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Pharmacophore identification by molecular modeling and chemometrics: The case of HMG-CoA reductase inhibitors

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SUMMARY

A methodology based on molecular modeling and chemometrics is applied to identify the geometrical pharmacophore and the stereoelectronic requirements for the activity in a series of inhibitors of 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme involved in cholesterol biosynthesis. These inhibitors present two common structural features – a 3,5-dihydroxy heptanoic acid which mimics the active portion of the natural substrate HMG-CoA and a lipophilic region which carries both polar and bulky groups. A total of 432 minimum energy conformations of 11 homologous compounds showing different levels of biological activity are calculated by the molecular mechanics MM2 method. Five atoms are selected as representatives of the relevant fragments of these compounds and three interatomic distances, selected among 10 by means of a Principal Component Analysis (PCA), are used to describe the three-dimensional disposition of these atoms. A cluster analysis procedure, performed on the whole set of conformations described by these three distances, allows the selection of one cluster whose centroid represents a geometrical model for the HMG-CoA reductase pharmacophore and the conformations included are candidates as binding conformations. To obtain a refinement of the geometrical model and to have a better insight into the requirements for the activity of these inhibitors, the Molecular Electrostatic Potential (MEP) distributions are determined by the MNDO semiempirical method.

INTRODUCTION

When only the structure of compounds characterized by a common biological activity is available and structural information on the receptor active site is lacking, the stereoelectronic requirements necessary to elicit the activity (the so-called pharmacophore) can be looked for through a

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comparative analysis of the physicochemical properties of the ligands; this is called the indirect approach.

If the molecules involved are characterized by conformational degrees of freedom, the first step of this approach is the search for common relative spatial disposition of selected functional groups considered relevant to biological activity [1, 2]. Energetically accessible conformations of each compound are evaluated and compared in the search for such a common spatial disposition. The resulting conformations are candidates as active, or binding, conformations, i.e. the conformations in which the drugs interact with the receptor site. The spatial disposition of the functional groups in the active conformations constitutes the geometrical pharmacophore.

Once the active conformations are determined their steric and electronic properties can be calculated and used in the evaluation of the similarity between compounds [3–5]. The analogies found between these properties allow the rationalization of the biological behavior in a family of compounds and the design of new drugs with optimized binding molecular parameters.

Figure 1 reports a series of eleven inhibitors of the 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme involved in cholesterol biosynthesis.

At present, the inhibition of endogenous cholesterologenesis is the most attractive method of lowering plasma cholesterol content. The biosynthetic pathway to cholesterol involves more than 25 different enzymes and the major rate-limiting step in this pathway is regulated by the HMG-CoA reductase, the enzyme which catalyzes the conversion of the HMG-CoA to mevalonic acid.

Compactin and mevinolin (compounds **3a** and **3b** of Fig. 1), two natural fungal metabolites, are potent inhibitors of HMG-CoA reductase at the nanomolar level. A considerable effort has been made by several laboratories to synthesize analogues related to mevinolin and compactin by modifying both the decalinic and the lactonic fragments. These studies have led to the definition of the

