Diltiazem-like Calcium Entry Blockers: A Hypothesis of the Receptor-Binding Site Based on a Comparative Molecular Field Analysis Model

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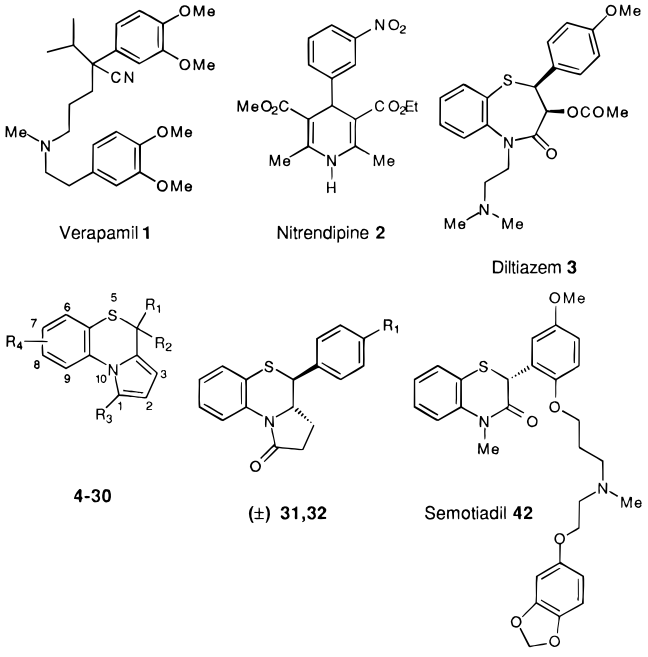
A series of 26 pyrrolo[2,1-*c*][1,4]benzothiazines, which have been already synthesized and reported to show calcium antagonist activity in both radioligand-binding assays and functional studies, were investigated using the comparative molecular field analysis (CoMFA) paradigm. Due to the lack of experimental structural data on these derivatives, the minimum energy conformers obtained by molecular mechanics calculations were used in the subsequent study. Structures were aligned following an alignment criterion based on the pharmacophoric groups of the studied compounds. The predictive ability of the CoMFA model was evaluated using a test set consisting of three representative compounds. The best 3D-quantitative structure−activity relationship model found yields significant cross-validated, conventional, and predictive r^2 values equal to 0.703, 0.970, and 0.865, respectively, the average absolute error of predictions being 0.26 log unit. The predictive capability of this model was also tested on a further test set of molecules consisting of diltiazem and nine pyrrolo[2,1-*d*][1,5]benzothiazepines endowed with calcium antagonist activity. The accurate results obtained also in this case revealed the robustness of the model. On the basis of the same alignment, the structural moieties of the studied calcium entry blockers which are thought to contribute to the biological activity were identified, and a possible receptor-binding site for all these compounds is presented taking into account the information derived from the analysis of the steric and electrostatic CoMFA contour maps.

Introduction

One of the most important achievements in the therapy of cardiovascular disorders has been the development, over the last 2 decades, of drugs which inhibit the entry of calcium ions into cells by blocking the calcium channels.[1] These calcium entry blockers (CEBs) are a group of structurally diverse compounds acting on several types of calcium channels.[2] As far as L-type voltage-operated channels are concerned, three distinct classes of selective, potent blockers are presently recognized,[3] which are typified by verapamil (**1**), nitrendipine (**2**), and diltiazem (**3**) (Chart 1). As in the case of other bioactive molecules, the available information about these drugs has been obtained through an essentially empirical approach, based on the synthesis of drug analogues having different functionalities and physicochemical properties.[4] This body of work has led to the clarification of the structure−activity relationships (SARs) of dihydropyridine and verapamil analogues, while not many effective CEBs related to diltiazem have so far been reported.[5] Accordingly, the available information concerning SAR of diltiazem[6] and related compounds[7] is still presently not conclusive.

In the last few years, an extensive investigation on a new class of CEBs, namely, 1-benzazepin-2-one derivatives, isosterically related to diltiazem, has been performed by Floyd and co-workers at Bristol Myers Squibb.[8] These studies elucidated the SAR of this class of CEBs and allowed to propose a possible receptor-binding mode for these molecules as well as diltiazem and desmethoxyverapamil.[9]

Chart 1



Recently we described the synthesis and the calcium antagonist activity of some pyrrolo[2,1-*c*][1,4]benzothiazine derivatives.[10−12] SARs for these new CEBs were also derived through a molecular modeling study taking into account their three-dimensional structural similarity to diltiazem.[12] However, for a rational drug design in this area, a model able to predict *a priori* the biological activity of new, possibly more active compounds would be welcome.

