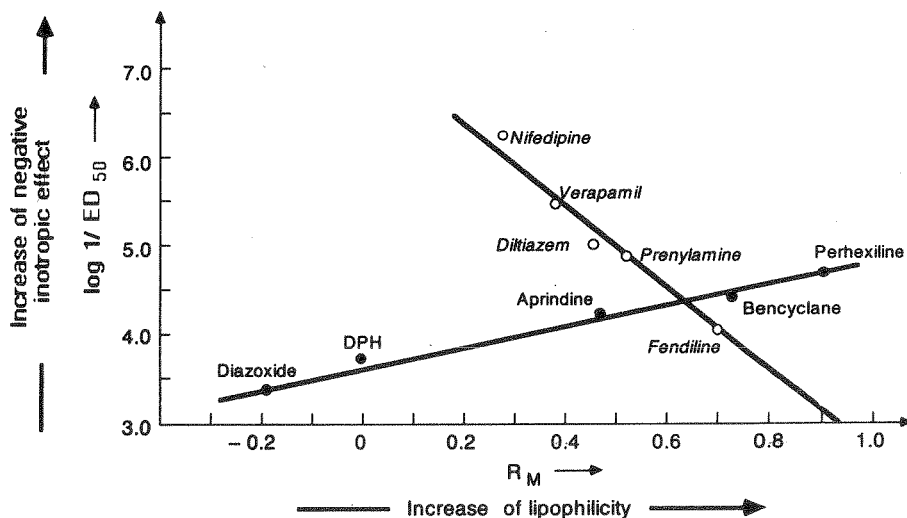
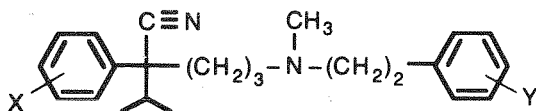


Figure 6. Correlations between lipophilicity and negative inotropic potency of calcium antagonists (open circles) and non-specific cardiodepressive agents (closed circles) [1,3]





**Negative Inotropic Activity** (Mannhold et al., 1981)

Group I  $\log 1/ED_{50} = 0.81 F + 4.77$   
 (n = 9; r = 0.81; s = 0.33)

Group I+II  $\log 1/ED_{50} = 0.93 F - 0.59 MR + \text{const.}$   
 (n = 13; r = 0.82; s = 0.35)

**Vasodilatory Activity** (Mannhold et al., 1987)

Group I  $\log 1/IC_{50} = 0.37 f + 4.46$   
 (n = 8; r = 0.94; s = 0.10)

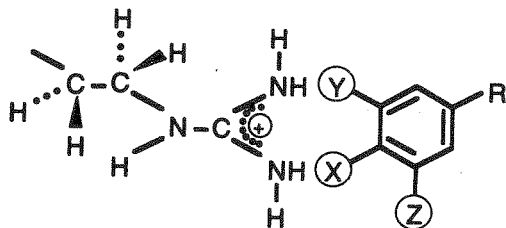
**Binding Affinity** (Kubinyi et al., 1985)

$\log 1/K_i = 0.49 [\text{OMe}] - 0.05 [\text{OMe}]^2 - 1.76$   
 (n = 23; r = 0.92; s = 0.19)

Figure 8. Structure activity relationships of verapamil analogs [19-22]

with lipophilicity, expressed by the hydrophobic fragmental constant  $f$  [21]. For a group of 23 analogs, including only methoxy-substituted compounds with different numbers and positions of the methoxy groups in the two aromatic rings, we derived a nonlinear equation which indicates that the optimal number of methoxy groups in the molecule is between 4 and 5; the binding affinity was used as biological activity parameter and we independently confirmed that there are close relationships between the binding affinity and other biological activities [22].

A very interesting approach was followed by Hölting to describe the biological activities of a series of ring-substituted verapamil analogs in a quantitative manner (figure 9) [23,24]. He looked



Geometry of the hypothetical interaction complex between verapamil and an arginine side chain (Höltje, 1982)

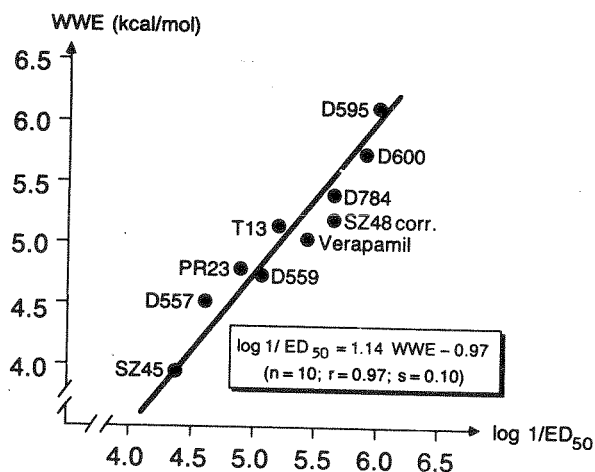


Figure 9. Interaction of verapamil analogs with an arginine side chain [23,24]

at different amino acid side chains and calculated interaction energies between these side chains and the part of the verapamil molecule which has been varied chemically. Different geometries and different distances were tested in order to obtain the best fit between these energy values and the observed biological activities. The resulting equation for the arginine side chain indicates that a basic group may be the binding partner of the verapamil analogs. Other amino acid side chains were tested but gave no corresponding correlations.

