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Structure–activity relationship of Ca^{2+} channel blockers: A study using conformational analysis and chemometric methods

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SUMMARY

A structure–activity relationship study has been done on 8 compounds with the activity known as 'Ca^{2+} channel blockers'. Conformational analysis was carried out using a molecular mechanics method. The 3D-QSAR approach was used and the most polar functional groups present in all the molecules were considered. Eight interatomic distances are necessary to define the relative spatial disposition of these relevant molecular fragments. The structure–activity relationship between interatomic distances and biological activity was performed using statistic and chemometric methods. In particular, with Principal Component Analysis, it was possible to reduce the number of interatomic distances: only six of the eight distances are sufficient to describe the system in a useful way. A classification method was iteratively used to select the most probable conformations linked to the biological activity and to build a model able to classify conformations according to their biological behaviour. Cluster analysis on the active selected conformations subsequently allowed the identification of two different geometrical patterns for the active compounds. Finally the validity of the model was verified by correctly predicting the activity of other molecules not used in the construction of the model but possessing known activity.

INTRODUCTION

Quite different classes of organic compounds act as 'Ca^{2+} antagonist' or 'Ca^{2+} channel blockers'. As therapeutic agents they have proved effective in the treatment of several cardiovascular diseases such as hypertension, angina pectoris, arrhythmia, ischemia and so on. These com-

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pounds affect the voltage-dependent Ca^{2+} channels and specifically reduce the influx of calcium ions through the channels; however, their detailed mechanism of action is largely unknown [1].

The chemical and pharmacological heterogeneity of Ca^{2+} channel ligands seem to suggest that they interact at different sites and by different mechanisms, i.e. that the calcium channels contain at least 3 different but allosterically linked binding sites for the most important classes of organic Ca^{2+} antagonists (phenylalkylamines, 1,4-dihydropyridines and benzothiazepines) [2].

In this research, only benzothiazepines and some structurally related compounds (benzothiazines and benzothiazocines) have been considered, the aim being to determine the structural features these compounds have in common.

The molecules shown in Fig. 1 constitute the training set and are representative of the structural class of compounds under study. As usual, to obtain a highly probable activity model the molecules must differ from each other in structure and part of them must be biologically active and part inactive.

The eight compounds considered (Fig. 1) were tested using different procedures (Table 1) [3]. The homogeneity of the pharmacological data was verified by testing compound **1** [4] using the three different procedures (A, B and C) and it is possible to refer to it. Compound **1** (Diltiazem) has been studied considering the S,S enantiomer which shows Ca^{2+} antagonist activity. It is interesting to note that, like compounds **5** [4], **6** [5], **7** [5] and **8** [5], the diltiazem R,R enantiomer does not present the 'Ca^{2+} channel blocker' effect, whereas compounds **2** [6], **3** [7] and **4** [7] show a significant Ca^{2+} antagonist activity.