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Table 1. Observed and Calculated Receptor-Binding Affinity Values of the Compounds Forming the Training Set

					pIC_{50}[a]		
compd	R_1	R_2	R_3	R_4	obsd[b]	calcd[c]	diff[d]
4	H	H	$(Me)_2NCH_2$	H	6.11	6.08	0.03
5	Ph	H	$(Me)_2NCH_2$	H	6.50	6.49	0.01
6	4′-$(OMe)C_6H_4$	H	$(Me)_2NCH_2$	H	9.37	9.26	0.11
7	2′,4′-$(OMe)_2C_6H_3$	H	$(Me)_2NCH_2$	H	6.82	6.89	-0.07
8	2′,4′-$(OMe)_2C_6H_3$	H	*s*-$C_4H_9NHCH_2$	H	7.82	7.89	−0.07
9	2′,4′-$(OMe)_2C_6H_3$	H	$O(CH_2CH_2)_2NCH_2$	H	5.91	5.87	0.04
10	4′-$(OMe)C_6H_4$	H	3,4-$(OMe)_2C_6H_3(CH_2)_2N(CH_3)CH_2$	H	7.28	7.16	0.12
11	Ph	Ph	H	8-Cl	5.36	5.39	−0.03
12	Ph	Ph	H	8-CF_3	5.69	5.82	−0.13
13	4′-$(OMe)C_6H_4$	OAc	H	H	7.52	7.57	−0.05
14	Ph	OAc	H	8-CF_3	7.19	7.21	−0.02
15	4′-$(OMe)C_6H_4$	H	$O(CH_2CH_2)_2NCH_2$	H	5.30	5.33	−0.03
16	Ph	Ph	$(Me)_2NCH_2$	6-Cl	7.09	7.18	−0.09
17	2′,4′-$(OMe)_2C_6H_3$	H	3,4-$(OMe)_2C_6H_3(CH_2)_2NHCH_2$	H	6.66	6.53	0.13
18	4′-$(OMe)_2C_6H_4$	4′-$(OMe)_2C_6H_4$	H	8-CF_3	5.30	5.24	0.04
19	4′-$(OMe)_2C_6H_4$	4′-$(OMe)_2C_6H_4$	H	H	5.30	5.22	0.08
20	Ph	Ph	H	6-Cl	6.50	6.38	0.12
21	Ph	Ph	$(Me)_2NCH_2$	H	5.30	5.46	−0.16
22	4′-$(OMe)_2C_6H_4$	4′-$(OMe)_2C_6H_4$	$(Me)_2NCH_2$	H	5.66	5.79	−0.13
23	Ph	OAc	H	6-Cl	5.80	5.94	−0.14
24	4′-$(OMe)_2C_6H_4$	H	*s*-$C_4H_9NHCH_2$	H	7.92	7.82	0.10
25	Ph	H	3,4-$(OMe)_2C_6H_3(CH_2)_2N(CH_3)CH_2$	H	6.70	6.80	−0.10
26	2′,4′-$(OMe)_2C_6H_3$	H	3,4-$(OMe)_2C_6H_3(CH_2)_2N(CH_3)CH_2$	H	5.80	5.90	−0.10
27	Ph	Ph	H	H	6.33	6.36	−0.03
31	OMe				8.36	8.36	0.00
32	H				9.11	9.12	−0.01

[a] IC_{50} is defined as the concentration (M) of the tested compounds that inhibited [^{3}H]nitrendipine binding on rat cortex homogenate by 50%. [b] Experimental data taken from refs 10−12. [c] Values calculated according to the calibration model. [d] Difference between observed and calculated values.

Table 2. Observed and Predicted Receptor-Binding Affinity Values of the Compounds Forming the Test Set

					pIC_{50}[a]		
compd	R_1	R_2	R_3	R_4	obsd[b]	pred[c]	diff[d]
28	4′-$(OMe)_2C_6H_4$	H	3,4-$(OMe)_2C_6H_3(CH_2)_2NHCH_2$	H	6.40	6.27	0.13
29	Ph	OAc	H	H	5.33	5.56	−0.23
30	Ph	Ph	$(Me)_2NCH_2$	8-CF_3	6.97	6.54	0.43

[a,b] See the corresponding footnotes of Table 1. [c] Values predicted by the CoMFA model. [d] Difference between observed and predicted values.