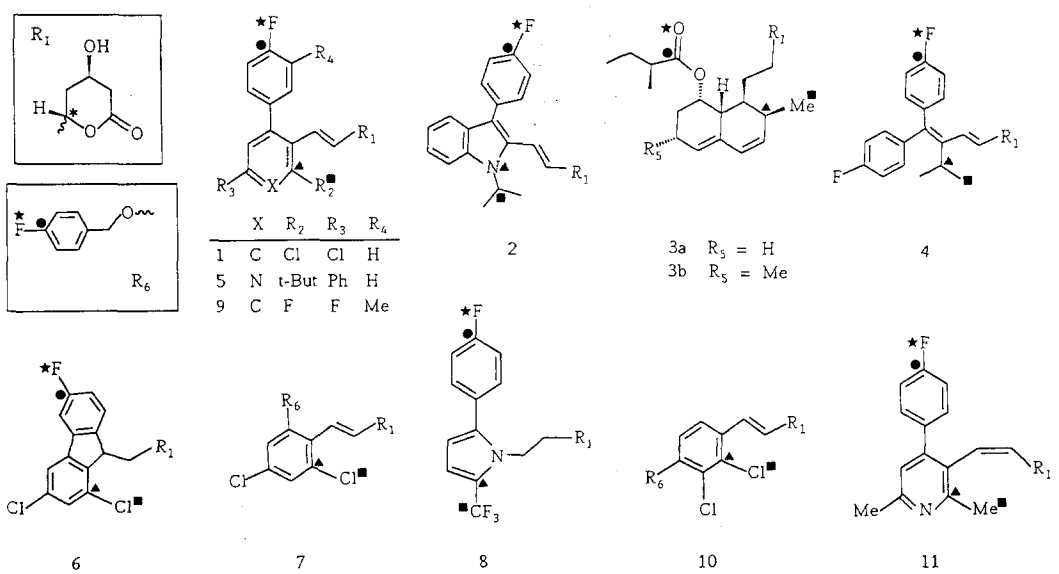

Fig. 1. Investigated HMG-CoA reductase inhibitors. The labelled atoms A (*), X (●), Y (★), M (▲) and L (■) are considered in the pharmacophore definition.

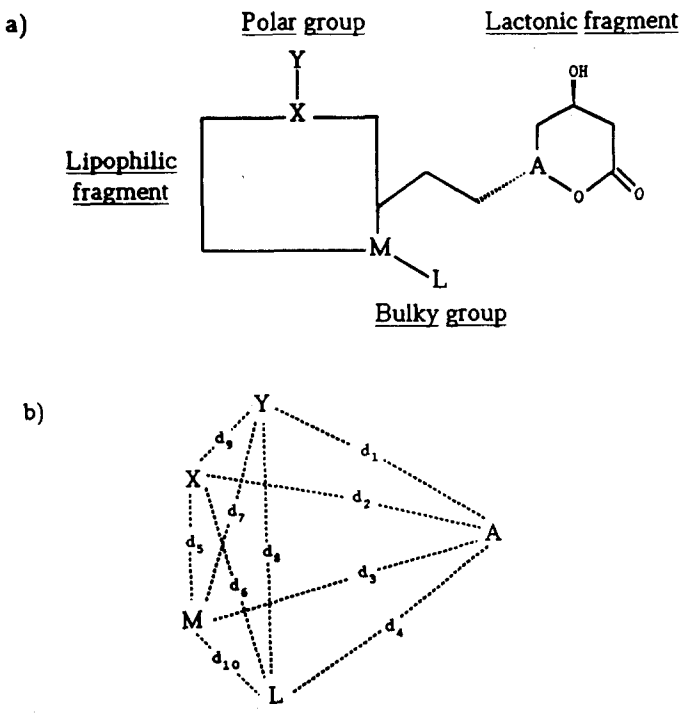

Fig. 2. (a) Main structural features connected with the activity of HMG-CoA reductase inhibitors and atoms included in the pharmacophore. (b) Interatomic distances considered as conformational descriptors.

main structural features (Fig. 2a) connected with the activity:

- a trans-stereochemistry of the lactone substituents with the hydroxy group in the (R) configuration;
- a large lipophilic region (decaline, biphenyl, imidazole);
- a two-carbon bridge (ethyl or ethenyl) as the optimum chain length between the lactone and the lipophilic region;
- the presence of a bulky group on the lipophilic fragment adjacent to the carbon bridge;
- the presence of a polar substituent about 8 atoms from the lactonic ring.

Structure–activity relationship (SAR) studies [13, 14], published during the development of this work, define some geometrical characteristics of the pharmacophore. These studies, performed on a partially different set of compounds, indicate (Fig. 3) that the torsional angle between the lipophilic fragment and the two carbon chain (τ) ranges between 60° and 90° [13] or between 110° and 130° [14] in the active compounds. In Ref. 13 the authors define the maximum extension of the substituents R_1 and R_2 (l_1 and l_2, respectively) and the overall width of the molecule measured as the distance between the farthest points of R_1 and R_2 (l_3). In the active compounds they find values of l_1, l_2 and l_3 less than 5.9, 3.3 and 10.6 Å, respectively.