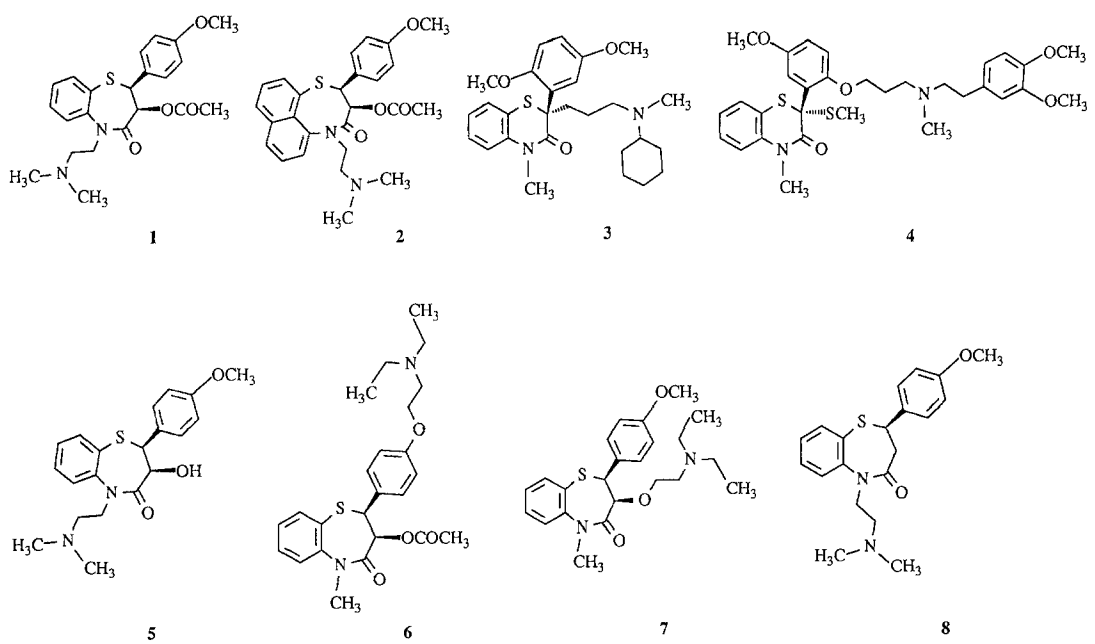


Fig. 1. Training set molecules.

TABLE 1
ACTIVITY DATA OF THE TRAINING SET COMPOUNDS AND PROCEDURES TO DETERMINE SUCH ACTIVITY

Compound	Procedure A[a]	Procedure B[b]	Procedure C[c]
1	6.77	7.40	7.10
2	6.60		
3	6.15		
4	6.20		
5		4.59	6.00
6		4.92	4.70
7		5.92	5.92
8		5.77	5.10

[a] Procedure A: pharmacological test in vitro measuring the ability to inhibit the tonic contractions of isolated organs expressed as pIC_{50}. IC_{50} is the concentration (μM) of the test compound required to produce 50% inhibition.

[b] Procedure B: biochemical test measuring the inhibition of specific [^{3}H]diltiazem binding to rat cerebral cortex expressed as pIC_{50}.

[c] Procedure C: biochemical test measuring the inhibition of specific [^{3}H]diltiazem binding to cardiac tissue expressed as pIC_{50}

METHODS AND RESULTS

Conformational analysis

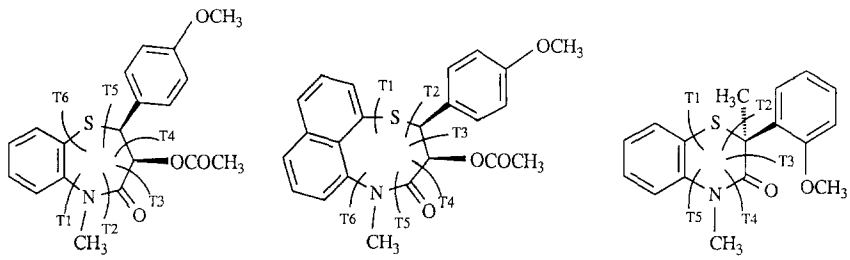
Conformational analysis was carried out using molecular mechanics calculations [8]. The target was to find the conformational minima of the training set molecules. Low-energy conformations are of course the most significant for predicting molecular properties by molecular modeling because, very probably, there is among them the conformation which interacts with the receptor.

The problem of locating the significantly populated conformers of molecules defined by a complex potential energy hypersurface undoubtedly is the most problematic and delicate question in molecular modeling studies. By addressing this question, a number of different conformational search techniques have been developed and proposed as solutions to the so-called conformational multiple-minimum problem.

In this work the search was performed with a tree-search procedure, as is implemented within the MULTIC submode [9] of the MacroModel interactive molecular modeling package [10,11].

The structures obtained by this conformation generator were subsequently used as starting geometries for optimization by molecular mechanics energy minimization to reach minimum energy conformers. In particular the starting geometries were subjected to block diagonal Newton-Raphson energy minimization (final RMS gradient <0.05 kJ/Å) using the MacroModel implementation of the MM2 molecular mechanics force field [12]. The nature of the stationary points was tested by computing the eigenvalues of the second derivative matrix. The minimum energy conformers were considered the same unless the least squares superimposition of the representative compared atoms finds one or more pairs of equivalent atoms separated by more than 0.25 Å.

The optimization of the starting geometries and the elimination of any duplicate conformer were performed by the Batchmin noninteractive molecular modeling program used in connection with MacroModel [10].



1a **2a** **3a**

Fig 2. Structures used to determine the minimum conformations assumed by the 6-, 7- and 8-membered cycles.

were performed by the Batchmin noninteractive molecular modeling program used in connection with MacroModel [10].

The conformational analysis was made in two different stages to localize the minima in the potential energy surface. Each stage was performed through the described combination between internal coordinate tree-search and molecular mechanics energy minimization.