In 1991, Scolastico and co-workers[13] proposed a pharmacophoric model for diltiazem and some structurally related compounds (benzothiazines and benzothiazocines) based on six interatomic distances between the most polar groups (descriptors) present in all the considered structures. Via selecting the most probable conformations linked to the biological activity, a model was built, able to classify conformations according to their biological behavior and to predict correctly the activity of other molecules not used in the construction of the model but possessing known activity. Most of our molecules do not possess some of the relevant molecular fragments considered by Scolastico and hence could not reasonably be predicted by that model. However, also in the case of compounds **7** and **17** (Table 1), which represent all the descriptors but the lactam moiety, substituted by the pyrrole ring (a nonisosteric bioanalogue[14]), the prediction capability of the model resulted to be unsatisfactory, the activity of both the compounds being completely mispredicted. This failure might be attributed to the fact that the Scolastico model takes into account only some structural features of the molecules and does not consider the steric and electrostatic properties, which are likely to be important for the biological activity of our compounds.

Herein, we describe a 3D-QSAR study on several pyrrolo[2,1-*c*][1,4]benzothiazines **4**−**32** (Chart 1) previously synthesized in our laboratories and found to be active as CEBs.[10−12] The comparative molecular field analysis (CoMFA) technique has been applied to develop a model able to explain and predict the activity of diltiazem-like compounds and helpful to design new and more selective calcium antagonists.

Methods

Biological Data. Tables 1 and 2 list the structures and the observed and calculated biological activity values of compounds **4**−**27**, **31**, **32**, and **28**−**30** forming the training set used to derive the CoMFA model and the set used to test the predictivity of the model itself, respectively. The calcium antagonist activity of these compounds was measured in rat cortex and heart homogenates by displacing [^{3}H]nitrendipine, while in functional studies their inotropic, chronotropic, and vasorelaxing effects were evaluated, as reported in our previous publications.[10−12] As regards this study, biological activity only refers to the binding affinity values expressed as pIC_{50}, that is, the −log of the concentration (M) of the tested compounds that inhibited [^{3}H]nitrendipine binding on rat cortex homogenate by 50%. Consequently, all the activity values are in the range of 5.30 (less active compound) to 9.37 (most active compound). The derivatives which have been found to be "inactive" were arbitrarily assigned an activity value equal to that of the compounds reported as the less active in their respective structural classes. Although all the compounds reported herein have been obtained and tested as the racemic form, they have been considered in this CoMFA study on the basis of examples in the literature where analogous decisions have been made in dealing with mixtures of stere-

oisomers.[15] However, the activity data for derivatives **31**−**32** refer to the diastereomerically pure *trans* compounds.

Molecular Modeling. It is well known that a given biological activity of a chiral compound is usually due to one of the enantiomers. Thus, in the case of 3-acetoxy-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one, only the 2*S*,3*S*-(+)-isomer **3** (diltiazem) is therapeutically useful.[16] Due to this fact and taking into account the presence of at least one stereogenic center common to many of the compounds considered in this study, it was arbitrarily decided to model these new CEBs according to the absolute stereochemistry of diltiazem. This decision can be justified on the basis that intramolecular distances and bond angles do not depend on the asymmetry of any single carbon atom and, consequently, the computational results are also independent of this choice.

Having already verified the accuracy of the generalized MM2 force field implemented in MODEL (version KS 2.99)[17] to describe our compounds,[12] their input geometries were generated and initially minimized by using that program. In order to evaluate the putative global minimum energy conformations of compounds **4**−**32**, a thorough conformational analysis (mixed search) was carried out with the program BKMDL[17] as reported in a previous publication.[12] We chose the mixed search conformational investigation since it is especially suited to provide all the possible conformers in the case of six- and seven-membered rings.[17] The search was stopped when duplicate geometries were mostly generated and the global minimum structure had been found several times. A detailed description of the conformational analysis procedure can be found in our previous publication.[12] Following this procedure a set of conformers was generated for each of the compounds **4**−**32**, but only their global minimum energy conformers were arbitrarily considered for the CoMFA study.

3D-QSAR Methodology. CoMFA calculations were performed using the QSAR module of Sybyl[19] and with the following characteristics: The grid in which the molecules were embedded was regularly spaced (1 Å) with dimensions of 23 × 22 × 22 Å (these values were determined by an automatic procedure performed by the Sybyl-CoMFA routine). Steric and electrostatic interaction energies were calculated using a carbon sp^3 probe atom with a +1 charge, a distance-dependent dielectric constant (1/*r*), and an energetic cutoff of 30 kcal/mol with no electrostatic interactions at steric bad contacts. The same three-dimesional grid was used in all the CoMFA studies.