In the present work we classify the 11 inhibitors as active (**1–5**), poorly active (**6–8**) and inactive (**9–11**) compounds according to the activity data reported in Table 1.

To search for the geometrical pharmacophore for these compounds a methodology developed in our laboratories and successfully applied to nootropic drugs [15] and Ca^{2+} channel blockers

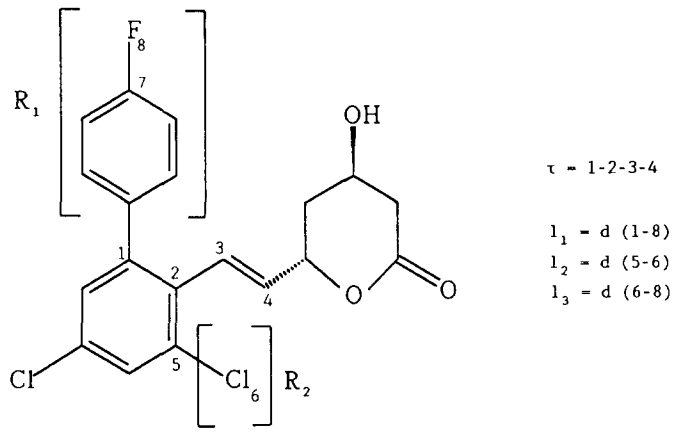

Fig. 3. Geometrical parameters reported in literature as relevant to the activity (compound **1** shown as example).

[16] has been used. This methodology is based on the combined use of molecular modeling and chemometrics: conformational analysis is used to evaluate the minimum energy conformations of each compound of the data set discarding minima with relative energies higher than a predefined threshold. Chemometrics techniques are used both to optimize the choice of the conformational descriptors and to search for geometrical similarities within the hundreds of conformations deriving from the conformational analysis.

Finally, the derived geometrical model is then analyzed according to the characteristics of the MEP distribution. MEP can be a useful descriptor of the recognition process between the ligand and the enzyme-active site; this is due to the long-range nature of the electrostatic force, the coulombic attenuation factor being related only to the first inverse power of the distance.

TABLE 1
ACTIVITY DATA (IC_{50}, nM), TOTAL STERIC ENERGY OF THE GLOBAL MINIMA (SE, kcal mol^{-1}) AND TOTAL NUMBER OF MINIMUM ENERGY CONFORMATIONS (n) FOR COMPOUNDS **1–11**

Compound	IC_{50}	Ref.	SE	n[a]
1	5	6	13.06	14
2	7	7	30.58	43
3a	10	8	13.82	49
4	14	9	23.77	17
5	18	10	17.93	13
6	85	11	14.13	9
7	200	12	11.34	108
8	250	13	24.10	27
9	860	6	10.55	16
10	> 1000	12	17.15	122
11	> 1000	10	17.93	14

[a] Within 6 kcal mol^{-1} above each global minimum.

METHODS AND RESULTS

Conformational analysis

Conformational analysis was carried out using the MM2 force field included in the molecular modeling software package MACROMODEL [17]. The reliability of this force field in determining molecular geometries and conformational relative energies was first confirmed on model compounds by comparing the results with semiempirical and ab-initio calculations (unpublished results).

For each compound a systematic search of minimum energy conformations was performed by the Multiconf option of MACROMODEL. The analysis was performed on the lactonic form of the 3,5-dihydroxy heptanoic acid moiety; the X-ray conformation of the lactonic fragment in compound **1** [6] was assumed as the starting conformation. The conformational behavior of the flexible open form of the acid had presumably no effects on the remaining part of the molecule and was not further considered.

Conformational isomers were generated increasing all the relevant torsional angles by 30° and fully relaxing the obtained geometries. All minimum energy conformations within 6 kcal mol^{-1} above each global minimum were retained for a total of 432 conformations. For each compound the number of minima and the total steric energy of the global minimum are reported in Table 1.

The SYBYL software package [18] was used for visualization of molecular models, for calculation of the least-square plane passing through a set of defined atoms and for evaluation of the distances of other atoms from this plane.