For the dependence of the binding affinities on the lipophilicity of the aliphatic side chain at the quaternary carbon atom a nonlinear relationship was derived (figure 10) [25]. The only outliers in this nice correlation were two different hydrogen-substituted compounds, which had a much lower affinity than predictable from the other analogs.

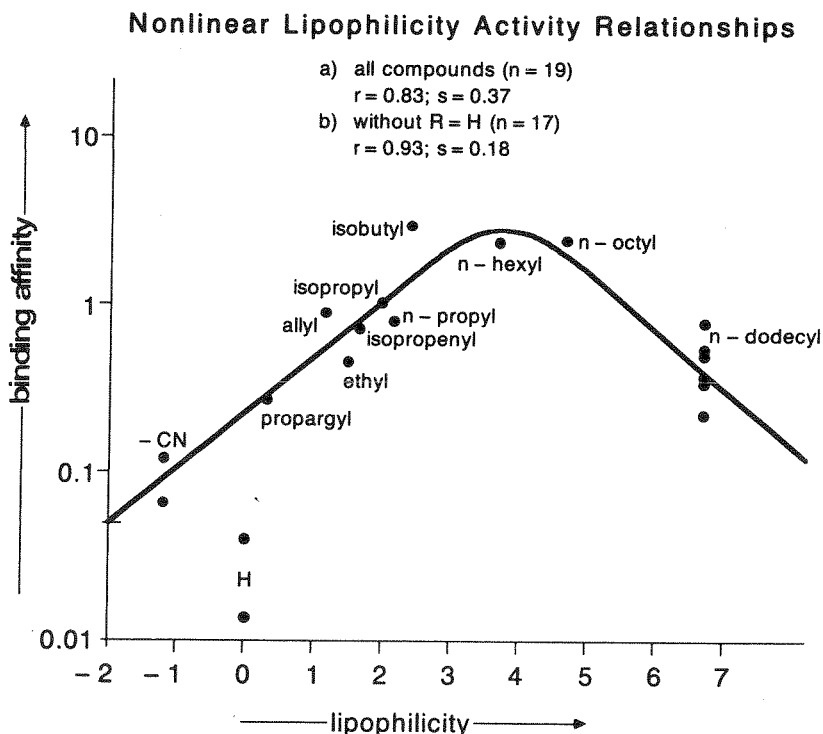


Figure 10. Nonlinear lipophilicity activity relationship of verapamil analogs [25]

In searching for the reasons of this unexpected inactivity of the hydrogen-substituted compound, we first looked at the X-ray structure of verapamil (figure 11) [26]. Verapamil is a molecule with many rotational degrees of freedom, thus its conformation at the receptor may be different from the X-ray conformation. As well, the conformation in solution, derived from two-dimensional nuclear magnetic resonance techniques, does not lead to a better

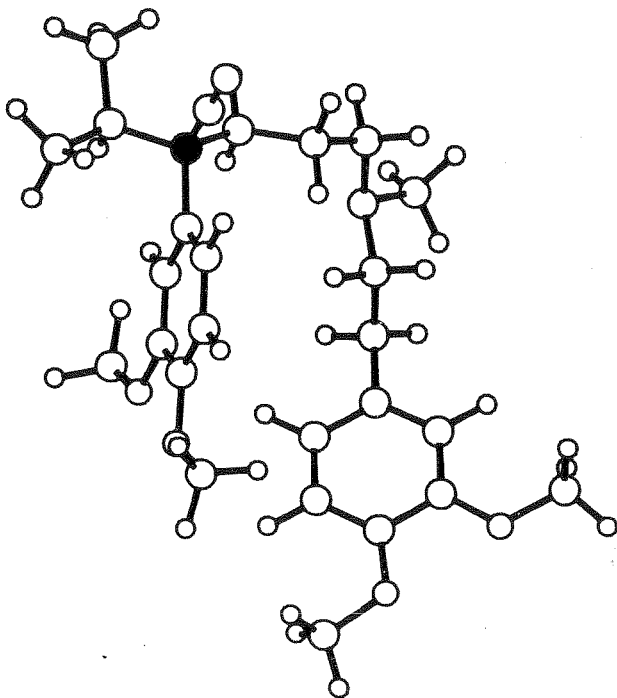


Figure 11. X-ray structure of verapamil [26]; the quaternary carbon atom bearing the nitrile group is indicated by a closed circle

prediction in the case of such a flexible molecule. The problem is: at the receptor site a flexible fit occurs, induced by the three-dimensional structure of the binding site. The resulting conformation is that one which allows the highest number of stabilizing interactions, irrespective whether this induced-fit conformation is indeed the lowest-energy conformation of the isolated molecule or not.