Regression analyses were done using the Sybyl implementation of the PLS[20] algorithm, initially with cross-validation[21] (the leave-one-out technique) to reduce the probability of obtaining chance correlations and six principal components (PCs). The number of groups of cross-validation was set equal to the number of components of the training set. The optimal number of latent variables (components) to be used in conventional analyses was chosen on the basis of the highest cross-validated r^2 (r^2_{cv}) value, the smallest standard error of prediction (SEP), and the minimum number of components. To improve the signal-to-noise ratio, all leave-one-out calculations were performed selecting a 2.0 kcal/mol energy column filter (the so called minimum_sigma option or field variance at each grid point was used for this purpose). The steric and electrostatic field columns were weighted according to the COMFA_STD default scaling option. In this method a field is considered as a whole and every CoMFA variable is affected by the overall field mean and standard deviation. Final PLS (non-cross-validated models) calibration equations were then derived using the optimal number of components so identified.

To assist selection among various 3D-QSAR calibration equations (models) and to test their utility as predictive tools, an external set (the so-called test set) of compounds with known activities not used in model generation was predicted. The predictive r^2 based only on molecules from the test set is normally reported as the most appropriate parameter to evaluate the predictive power of a CoMFA model. Predictive r^2 is calculated using the following equation:

$$\text{predictive } r^2 = 1 - (\text{“press”}/\text{SD})$$

where SD is the sum of the squared deviations between the actual activities of the compounds in the test set and the mean activity of the training set compounds and “press” is the sum of the squared deviations between predicted and actual activities for every compound in the test set. It is obvious from the equation that prediction of the mean value of the training set for every member in the test set yields a predictive $r^2 = 0$, while negative values are possible when the predictions are worse than predicting the mean value of the training set. This procedure was followed to evaluate the predictive ability of our CoMFA models. As a further attempt to validate the best CoMFA model obtained, the bootstrap validation method[21] was used as implemented in Sybyl. This technique is used to estimate the stability of the parameters (mean and standard deviation) associated with the statistical models.

CoMFA coefficients contour maps of the coefficients of each grid point were also generated by following the standard procedure in Sybyl. These maps show lattice points where the QSAR strongly associates changes in steric and electrostatic field values with changes in biological activity in order to obtain chemical information.

CoMFA Model. CoMFA was used to examine the correlation between calculated physicochemical properties (steric and electrostatic) and measured *in vitro* receptor-binding affinities of a training set of 26 compounds. The “alignment rule”, that is, the superimposition of the molecular models within a three-dimensional fixed lattice, is one of the most important input variables in CoMFA. To define the alignment rules one can use a variety of methods that are generally dependent on whether or not crystallographic data are available. Since no X-ray structural information for any of the receptor−CEB complexes is available, we resolved on defining an alignment criterion based on the pharmacophoric groups of the studied compounds. The key substructures in diltiazem-like CEBs, which are thought to play an important role in the interaction with the binding site, are (i) the centroid of the condensed benzene ring, (ii) the sulfur atom, (iii) the basic nitrogen atom in the side chain, (iv) the amide moiety, and (v) the ester function. Thus, it was reasonable to align our compounds by following a pharmacophoric scheme proposed earlier for benzothiazepinones and benzazepinones,[8c] binding at L-type calcium channels. Accordingly, the minimum energy conformers of the molecules forming the training set were aligned keeping the pharmacophoric groups superimposed (oxygen of the methoxy group, bridgehead nitrogen atom, fused aromatic ring, remote exocyclic nitrogen, and C1′ and C4′ atoms of the 4β-phenyl ring). Table 3 reports the statistical results of the cross-validated PLS-CoMFA experiments according to this alignment rule. The statistics of the final “best correlation” model with the optimal number of components are given in Table 4.

Another variable in the CoMFA procedure is the calculation of the point charges. However, from recent publications there

Table 3. Statistics of the Cross-Validated CoMFA Analyses[a]

principal components	r^2_{cv}	SEP
1	0.474	0.800
2	0.524	0.782
3	0.498	0.826
4	0.640	0.721
5[b]	0.703	0.655
6	0.698	0.676

[a] Minimum $\sigma = 2.0$; number of cross-validation runs = 26. [b] Optimal number of components found.

Table 4. Statistics of the Calibration CoMFA Model

r^2	0.970
SEE	0.215
F-test	96.608
steric and electrostatic contributions (%)	56.1, 43.9
r^2_{boot}[a]	0.996 ± 0.003
SEE_{boot}[a]	0.075 ± 0.058

[a] Results of the bootstrapped analysis (15 samplings, minimum $\sigma = 2.0$).