Pharmacophore definition

On the basis of the indicated general features of these inhibitors, atoms A, M and X, L and Y were respectively selected as representative of the spatial disposition of the lactonic (A), lipophilic (M and X), bulky (L) and polar (Y) groups. The atoms considered in the pharmacophore definition in each compound are labelled in Fig. 1 and a general scheme of the pharmacophore elements is depicted in Fig. 2a. The 10 interatomic distances defined by the 5 atoms (Fig. 2b) were initially considered as conformational descriptors.

Multivariate data analysis

A Principal Component Analysis (PCA) [19] was performed to select which distances were necessary for a description of the total variability of the system without losing useful information. The 10 distances were evaluated for all the conformational minima and the correlation matrix of their autoscaled values was submitted to PCA.

The cumulative percent variance and the loading values of the first 3 principal components are reported in Table 2; the loading projections on these principal components are shown in Fig. 4.

The conformational minima of compounds **1**–**11**, described by the autoscaled values of the 3 interatomic distances d_1, d_4 and d_5 selected by PCA (see Discussion), were submitted to a cluster analysis using the nonhierarchical Jarvis–Patrick clustering method (for a description of this method see Refs 15 and 20). The cluster procedure was carried out for different values of the R parameter which affects the clustering selectivity. In the Jarvis–Patrick method the number of nearest neighbors to be considered for each object is represented by K. In this case K = 50 has been assumed. As R ranges between 1 and K, the clustering procedure was scanned by increasing R by

TABLE 2
NUMBERING OF THE COMPONENTS, CUMULATIVE PERCENT VARIANCE AND LOADINGS OF THE FIRST THREE PRINCIPAL COMPONENTS OBTAINED BY PCA

Component id.	PC1	PC2	PC3
Cumul. % var.	51.85	69.64	85.51
d_1	0.336	0.183	−0.399
d_2	0.316	0.168	−0.451
d_3	0.111	−0.652	−0.247
d_4	0.046	−0.712	−0.050
d_5	0.408	−0.037	0.215
d_6	0.420	−0.003	0.186
d_7	0.402	−0.030	0.301
d_8	0.403	0.005	0.294
d_9	0.271	0.028	0.004
d_{10}	0.178	0.056	−0.562

tens till 50. For our purpose the best solution is a cluster containing conformations of the maximum number of the active compounds **1**–**5**. Such a solution was located between R = 30 and R = 40 and R was further scanned within these values. For each R value, the total number of clusters and detailed results of the most significant ones are reported in Table 3.