On the other hand, one conformational feature seems to be of special importance for the binding of verapamil to its receptor: the nitrile group and the aromatic ring are arranged in a plane, induced by the bulky isopropyl group and by the chain bearing the rest of the molecule [23,27]. The rotational barrier of the bond involved seems to be high enough to stabilize this conformation also at the receptor site [28].

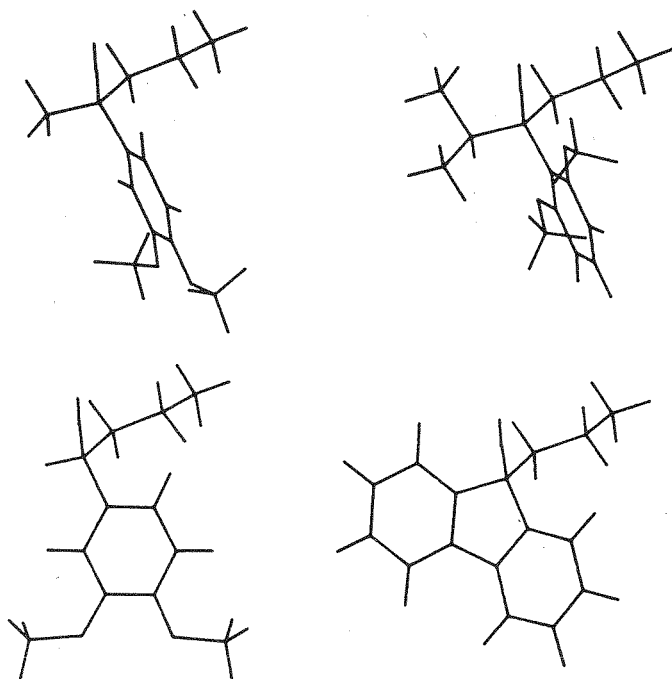
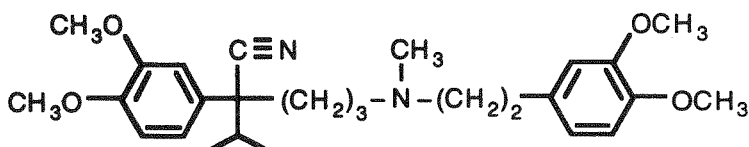


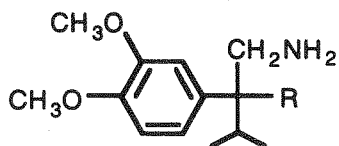
Figure 12. Lowest energy conformations of verapamil analogs. Upper left: methyl (instead of isopropyl) analog [28]. Upper right: 2,6-dimethoxy (instead of 3,4-dimethoxy) analog [29]. Lower left: hydrogen (instead of isopropyl) analog [28]. Lower right: fluorene analog [30]

Even for an analog with methyl instead of isopropyl (figure 12) [28] this barrier seems to be high enough and even for a 2,6-disubstituted analog the coplanar conformation is the most probable one, as demonstrated by Höltje [29]. In contrast to this, in the analog with hydrogen instead of isopropyl such a conformation is no longer the most stable one [28]. In the lowest-energy conformation the plane of the aromatic ring intersects the angle between the C-H bond and the C-C≡N bond and we take this as an explanation for the much lower than predicted affinity of this analog. In addition, a fluorene analog, synthesized and tested by Gualtieri [30], where the nitrile group and the benzene rings are in different planes, is completely devoid of calciumantagonistic activity.

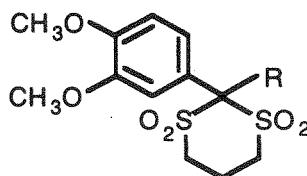
There has been some discussion, whether the nitrile group of verapamil is essential for biological activity or not (figure 13). From the inactivity of an amino analog, Mannhold and Höltje concluded the nitrile function to be essential [31]. But tiapamil [32], a cyclic disulfone with moderate calciumantagonistic activity, gives evidence that groups with comparable electronic properties are able to substitute the nitrile group to a certain extent. From our own experience, exchange of the nitrile group by an aldehyde or azido group also leads to comparable binding affinities [25].