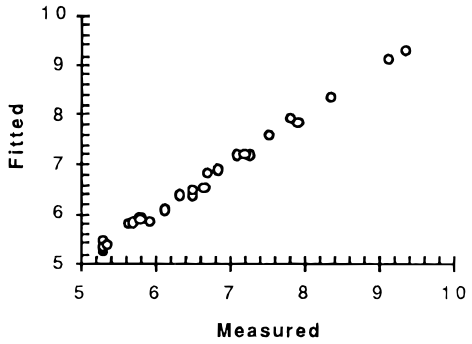


Figure 1. Fitted vs measured pIC_{50} values for the CoMFA analysis of the 26 compounds of the training set. The model was derived using five principal components yielding a cross-validated $r^2 = 0.703$.

is strong evidence to suggest that in CoMFA the overall influence of the point charges is not sensitive to the method of how these are calculated.[15c] Therefore, the partial atomic charges for all the molecules were computed with Sybyl using the GAST-HUCK method of this program and were used without further refinement.

The Test Set. The test set consisted of three molecules (**28**−**30**) whose affinities spanned 2 orders of magnitude covering a range of 1.6 log units (from 6.97 to 5.33). These derivatives were chosen among active and inactive compounds to maximize a uniform sampling of biological activity. All predicted activities for the test set molecules were calculated using the optimal CoMFA model. The predictive power results of the non-cross-validated calibration model on the test set are summarized in Tables 2 and 4. The prediction statistics are given in Table 4, while Table 2 reports the observed and predicted affinity values along with their difference.

Results and Discussion

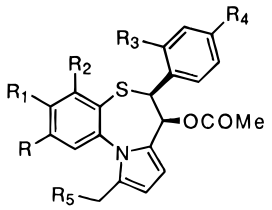
The alignment chosen yielded good cross-validated results ($r^2_{cv} = 0.703$, SEP = 0.676) (Table 3) and conventional results ($r^2 = 0.970$, *F*-test value = 96.608) (Table 4), with the optimal number of components found equal to 5. The corresponding calibrated model satisfies 97% of the total variance in receptor-binding affinity found in the training set with a standard error of estimate of 0.215 log unit. In this model, both steric and electrostatic fields contribute to the QSAR equation by 56.1% and 43.9%, respectively. A high bootstrapped (15 samplings) r^2 value of 0.996 ± 0.003 with a correspondingly small standard error of estimate (0.075 ± 0.058 log unit) adds a high degree of confidence to this analysis. Figure 1 depicts a plot of fitted vs measured affinity values of compounds **4**−**27**, **31**, and **32** using the optimal non-cross-validated model.

As already noted, the three compounds **28**−**30** (test set) were used to evaluate the predictive power of this CoMFA model. As in the calibration experiments, a good predictive ability with an r^2_{pred} of 0.865 for the compounds in the test set was obtained. It can be seen in Table 2 that the affinities of all the examined compounds are predicted within 0.43 log unit of their experimentally measured affinities with an average absolute error of 0.26 log unit across a range of 1.6 log units.

A further test of robustness of our CoMFA model dealt with the assessment of its general applicability in predicting the activity of compounds belonging to different classes, such as diltiazem (**3**) and a related new family of CEBs (**33**−**41**).[22] Structures were minimized, and the corresponding minimum energy conformers were aligned as described above. The observed and predicted pIC_{50} values for diltiazem and **33**−**41** are listed in Table 5 and plotted in Figure 2. As can be seen, our CoMFA model was able to forecast within 0.72 log unit the biological activity of these compounds with a $r^2_{pred} = 0.680$ and an average absolute error of 0.33 log unit. These parameters are comparable to those obtained in predicting the affinity of the test set molecules **28**−**30**, thus demonstrating that this CoMFA model can be applied to structurally different compounds. It is particularly interesting to observe that even the affinity of diltiazem has been correctly predicted (within 0.24 log unit). On the other hand, it was impossible to extend our study to other representatives of CEB classes, such as semotiadil (**42**; Chart 1)[23] and related compounds because no data concerning the displacing of [^{3}H]nitrendipine in rat cortex or heart homogenates are available in the literature for such compounds.