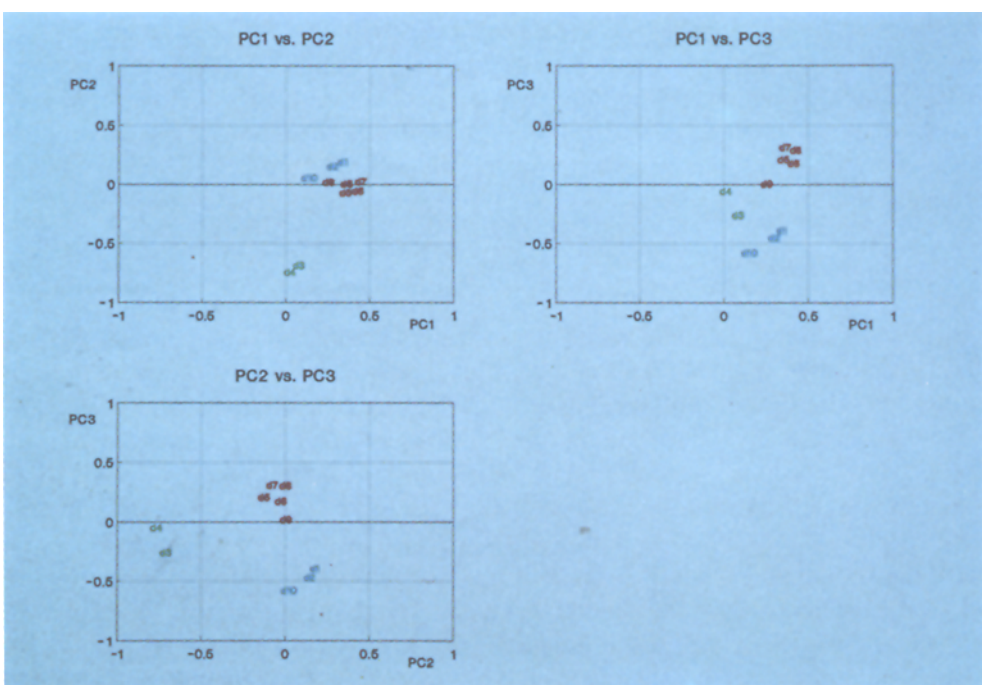

Fig. 4. Loading projections of the first 3 principal components.

TABLE 3
TOTAL CLUSTERS FOR EACH R VALUE[a]

R	Total clusters	Conf.	Molecules											d_1	d_4	d_5	SD
			1	**2**	**3a**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**				
10	7																
		82	Y	Y	–	Y	Y	–	Y	Y	Y	Y	Y	6.28	5.10	6.50	0.57
20	7																
		78	Y	Y	–	Y	Y	–	Y	Y	Y	–	Y	6.18	5.10	6.51	0.55
30	13																
		65	Y	Y	–	Y	Y	–	–	Y	Y	–	Y	6.00	5.13	6.51	0.53
40	33																
		43	Y	–	–	Y	Y	–	–	Y	Y	–	–	5.94	5.03	6.49	0.34
50	307[b]																
35	19																
		66	Y	Y	–	Y	Y	–	–	Y	Y	–	Y	6.03	5.13	6.49	0.52
37	24																
		64	Y	Y	–	Y	Y	–	–	Y	Y	–	–	6.03	5.14	6.50	0.50

[a] For the most significant cluster, total number of conformations (Conf.), molecules included (Y), centroid value (Å) and cluster standard deviation (SD) (Å) are reported.
[b] No significant clusters are identified.

All calculations were performed by using the Software for Chemometric ANalysis (SCAN) package [21]. This software collects several chemometric methods and deals with classification, clustering and regression problems in a unified framework; furthermore a deep exploratory data analysis can be performed with several incorporated graphics tools.

Molecular Electrostatic Potential (MEP) evaluation

The MEP distribution of the lowest energy conformation of each compound present in the 'active' cluster (see Discussion) was analyzed. For comparison, the MEP analysis was also performed on representative conformations of the compounds not included in this cluster. For each of such compounds the conformation closest to the geometrical model represented by the active cluster, i.e. the conformation with the lowest distance in the d_1, d_4 and d_5 space from the centroid of the active cluster, was selected.

All MEP calculations were performed on model compounds in which a methyl group substitutes the common lactonic fragment (R_1 = Me in Fig. 1).

The MEP distributions were calculated from the MNDO semiempirical wave function [22] in the plane containing part of the common lipophilic fragment of the molecules, i.e., in almost all the cases, the least-square plane defined by atoms L, M, X and Y. The ability of the MNDO method to reproduce the MEP characteristics evaluated at the ab-initio HF 6-31G* level has been reported [23].

Figure 5 reports the MEP isocontour maps for compounds **1**, **3a**, **4**, **6**, **8** and **9**.