In the first stage the lower energy spatial dispositions, assumed by the 6-, 7- and 8-membered cycles present in the simplified structures shown in Fig. 2, were determined. This determination

TABLE 2
MINIMUM ENERGY CONFORMATIONS ASSUMED BY THE 6-, 7- AND 8-MEMBERED CYCLES

1a

C	E	ΔE	T1	T2	T3	T4	T5	T6
1	18.66	0.00	−53.8	1.5	75.3	−37.6	−44.1	75.6
2	19 25	0.59	52.4	−1.9	−79.2	49.5	31.5	−69.7
3	20.40	1.74	54.4	−2.8	−78.4	48.7	32.2	−68.7
4	24 26	5.60	55.4	0.6	−27.7	−32.3	86.7	−61.9

2a

C	E	ΔE	T1	T2	T3	T4	T5	T6
1	26.50	0.00	78.1	−70.2	−34.8	82.6	21.3	−74.0
2	27.76	1.26	−76.1	65.6	38.6	−84.5	−19.2	73.0
3	28.61	2.12	−69.6	66.3	36.6	−84.1	−19.3	77.9
4	31.58	5.08	80.1	−67.0	−38.7	84.4	21.3	−71.5

3a

C	E	ΔE	T1	T2	T3	T4	T5
1	22.54	0.00	−38.4	63.7	−53.1	1.0	34.2
2	26.66	4.12	31.1	−50.6	39.1	5.3	−31.6

was performed using the NCLOSE option of the MULTIC submode of the MacroModel software. Through this option a bond in each cycle was defined as a ring-closure bond and was temporarily broken to produce a pseudoacyclic molecule, to whose rotatable bonds was applied a 30° dihedral angle resolution.

Among the generated conformations, the minimization was performed only for those in which the distance between the two atoms forming the ring-closure bond was between 1 and 2 Å. Two minimum conformations for the 6-membered ring, three for the 7-membered ring and two for the 8-membered ring were obtained within 10 kcal/mol above the global minimum (Table 2).

In the next stage the conformational isomers of the complete structures were generated starting from optimized geometries in which the cycle was placed in each previously determined minimum situation. A 60° dihedral angle resolution was used for all acyclic rotatable bonds; after optimization a total number of 485 minimum conformations was obtained for the eight compounds of the training set. The number of minima found for each molecule and the steric energy of the global minimum are reported in Table 3.

The general protocol of the conformational search and the method of calculation chosen proved to be effective in solving the conformational multiple minimum problem for the diltiazem-like Ca^{2+} antagonists. For diltiazem itself a conformation analogous to the one described in the crystal structure [13] was found 1.82 kcal/mol above the global minimum (Fig. 3 and Table 4).

Statistic and chemometric methods

The results derived from conformational analysis provide different molecular three-dimensional descriptors suitable for the characterization of the examined compounds.

In this work interatomic distances were chosen as conformational descriptors; from among all the possible distances we considered those necessary to define the relative spatial disposition of the most polar functional groups present in all the molecules examined. It is assumed that these molecular fragments are responsible for the most significant interactions with the biological receptor.

We identified, as significant common groups for the Ca^{2+} antagonists under study, an endocyclic amidic group, a tertiary aminic nitrogen atom and a phenolic ether, a total of five atoms.

TABLE 3
TOTAL MINIMUM ENERGY CONFORMATIONS AND STERIC ENERGY OF GLOBAL MINIMUM (kcal/mol) FOR COMPOUNDS **1–8**

Compound	Total minimum energy conformations	Steric energy of global minimum (kcal/mol)
1	41	22.00
2	51	29.30
3	57	45.74
4	80	46.12
5	37	11.04
6	48	27.04
7	54	17.47
8	37	22.52