The CoMFA steric and electrostatic fields are shown as contour maps in Figures 3 and 4. The field values were calculated at each grid point as the scalar product of the associated QSAR coefficient and the standard deviation of all values in the corresponding column of the data table (STDEV*COEFF) and always plotted as the percentage of contribution to the QSAR equation. These surfaces are to be considered as a representation of the lattice points, where differences in field values

Table 5. Observed and Predicted Receptor-Binding Affinity Values of Diltiazem and Compounds **33**−**41**



(±) **33-41**

							pIC_{50}[a]		
compd	R	R_1	R_2	R_3	R_4	R_5	obsd[b]	pred[c]	diff[d]
33	H	H	H	H	H	$(Me)_2N$	5.30	5.88	−0.58
34	H	H	H	H	OMe	$(Me)_2N$	6.87	6.89	−0.02
35	CF_3	H	H	H	H	$(Me)_2N$	6.52	5.87	0.65
36	CF_3	H	H	H	OMe	$(Me)_2N$	5.30	5.92	−0.62
37	Cl	H	H	H	H	$(Me)_2N$	5.91	5.66	0.25
38	Cl	H	H	H	OMe	$(Me)_2N$	8.48	8.43	0.05
39	H	H	Cl	H	H	$(Me)_2N$	7.54	7.45	0.09
40	H	H	Cl	H	OMe	$(Me)_2N$	7.66	7.70	−0.04
41	H	Cl	H	H	OMe	$(Me)_2N$	5.30	6.06	−0.76
diltiazem							7.34	7.58	−0.24

[a−d] See the corresponding footnotes of Table 2.

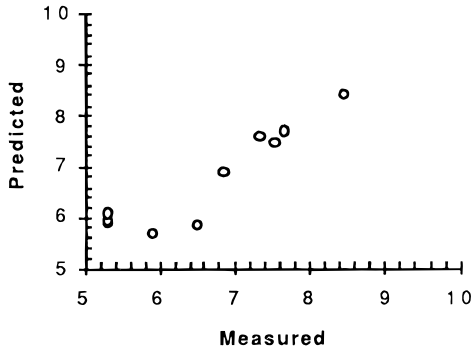


Figure 2. Predicted vs measured pIC_{50} values for the CoMFA analysis of compounds **3** and **33**−**41**. Predictive $r^2 = 0.680$.

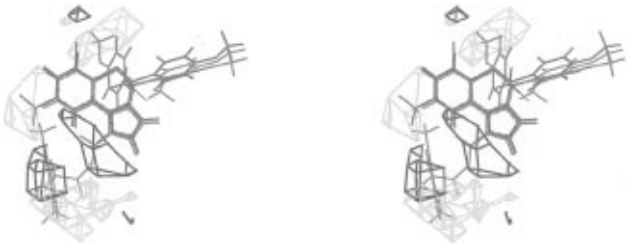


Figure 3. CoMFA steric STDEV*COEFF contour plot from the analysis based on the 3D-QSAR model with no cross-validation. Sterically favored areas are represented by green polyhedra. Sterically unfavored areas are represented by yellow polyhedra. Compounds **17**, **18**, and **23** are also represented as magenta, orange, and cyan structures, respectively.

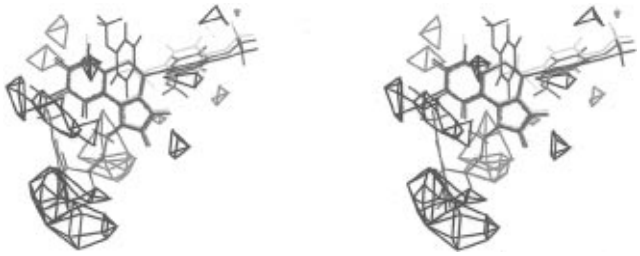


Figure 4. CoMFA electrostatic STDEV*COEFF contour plot from the analysis based on the 3D-QSAR model with no cross-validation. Negative charge unfavored areas are represented by red polyhedra. Negative charge favored areas are represented by blue polyhedra. Compounds **17**, **18**, and **23** are also represented as cyan, green, and yellow structures, respectively.

are strongly associated with differences in receptor-binding affinity. Though the interpretation of these maps is primarily intuitive and highly subjective, the absence of the lattice points does not mean that a given pharmacophore element has no influence on the biological activity. It can also indicate that all the examined compounds exert the same steric and/or electrostatic influence in a certain area or, as suggested by Cramer,[24] can help to delineate the less explored volume of a lattice. In Figure 3 are shown the steric contour maps plotted using compounds **17** (magenta), **18** (orange), and **23** (cyan) as reference structures. The green and yellow polyhedra represent regions of space whose occupancy by the ligands respectively increases or decreases the receptor-binding affinity. The yellow contours surround basically three different portions of the superimposed molecules, namely, the pyrrole side chain, the fused benzene ring in correspondence of C-8, and the *para* position of the 4α-phenyl ring. The side chain is the conformationally more flexible part of the molecules, and the corresponding steric contour maps are dependent on the conformers chosen for the construction of the CoMFA model. The reported contour maps were calculated using the minimum energy conformers. Substantially, the model suggests the presence of no more than one bulky substituent on the basic nitrogen: In fact, both the morpholino derivatives **9** and **15** are among the less active compounds.