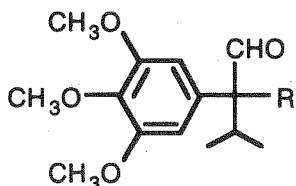
### Verapamil



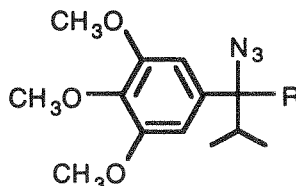
Amino analog, inactive



Tiapamil (Hoffmann - La Roche)  
active



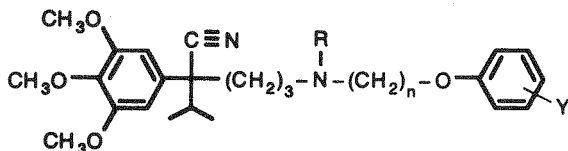
Aldehyde analog, active



Azido analog, active

Figure 13. Nitrile group analogs of verapamil [25,31,32]

An interesting perspective was opened by Japanese workers, who found that phenoxy analogs of verapamil have different selectivities as calcium antagonists and as  $\alpha$ -antagonists (figure 14)



$\text{Ca}^{++}$  - Antagonistic Activity     $\text{R}=\text{CH}_3, n=3$

$$\begin{aligned} pA_2 = & -0.35 \pi^2 - 0.58 B - 5 \text{ para} + 0.43 \pi \text{ para} - \\ & 1.40 F_{\text{ortho}} + 8.39 \\ & (n=28; r=0.92; s=0.26) \end{aligned}$$

$\alpha$ - Antagonistic Activity     $\text{R}=\text{H}, n=2$

$$\begin{aligned} pA_2 = & -0.18 \pi^2 - 0.63 \sigma_{\text{ortho}} + 0.40 E_s^{\text{ortho}} + \\ & 1.08 I_{\text{ortho}} + 0.79 E_s^{\text{meta}} + 0.69 \pi_{\text{meta}} - \\ & 0.71 L_{\text{para}} + 8.72 \\ & (n=28; r=0.93; s=0.38) \end{aligned}$$

T. Fujita et al., 1987, in press

Figure 14. Structure activity relationships of verapamil analogs [33]

[33], dependent on the nitrogen substitution, on the chain length at the right side of the molecule, and on the substitution pattern in the right aromatic ring. The large numbers of parameters in both equations reflect the complexity of the drug receptor interactions.

The structure of nifedipine differs drastically from the structure of verapamil and its analogs. Consequently different structure activity relationships are derived for this series (figure 15) [19,34]. Essential for high biological activity are: the dihydro-

**Essential:**

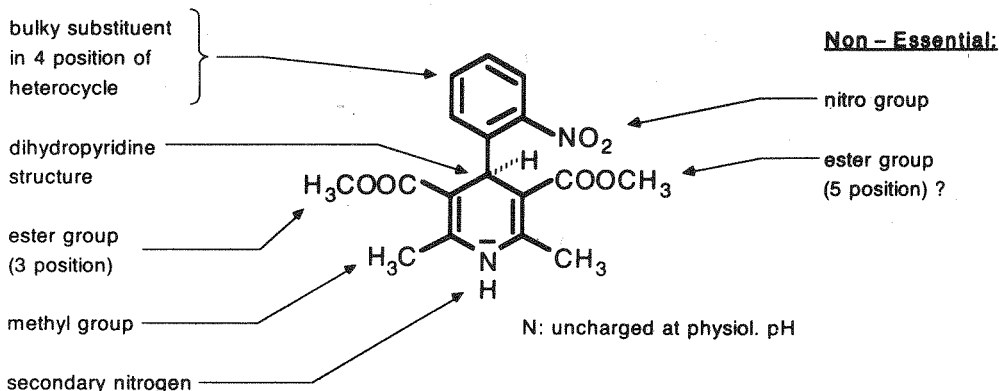


Figure 15. Essential and non-essential structural moieties of nifedipine [1,3,19]

pyridine ring with its secondary nitrogen, uncharged at physiological pH values; the methyl groups in the 2 and 6 positions; at least one ester group, while the other one is favourable for biological activity, but not essential; a bulky substituent in the 4 position, e.g. a phenyl group (ortho-substitution of this ring and, to a certain extent, meta-substitution increase biological activity, while para-substitution decreases the biological activity). These relationships can be formulated quantitatively (figure 16), in the first two equations for ortho-substituted analogs, where  $B_1$  is a steric parameter, and in the third equation for 18 ortho-, meta- and para-substituted compounds, where  $L$  is another steric parameter and  $\sigma$  is the electronic Hammett parameter [35]. We were able to improve this relationship by considering only one parameter for each position of substitution [36]; the message from this equation is: bulky ortho-substituents and electron attracting meta-substituents increase the biological activity, while large para-substituents decrease it.