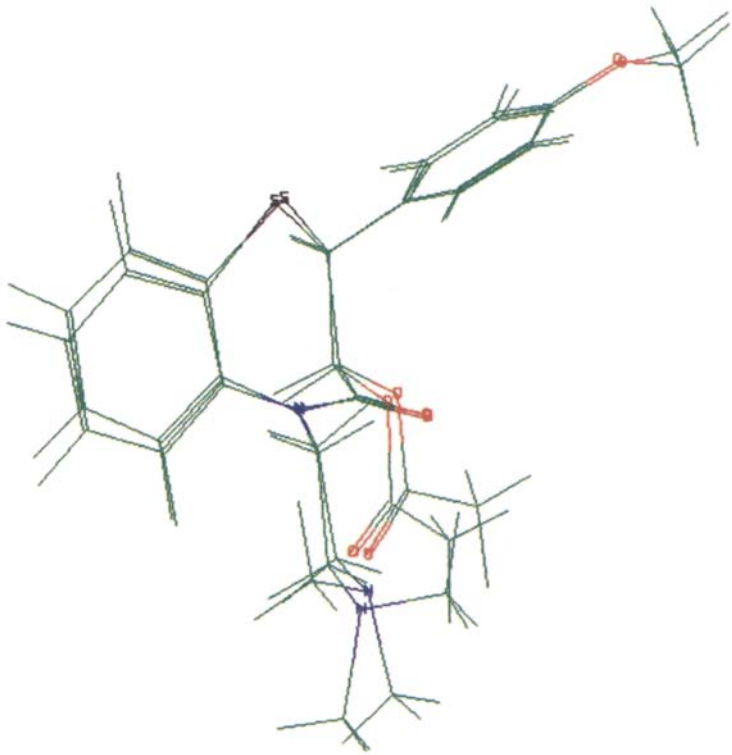


Fig. 3. Molecular superimposition between a calculated conformational minimum for diltiazem and its X-ray structure.

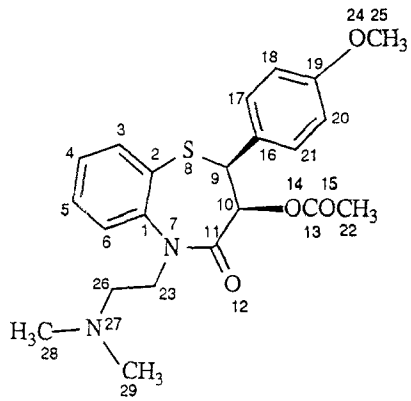
For a body defined by five points there are ten possible distances (N(N − 1)/2) but nine degrees of freedom (3N − 6). As two of the ten distances are bond distances in the studied system only eight interatomic distances were considered when defining the relative spatial disposition of the five chosen atoms (Fig. 4).

It is worth noting that for molecules **3** and **4** there was an ambiguous choice for the oxygen atom in the aromatic ring since more than one phenolic ether is present in these structures. For compound **3** we chose the *meta* oxygen. This, more than the oxygen in the *ortho* position, seems to occupy a position equivalent to the one assumed by the oxygen atoms in the other molecules. This assumption was confirmed by molecular superimposition tests carried out with the software MacroModel.

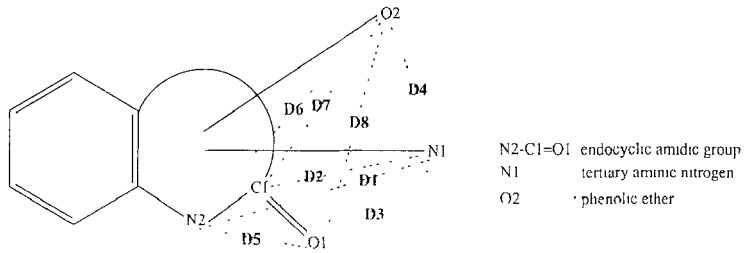
In molecule **4** two aromatic rings are present, each carrying two oxygen atoms. We could not choose a priori which one of the rings is involved in the interaction with the receptor since we know neither the site nor the mechanism of action; therefore we adopted two different descriptions for the same molecule, using in one case the *meta* oxygen on the ring furthest from the benzothiazinic system (description named **4A**) and, in the other case, the *meta* oxygen in the nearest ring (description named **4B**).

We adopted a reasoned sequence of different chemometric methods [14] to relate the biological behaviour of the compounds in the training set to the chosen molecular descriptors (interatomic distances).