As far as the C-8 position is concerned, the steric contour maps suggest that the presence of substituents is detrimental to the activity, as demonstrated by the lower affinity of **11** and **12** with respect to **27**. Finally, with regard to the substitution at C-4, it is interesting to note that while the β-substituent lies in an empty area (as far as our compounds are considered), the α-substituent is located in a well-defined region that can

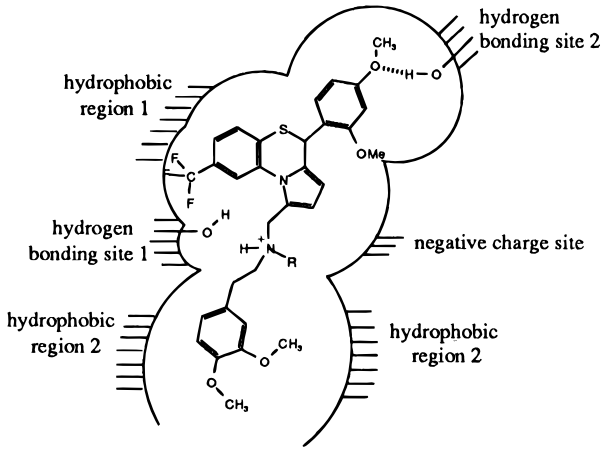


Figure 5. Proposed model of the receptor-binding site for diltiazem-like CEBs shown with a hypothetical compound interacting at hydrogen-bonding sites 1 and 2, hydrophobic regions 1 and 2, and the negative charge site on the receptor (top view).

easily accommodate either the acetoxy or phenyl groups but not the *p*-methoxyphenyl ring (the methoxyl falls in a yellow polyhedron).

Figure 4 shows ligands **17** (cyan), **18** (green), and **23** (yellow) embedded in the CoMFA electrostatic contour maps. The red and blue polyhedra describe regions where a high electron density within the ligand structure enhances or diminishes, respectively, affinity. A rather large red area surrounds either the basic nitrogen of the side chain or the oxygen of the lactam carbonyl (compounds **31** and **32**), showing that both these structural elements are important for the binding. Less extended red areas are found in correspondence of C-6 and C-7, but such contours should not be overemphasized, since there are exceptions to this pattern. Conversely, the presence of a blue polyhedron in proximity of C-8 is in accordance with what we have described about the steric contours, which do not allow for the presence of substituents at this position. Another blue area surrounds the 2′-methoxyl on the pendent 4β-phenyl ring, which generally causes a drop in affinity. Finally, the large blue area corresponding to the side chain suggests that optimal substituents on the basic nitrogen would be lipophilic groups lacking heteroatoms, in agreement with the steric contour maps that do not exclude the possibility of one long alkyl chain on the basic nitrogen.

According to the findings from this 3D-QSAR study, the following hypothesis on the mode of binding of diltiazem-like CEBs can be proposed as illustrated in Figures 5 and 6. CEBs are thus suggested to interact with their receptor through a negative charge site, two hydrogen-bonding sites, and three hydrophobic regions. As can be seen in Figure 5 (top view), the hydrophobic region 1 surrounds the polycyclic core quite closely, so that it does not accept substituents at carbons 6−8. The second region (hydrophobic region 2) surrounds less tightly the side chain, allowing the presence of at most one bulky substituent, and is closely adjacent to both the negative charge site and one hydrogen-bonding site which interact, respectively, with the protonated basic nitrogen and the lactam carbonyl oxygen, depending on the molecules. A second hydrogen-bonding site is located in the pocket which accommodates the 4β-phenyl ring and interacts with the oxygen of the *p*-methoxyl.

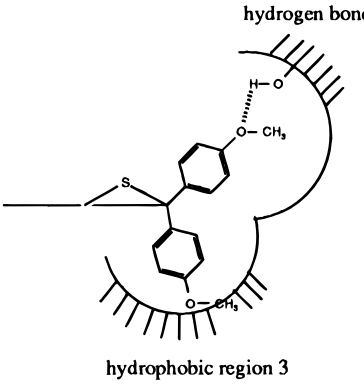


Figure 6. Proposed model of the receptor-binding site for diltiazem-like CEBs shown with a hypothetical compound interacting at hydrogen-bonding site 2 and hydrophobic region 3 on the receptor (edge view).