## Nifedipine Analogs

Negative Inotropic Activity (Rodenkirchen et al., 1979)

$$\log 1/EC_{50} = 0.80 B - 1_{ortho} + 5.06$$

(n = 8; r = 0.91; s = 0.12)

Binding Affinity (Mahmoudian and Richards, 1986)

$$\log 1/IC_{50} = 2.41 B - 1_{ortho} + 5.15$$

(n = 7; r = 0.91; s = 0.32)

$$\log 1/IC_{50} = 2.24 B - 1_{ortho, meta} - 0.48 L_{meta} -$$
$$1.29 B - 1_{para} + 1.95 \sigma_{meta} + 7.57$$

(n = 18; r = 0.93; s = 0.45)

Binding Affinity (Kubinyi, 1987)

$$\log 1/IC_{50} = 2.52 B - 1_{ortho} + 3.21 \sigma_{meta} -$$
$$0.71 L_{para} + 6.45$$

(n = 18; r = 0.95; s = 0.38)

Figure 16. Structure activity relationships of nifedipine analogs [19,34-36]

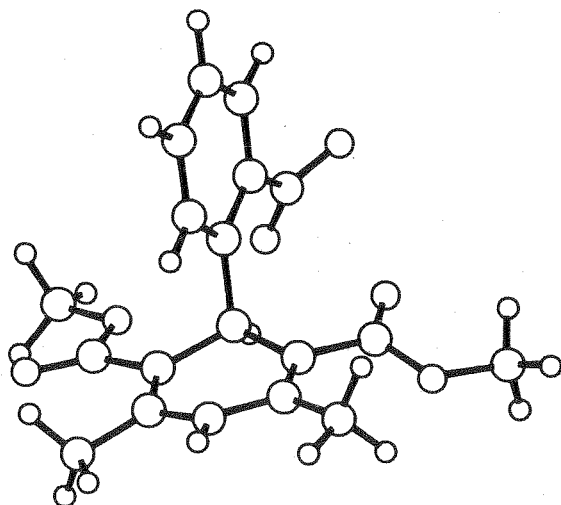
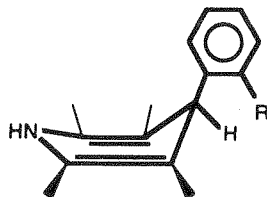
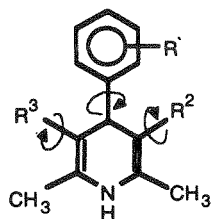
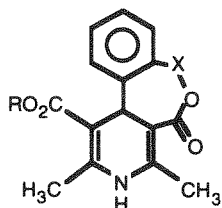


Figure 17. X-ray structure of nifedipine [37]

As in the case of verapamil, valuable information can be derived from the X-ray analysis (figure 17) [37]: nifedipine is a much more rigid molecule, a fact which may partially explain its high affinity to the binding site (this is an entropy effect, because only few degrees of rotational freedom are lost when going from the free state to the bound ligand). An important conformational restriction can be seen: due to the bulky alkoxycarbonyl groups at both sides of the dihydropyridine ring, and furthermore stabilized by the nitro group in the ortho-position of the phenyl ring, the plane of the aromatic ring has an angle of nearly 90 degrees to the plane of the dihydropyridine ring. That this perpendicular arrangement of both rings is essential for the binding affinity and thus for the biological activity, was proven by Seidel and his coworkers (figure 18), who synthesized lactones with 6- to 12-membered rings, where the phenyl ring is fixed at different angles to the dihydropyridine ring [38].



Conformational flexibility of DHPs



Lactones as conformationally restricted analogs

Figure 18. Conformational analysis of nifedipine [38]

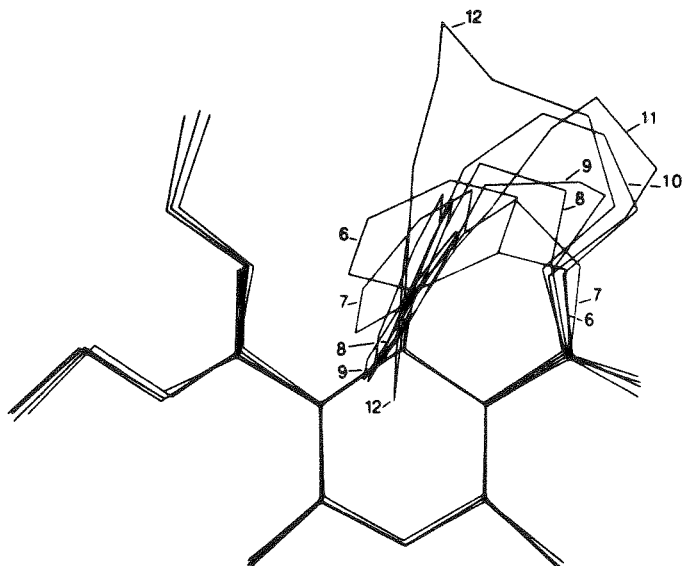


Figure 19. Superimposed X-ray structures of the dihydropyridine lactones [38]