TABLE 4
INTERNAL COORDINATES (NON-H-ATOMS) FOR THE DILTIAZEM CALCULATED CONFORMATIONAL MINIMUM 1.82 kcal/mol ABOVE THE GLOBAL MINIMUM



Atom number (I)	Chemical symbol	Bond length (Å) NA I	Bond angle (degrees) NB:NA:I	Twist angle (degrees) NC:NB:NA.I	NA	NB	NC
1	C						
2	C	1.40126			1		
3	C	1 39587	120.54750		2	1	
4	C	1.39310	120.32762	0.48930	3	2	1
5	C	1.39254	119 59604	0.58166	4	3	2
6	C	1.39380	119.97335	−0 77843	5	4	3
7	N	1.35306	120.90450	177.03111	1	2	3
8	S	1.76945	120.49458	174.82940	2	1	3
9	C	1.82382	98.14205	74.85597	8	2	1
10	C	1.53833	112.94546	−40 95115	9	8	2
11	C	1 39885	124.55634	−49 97522	7	1	2
12	O	1.23130	123.54013	176.29460	11	7	1
13	C	2 35550	137.98971	−171.83237	10	9	8
14	O	1.34616	32.23061	12.82282	13	10	9
15	O	1 20910	92.74211	−162 93938	13	10	9
16	C	1.51677	108.91609	−166 14978	9	8	2
17	C	1.39740	122.15333	53.10718	16	9	8
18	C	1.39489	121.37901	177.61252	17	16	9
19	C	1.39747	120.65582	−0.29347	18	17	16
20	C	1 39527	118.06664	−0.10670	19	18	17
21	C	1.39361	121.18355	0.08161	20	19	18
22	C	1.51207	141.47927	21.13410	13	10	9
23	C	1.49578	119.12472	129.62747	7	1	2
24	O	1.37151	124.68144	−179.91801	19	18	17
25	C	1.40605	119.26823	−0.09840	24	19	18
26	C	1.53902	114.11549	107.26593	23	7	1
27	N	1.45944	113.62256	170.98471	26	23	7
28	C	1.45750	110.49103	−173.21550	27	26	23
29	C	1.45611	112.57068	63.26139	27	26	23



D1= N1-N2 D2= N1-C1 D3= N1-O1 D4= N1-O2 D5= N2-O1 D6= N2-O2 D7= C1-O2 D8= O1-O2

Fig 4. Labels of the five atoms and of the eight distances.

The starting data set, made up of 485 minimum conformations identified by the different molecules of the training set and described by eight interatomic distances, was autoscaled. A Principal Component Analysis (PCA) [15] was performed on these data to verify whether all eight distances were required for a complete description of the system.

From Table 5 we can observe how each PC explains a part of the total variance of the system, decreasing from the first to the last; a very important point to note is how the first four components alone are able to describe about 97% of the total variance of the data set.

It was therefore assumed that PC1, PC2, PC3 and PC4 would be sufficient to correctly describe the system and they were then used to find the most significant interatomic distances.

Analysis of the coefficients of the original variables in the relevant PC (Table 6) allowed the evaluation of each variable's information and the determination of a cluster formed by the distances D1, D2 and D3, all strongly correlated, contributing the same information. In view of the characteristic role played by distances D4 and D5 in the PC4 and PC3, respectively, these distances have also been considered. Although the distances D6, D7 and D8 behave similarly, due to their splitting in PC3 and considering the reduced number of total variables, it was decided to use all three in the description of the system. Thus, a total of six distances was considered sufficient for a full and complete description of the 485 minimum conformations.

A first classification of the objects under study was then performed. Two biological classes were used: the conformers of the active molecules were assigned to the active class and the conformers

TABLE 5
PERCENTAGE OF VARIANCE EXPLAINED BY EACH PRINCIPAL COMPONENT AND CUMULATIVE PERCENTAGE VARIANCE

Principal component	% variance	Cumulative % variance
1	48.96	48 96
2	30.36	79.33
3	10.71	90.04
4	6.70	96 74
5	1.91	98.64
6	0 99	99.63
7	0.22	99 85
8	0.15	100.00

TABLE 6
LINEAR COMBINATION OF THE STARTING VARIABLES (INTERATOMIC DISTANCES) IN THE PRINCIPAL COMPONENTS

	D1	D2	D3	D4	D5	D6	D7	D8
PC1	0.463	0.450	0.438	−0.326	−0.255	−0.274	−0.276	−0.264
PC2	0 211	0.234	0.224	−0 260	−0.104	0.494	0.534	0.493
PC3	0 081	0 115	0.044	−0 300	0.884	0 203	−0 028	−0 254
PC4	0.072	0 315	0 403	0.782	0 275	−0 101	0 018	0.188
PC5	0.013	0 007	0.086	0.278	−0 248	0.683	−0.013	−0.622
PC6	−0.730	−0 082	0.642	−0.214	−0.032	−0.015	−0.017	−0.026
PC7	−0 349	0.624	−0.326	−0.004	−0.050	−0.237	0 484	−0.297
PC8	−0.272	0.483	−0 260	−0.045	−0.040	0.328	−0.635	0 333

of the inactive molecules to the inactive class. After this assignment of the objects to classes of different activity it was possible to use the classification method K-Nearest Neighbours (KNN) [16].