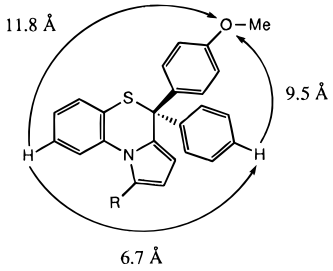


Figure 7. Distance map consisting of three distances and three pharmacophoric points.

This last interaction seems to be of particular importance in order for CEBs to show high affinity values.

Figure 6 presents an edge view of the compounds in the putative binding site. This view clearly shows that the 4α-substituents lie almost perpendicularly to the plane of the tricyclic system and occupy a lipophilic cleft (the hydrophobic region 3) that in turn can accept substituents as long as a phenyl group but is shaped to prevent *para*-substituted analogues from fitting. This important finding may account for the lower activity of the 2*R*,3*R*-(−)-isomer of diltiazem.

In addition, we can suggest that, in order to be accepted by their receptor-binding site, diltiazem-like CEBs should not exceed the following dimensions (see Figure 7): 11.8 Å (distance between the hydrogen at C-8 and the oxygen at C-4′ of the 4*β*-phenyl ring), 6.7 Å (distance between the hydrogen at C-8 and the hydrogen at C-4′ of the 4α-phenyl ring), and 9.5 Å (distance between the oxygen at C-4′ of the 4*β*-phenyl ring and the hydrogen at C-4′ of the 4α-phenyl ring).

Floyd and co-workers[9] identified only two pharmacophores in benzazepinone and benzothiazepinone CEBs, i.e., the basic nitrogen and the phenyl methyl ether, and proposed that the polycyclic core of these compounds is serving as a scaffold and functions essentially to position the two pharmacophores in an optimal spatial situation. Accordingly, CEBs would bind to the calcium channel protein in an "inboard" binding conformation in which the side chain amine is placed over the mean plane of the molecule and in proximity to the phenyl methyl ether pharmacophore. Although our model takes into consideration also other structural features of the molecules as possible relevant pharmacophores, it is consistent with the hypothesis that the basic nitrogen and the 4′-methoxyl should lie at a certain mutual distance. However, Floyd's hypothesis on the bioactive conformation of CEBs can not be extended to our molecules that, because of the presence of the pyrrole ring, are much more rigid and can not present the two pharmacophores in the bound conformation described above. We feel that both Floyd's and our binding models could be valid, but only for the respective class of compounds. The fact that diltiazem seems to fulfill both the models suggests that more work is necessary to evaluate the possibility of existence of a single, more complex binding mode for CEBs.

Conclusions

A 3D-QSAR model has been developed using the CoMFA methodology for a set of 26 CEBs showing different binding affinity values. The model is able to predict accurately not only the receptor affinity of three structurally similar compounds not used in the construction of the cross-validated model but, more interestingly, also the affinity values of diltiazem itself and of nine new diltiazem-like CEBs. The results indicate a correlation between the receptor affinity of these compounds and the steric and electrostatic fields around them. We chose the CoMFA alignment as a criterion for the superimposition of the molecules, even though the decision to use the minimum energy conformers is subjective. However, we feel that such a decision is justified in this case by the absence of structural information about the actual biologically active conformation of the studied molecules and the great rigidity of their structures. On the basis of this alignment, we have proposed a hypothesis of the receptor-binding site for CEBs. This model justifies the importance of the main pharmacophoric groups (*p*-methoxyl on the 4*β*-phenyl ring, either the basic nitrogen or the lactam carbonyl, and the fused aromatic ring) as well as of their relative distances. In fact, since the hydrogen-binding interaction is extremely dependent on the distance and the relative orientation of the acceptor and donor groups, spatial considerations are particularly important for the methoxy and carbonyl groups, their spatial locations being critical for the biological activity. We found that the distances among pharmacophoric groups in our model are in excellent agreement with those described by Scolastico for the cluster containing diltiazem and related compounds.[13] On the other hand, the receptor-binding mode suggested by Floyd[9] for benzazepinones does not seem to be in complete agreement with that here proposed, mostly because of the different steric requirements of our pyrrolobenzothiazine and pyrrolobenzothiazepine derivatives. In conclusion, the CoMFA model here described could be useful to design and synthesize new diltiazem-like CEBs, which in turn will further validate the model itself and allow refinement of the receptor topological model.

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