The angles were derived from the X-ray analyses (figure 19); only the largest lactone with a 12-membered ring is able to adopt a conformation in which the aromatic ring plane almost ideally bisects the dihydropyridine ring. Shorter lactone bridges force the aryl substituent to deviate from the bisecting plane. The deviations of the lactones with ring sizes between 8 and 11 are rather small, whereas the 6- and 7-membered rings are twisted out of the perpendicular plane dramatically; the deviation of the aromatic ring plane from the bisecting plane amounts to 80 degrees in the case of the 6-membered ring lactone, leading to a nearly coplanar arrangement of both rings.

In a receptor binding assay,  $K_i$  values for the lactones were observed which correlated well with the degree of deviation of the aromatic ring from the perpendicular orientation (figure 20) [38]. The 6-membered ring compound with its largest deviation is completely inactive; for the other compounds the activity increases with decreasing deviations from the 90 degrees angle. The

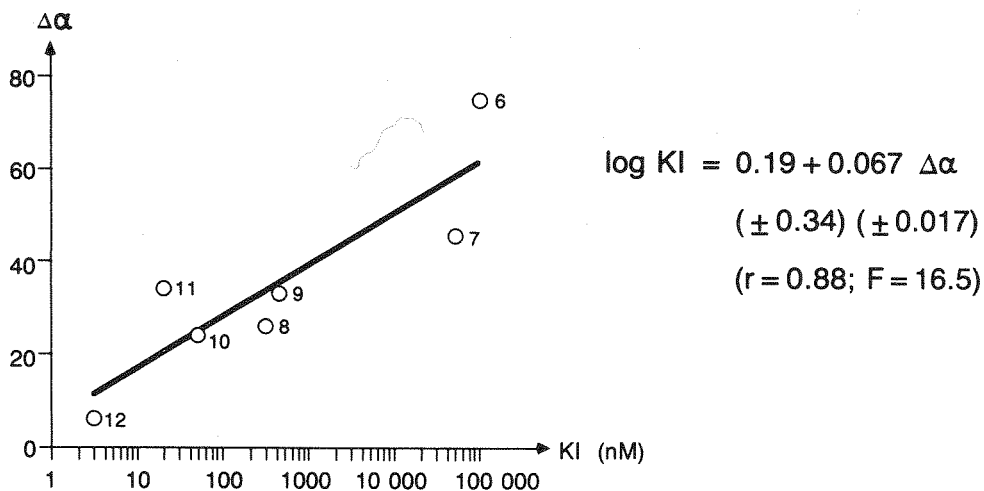


Figure 20. Structure activity relationship of the dihydropyridine lactones [38]

11-membered ring does not fit well, possibly due to some conformational flexibility. The binding data also correlate well with in vitro-data from the  $K^+$ -induced contraction of rabbit aorta. Independent evidence for the optimal conformation at the receptor site was derived from a sulfur-bridged analog of nitrendipine (figure 21) [39].

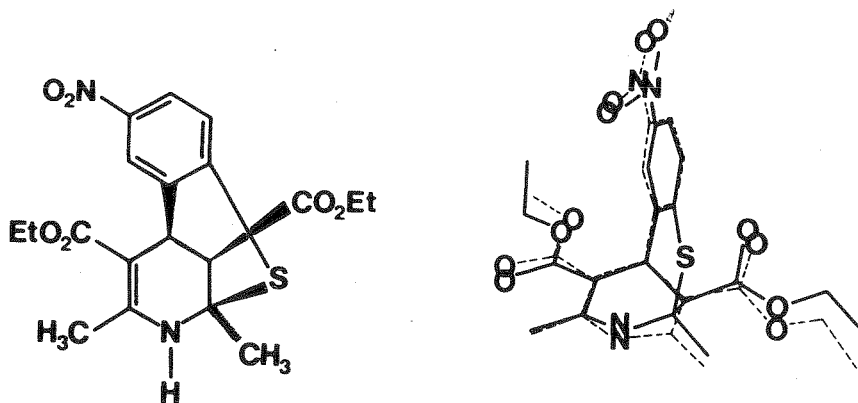
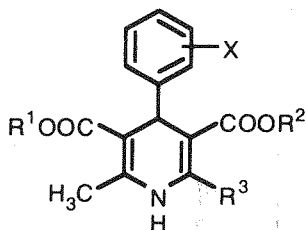


Figure 21. Sulfur-bridged nitrendipine analog [39]; matching with nitrendipine

Higher selectivity is claimed for a number of second generation dihydropyridines (figure 22); to mention only a few of them: Nitrendipine [40], Nimodipine [41], Nisoldipine [42], Felodipine [43] and Amlodipine [44].