For each object to classify, the method measures the distances from it to the others, considers the K-nearest objects and classifies the object under study by assigning it to the most represented class among the K.

This method was applied in an original way to the 485 minimum conformations described by six interatomic distances (we arbitrarily chose the D1 distance between the three correlated ones) and distributed in two biological classes, the aim being to eliminate the spatial dispositions shared by the active and inactive compounds and to isolate the conformational minima typical of only the biologically active compounds. To obtain this the KNN classification method was performed iteratively using the autoscaled data, the Euclidean distance and a K-value of 3. At each step we focused our attention on the conformations of the active molecules incorrectly classified and reassigned them to the inactive class. In this way we could exclude the ambiguous or uncertain cases

TABLE 7
VALUES OF THE CLASSIFICATION PARAMETER (NER %)[a] AND NUMBER OF POORLY CLASSIFIED CONFORMERS FOR EACH COMPOUND OF THE TRAINING SET AT EACH STEP OF THE ITERATIVE CLASSIFICATION PROCEDURE. THE PERCENTAGE OF CONFORMATIONS CLASSIFIED AS ACTIVE FOR EACH COMPOUND AT THE END OF THE PROCEDURE IS SHOWN ON THE LAST LINE

KNN (K = 3) method		Compounds								
Step	NER%	1	2	3	4A	4B	5	6	7	8
1	89.9	19	8	0	2	0	8	0	1	11
2	96 7	5	3	0	0	0	5	0	0	3
3	98 4	1	0	0	0	0	4	0	0	3
4	98.6	0	0	0	0	0	4	0	0	3
Active conf %		39	78	100	98	100	11	0	0	8

[a] The classification parameter Non Error Rate % is expressed as percentage ratio between the number of correctly classified conformations and the total number of conformations.

and we could obtain a group of conformations for the active molecules recognized as such without doubts or mistakes (Table 7). When no further erroneous classification of the active conformations was found we had the desired structure–activity relationship in the form of a model of class prediction, that is to say a rule able to separate the objects into two classes of different activity, depending on the values assumed by the six variables in each conformer.

Of the various different classification methods, KNN was revealed to have a high prediction power and a high classification ability [17]. This technique in fact was able to point out hidden regions of activity located in the multivariate space and not detectable by other methods.

On the 271 spatial dispositions isolated as active by the iterative classification procedure, a cluster analysis [18, 19] was made using the K-Means technique (using autoscaled data, Euclidean distance and an optimized K-value); here K represents the desired number of clusters. This led to the determination among these objects of three natural clusters; their numeric characterizations

TABLE 8
NUMBER OF OBJECTS, MEAN (Å), STANDARD DEVIATION, MINIMUM AND MAXIMUM (Å) OF THE SIX SIGNIFICANT VARIABLES IN EACH CLUSTER DETERMINED WITH THE K-MEANS TECHNIQUE

Cluster 1 (174 objects)

Distance	Mean	Std.Dev.	Minimum	Maximum
D1:N1-N2	6.962	1.275	4.252	9.781
D4:N1-O2	7 822	1.164	4.701	10 282
D5:N2-O1	2.297	0.006	2.273	2.306
D6:N2-O2	6.148	1.106	4.776	9.141
D7:C1-O2	5 789	0.818	4 868	7.951
D8:O1-O2	6.154	0.936	4.306	8 865

Cluster 2 (37 objects)

Distance	Mean	Std.Dev.	Minimum	Maximum
D1.N1-N2	6.647	1.365	4.454	9 781
D4:N1-O2	6 866	0.101	6.630	7.129
D5:N2-O1	2.296	0.003	2.291	2.305
D6:N2-O2	10.413	1.658	7.832	13.638
D7:C1-O2	10.242	1.486	7 812	12.966
D8:O1-O2	10.630	1.519	8.274	13.635

Cluster 3 (60 objects)

Distance	Mean	Std.Dev.	Minimum	Maximum
D1:N1-N2	3.591	0 530	2.955	5.398
D4:N1-O2	10.002	1 098	7.215	11.931
D5:N2-O1	2.311	0.006	2.293	2 322
D6:N2-O2	8.025	0.574	6 230	8.609
D7.C1-O2	7.189	0.517	6.101	8.010
D8:O1-O2	7 310	0.976	6.263	8.992

(Table 8) define the possible molecular geometries responsible for the biological activity and give an activity geometrical model.

The first cluster contains 174 objects deriving from molecules **3**, **4A** and **4B**; the second cluster contains 37 objects deriving from molecule **4A** and characterized by a completely extended spatial disposition of the alkyl chain. The third cluster contains 60 objects deriving from molecules **1**, **2** and **4B**.

Considering the nature of the three clusters, there are only two certain geometries; these are the geometry described by the centroid of the first cluster that is typical of the systems containing a 6-membered ring and the one described by the centroid of the third cluster and typical of the systems containing 7- and 8-membered rings.

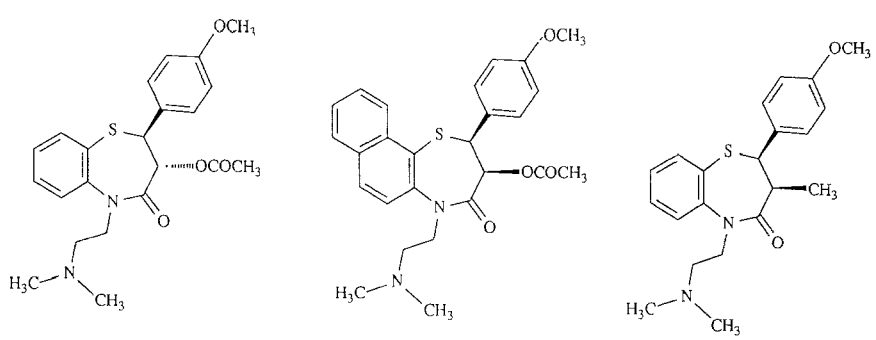
The second cluster is not particularly significant because it contains only the conformations deriving from compound **4A**, characteristic of this molecular structure or its specific description.

From the chemometric elaboration of the data it was possible to obtain a model that enables the classification of the conformations of a molecule of this structural class which has unknown calcium antagonist activity.

We have not proposed the cross-validation of the classification rule as a validation method within the elaborated strategy. This is because of the dimensions of the problem and of the necessary time-consuming computational requirements. Studies should be carried out to obtain validation criteria suitable for this type of problem. In the meantime we have conducted very simple tests on three compounds not present in the training set but having known calcium antagonist activity (Fig. 5).

The conformational minima derived from compounds **9** [4], **10** [20] and **11** [21] with the procedure already described were classified using the KNN method. This classification was made on the basis of the rule obtained from the analysis of the training set and on the values assumed by the six significant interatomic distances in each conformer.

On this basis 15% of the conformers deriving from compound **9**, 32% of the conformers deriving from compound **10** and 41% of the conformers deriving from compound **11** were assigned to the



9		**10**	**11**
Procedure B	Procedure C	Procedure A	Procedure A
4.44	5.35	6.64	7.1 (racemic compound)

Fig. 5 Molecules used to verify the model and their activity data (the A, B and C procedures are the same as in Table 1).

active class. These percentages of conformations belonging to the active class were, however, compared with the relative percentages obtained for the different training set compounds after the iterative application of the KNN classification method (Table 7). This comparison suggested the biological inactivity of compound **9** and the biological activity of compounds **10** and **11**. These conclusions are in full agreement with pharmacological data.

Thus we can conclude that the performance of the classification rule was more than satisfactory. The structure–activity relationship can then be used to predict the biological behaviour of molecules belonging to the considered structural class possessing unknown pharmacological activity, and to project new calcium antagonists.

It was not considered necessary to suggest what percentage of conformations would have to be active to recommend that a new compound be synthesized. Such a decision would depend not only on the model but also on the economical risk than can be taken. However, a simple and reasonable criterion could be the comparison of the percentage of active conformations obtained for the new compound with the relative percentage obtained for a structurally analogous training set compound after the iterative application of KNN.

The structure–activity relationship obtained in this work has already been used for the rational drug design of new calcium antagonists; the structures of interest, according to the model, have been synthesized and were shown to be biologically active. The data concerning this research will be discussed in a future paper.

DISCUSSION AND CONCLUSIONS

The combined use of molecular modeling and chemometric methods allows the rationalization of the biological behaviour of a class of organic calcium antagonists and the construction of a structure–activity relationship of great interest for the determination of new active structures in this sector.