	X	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>
Nifedipine	2 - NO <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>
Nitrendipine	3 - NO <sub>2</sub>	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>
Nimodipine	3 - NO <sub>2</sub>	CH <sub>2</sub> CH <sub>2</sub> OCH <sub>3</sub>	CH(CH <sub>3</sub> ) <sub>2</sub>	CH <sub>3</sub>
Nisoldipine	2 - NO <sub>2</sub>	CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>
Felodipine	2,3 - Cl <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	CH <sub>3</sub>
Amlodipine	2 - Cl	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	CH <sub>2</sub> O(CH <sub>2</sub> ) <sub>2</sub> NH <sub>2</sub>

Figure 22. Nifedipine analogs [40-44]

There is sufficient evidence that verapamil and nifedipine analogs bind in a different manner and most probably at different sites. However, if one compares the conformational restrictions of both structures, there results a striking similarity (figure 23) [28]: superposition of both molecules shows a perfect fit of the plane of the aromatic rings and of the nitril nitrogen atom with the dihydropyridine nitrogen atom. Whether this correspondence is fortuitous or not, cannot be decided by the evidence on hand. Hopefully affinity labelling, which is in progress and structure determination of the labelled channel proteins will clarify the relationships and differences between both structural types [45,46].

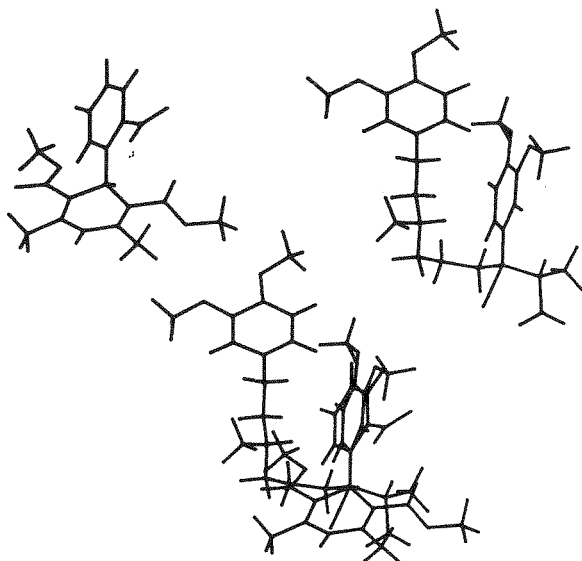


Figure 23. Matching of verapamil (upper right molecule) and nifedipine (upper left molecule) [28]

The third structural type of highly active calcium antagonists is represented by diltiazem (figure 24) [9]. Only a few details are known about the structure activity relationships in this series, because only a few analogs were published together with biological data [47,48]. The most important structural feature is the *cis*-arrangement of the aromatic substituent with the acetoxy group, which is a prerequisite for biological activity.

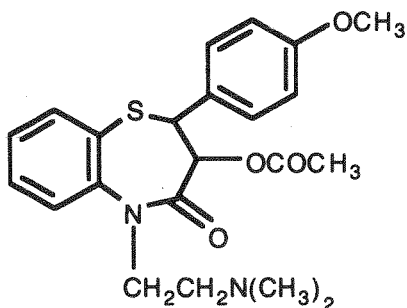


Figure 24. Structure of diltiazem [9,47,48]

Hoe 166, a new structure developed at Hoechst (figure 25) [49], is up to date the most potent non-dihydropyridine calcium antagonist. This compound, which is under clinical investigation, and the closely related Japanese compound SA-2572 [50] can be seen as hybrid structures between diltiazem and verapamil.