From the detailed description of the work presented here it is possible to understand the general scheme of the methodology elaborated, used and proposed. This approach reveals itself to be an original treatment of the general QSAR strategy for computer-assisted drug design.

At present the individuation of new drugs possessing a more selective biological activity avails itself of planning methodologies with the use of an elaborator to establish relationships between the structure of a molecule and its pharmacological activity. These methodologies are indicated as QSAR (Quantitative Structure–Activity Relationship) methods and follow a logical trace composed of five phases.

The first phase considers the identification of a starting set of molecules possessing homogeneous pharmacological data that characterize the activity. This set of compounds, representative of a whole class of drugs is the so-called 'training set' and is built according to the criteria of the maximum structural differentiation between compounds and the maximum comparability of the correspondent data of activity. Following these criteria we built the training set of the calcium antagonists shown in Fig. 1.

The second phase in the study of the structure–activity relationships consists in the choice and in the determination of suitable descriptors of the molecular systems, that can be correlated to the activity. This means that calculated or experimentally determined parameters must be chosen to describe and identify the compounds. The molecular descriptors can be divided in two categories:

those dependent on the spatial disposition adopted by the molecule and those completely independent of this. It is therefore evident how this second phase can be performed in many different ways, depending on the kind of chosen descriptor and on the computational or experimental method adopted to determine it. This work adopts three-dimensional molecular descriptors, in particular interatomic distances. The second stage of the QSAR strategy was carried out by an appropriate conformational analysis of the considered compounds and, subsequently, by choosing the atoms whose relative spatial disposition it is necessary to know. In this way interatomic distances to use as three-dimensional molecular descriptors were obtained.

The third phase consists in the construction of the quantitative structure–activity relationship, that is to say the values of the descriptors chosen in the different molecules of the training set must be related to their biological activity. To do this it is possible to use different techniques, depending on the kind of descriptor adopted, on the available data activity and on what is going to be obtained: a mathematical model, a geometrical model, a cataloguing in activity classes and so on. In this work an interesting structure–activity relationship was obtained with the help of chemometric methods, taking advantage of the potentiality and versatility that they offer to the chemist. In particular it was possible to obtain, by the reasoned sequence of the chemometric methods described, a discriminant model for objects belonging to different classes and a predictional class model for a conformer of a given molecule belonging to the structural category under study.

The fourth phase considers the validity check of the structure–activity relationship built with the compounds of the training set in the previous stage. Only after this check is it advisable to use the information deriving from the model to suggest new pharmaceutical leaders. At present the performance of the quantitative structure–activity relationship is examined to assess whether it is able to correctly predict the activity of other molecules, molecules not used in the training set but possessing known biological activity.

The model proposed in this work gave a positive answer to this simple and not strict validation procedure and let us hope for good results in the rational projection of new drugs, the target of the fifth and last phase in the QSAR strategy.

Each phase of the QSAR approach can be faced in different ways, depending on the kind of problem to be solved. Combinations of different scientific trends can be involved and consequently methodological developments of different originality are generated. For example the structure–activity methodology adopted in the study on the calcium antagonists is based on the combined use of molecular modeling and chemometrics for indirect drug design. Even if the receptor is unknown, through a comparative analysis of molecular conformations of compounds showing a common biological activity, it is possible to identify the 'pharmacophore', i.e. to recognize a group of atoms in a definite spatial arrangement which confers to a molecule one or more physical-chemical and stereo-electronic properties responsible for the receptor activation.

The common feature of every QSAR approach leading to the design of new drugs is the possibility of addressing, in a rational and intelligent way, the next synthetic effort, limiting it to compounds having a high probability of being active.

DETAILS OF THE CALCULATIONS

The conformational analysis was carried out performing molecular mechanics calculations with the MacroModel Version 2.5 and the Batchmin Version 2.1 [10,11]. Force-field parameter

sets used in the present work are those included in these versions of this integrated software system. The MacroModel version of MM2 differs from the original MM2 developed by Prof. N.L. Allinger mainly in the electrostatic contribution: the former uses partial charges, the latter dipole–dipole interactions. Partial charges are derived from the original MM2 bond dipoles; however, the electrostatic energies are somewhat different. The model used in this study for the dielectric medium is the distance-dependent model and the calculations were performed 'in vacuo' (bulk dielectric constant = 1.0).

The chemometric elaboration of the data was performed entirely with the software package SCAN [22].

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