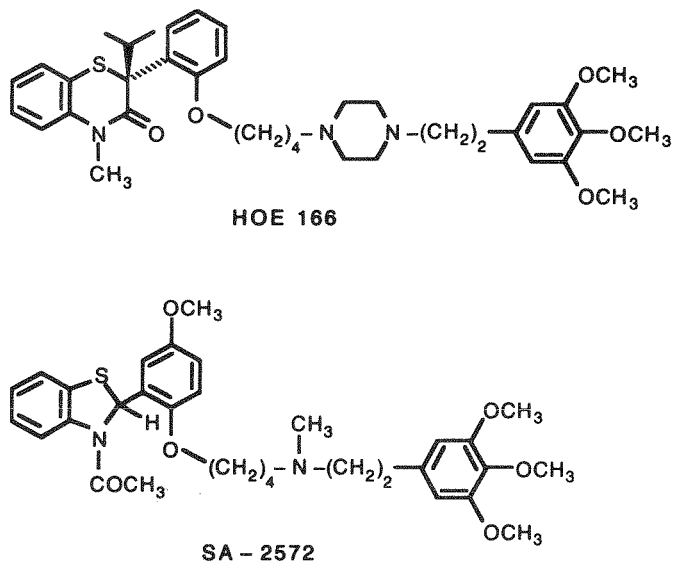
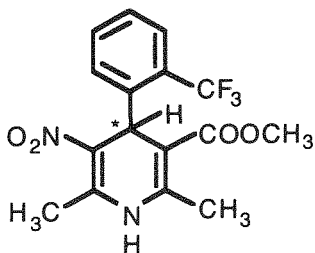


Figure 25. Structures of HOE 166 [49] and SA-2572 [50]

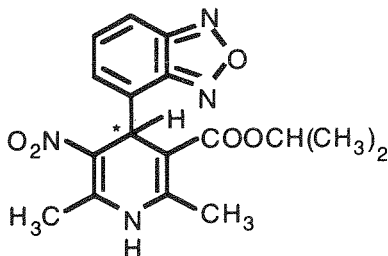
Nevertheless, the binding characteristics of Hoe 166 indicates that the inhibition at the nifedipine, the verapamil and the diltiazem binding site is in all three cases non-competitive. It looks like nature has reserved at least one binding site for every successful drug company. Structure activity relationships for Hoe 166 and its analogs have been discussed [49] but no quantitative relationships were derived until yet.

A review on the structure activity relationships of calcium channel blockers would be incomplete without mentioning the puzzling fact that some structurally very closely related analogs of the most active dihydropyridines are calcium agonists instead of antagonists (figure 26). Calcium agonists stabilize



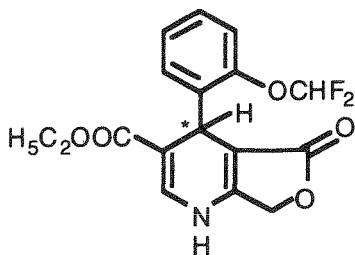
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S-[-]- form: agonist  
R-[+]- form: weak antagonist

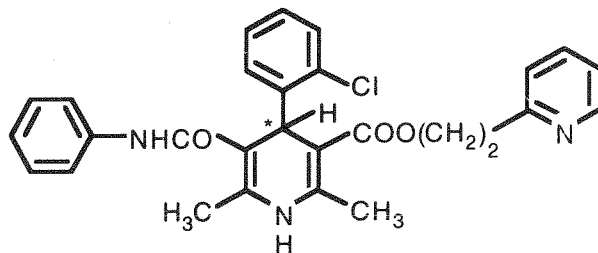


202 - 791

S-[+]- form: agonist  
R-[-]- form: antagonist



CGP 28 392



YC 170

Figure 26. Structures of calcium agonists [51-55,60-64]

the calcium channel in its open state, thus they facilitate the influx of calcium ions and show positive inotropic activity. The first member of this class of compounds was found when binding experiments indicated that BAY K 8644 [51] had high affinity to the dihydropyridine receptor but no corresponding negative inotropic activity. The SANDOZ compound 202-791 was separated into the stereoisomers: the R-[-]-form proved to be a calcium antagonist, while the S-[+]-form was a calcium agonist [52-54]. Shortly afterwards the same effect was found for BAY K 8644. The R-[+]-form is a weak antagonist, while the S-[-]-form is the calcium agonist [55]. Structurally the only difference between these compounds and the classical dihydropyridines is a nitro group at the dihydropyridine ring instead of a second ester group. In one enantiomer this nitro group makes the difference to the antagonists while in the other enantiomer

the nitro group obviously binds in a similar manner as an ester group, without any influence on the mode of interaction with the receptor [56-58].

There are only few examples in the literature [59], where a racemate is made up from an agonist and an antagonist; however, one should remember that the calcium channel blockers do not fulfill the classical definition of competitive antagonists. In truth both forms, agonists as well as antagonists, induce changes of the receptor kinetics, most probably by different allosteric effects on the channel proteins.

CGP 28 392 [60-62] and YC 170 [63,64] are two further examples of calcium agonists in the dihydropyridine series.

At the moment the calcium agonists are pharmacological tools only. However, they provide a new mechanism of positive inotropic activity which may lead to new drugs for the treatment of congestive heart failure in the future.

Dedicated to Prof. Dr. Helmut Dörfel on the occasion of his 60<sup>th</sup> birthday

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