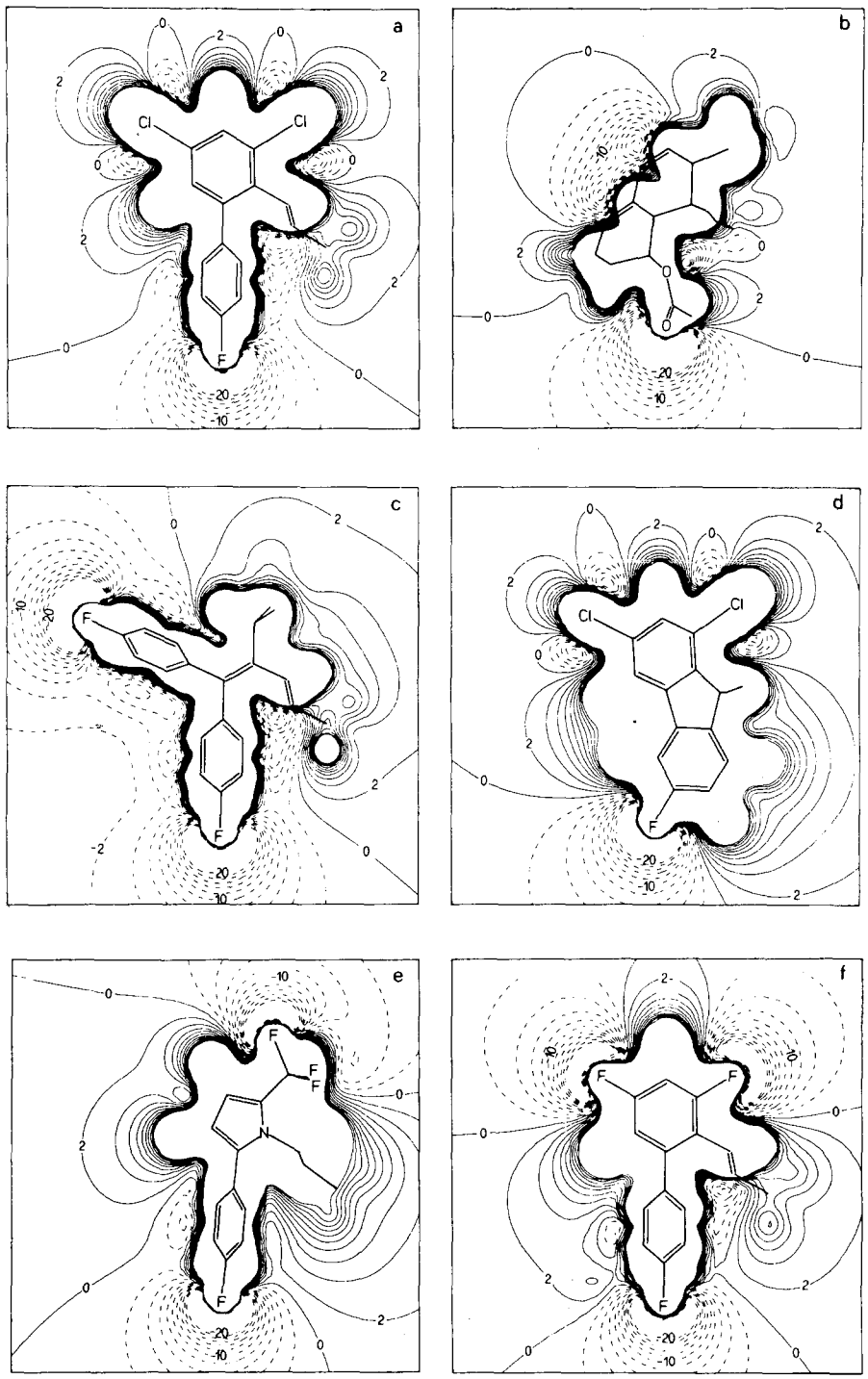

Fig. 5. Molecular electrostatic potential maps in the lipophilic plane for compounds **1** (a), **3a** (b), **4** (c), **6** (d), **8** (e) and **9** (f). Solid and dashed lines correspond to positive and negative values, respectively. Isocontour levels every 2 kcal mol^{-1}.

DISCUSSION

Results of PCA (Table 2) show that the first 3 components explain about 85% of the total variance of the data set. The loading projections of the 10 original variables on the planes defined by each pair of the 3 components (Fig. 4) clearly show 3 main groups in which the original variables carry the same information. Thus, only 3 interatomic distances, one from each group, suffice to represent the variability of the system and the 3 distances d_1, d_4 and d_5 have been chosen as conformational descriptors.

Table 3 reports the results of the cluster analysis. For each R value the simultaneous presence of conformations of the greatest number of active compounds (i.e. compounds **1**–**5**) is adopted as the leading criterion to select the meaningful clusters. For each R value, there is only one cluster that fulfills this criterion. Moreover, the cluster obtained for R = 37 (d_1 = 6.03, d_4 = 5.14, d_5 = 6.50, SD = 0.50) contains not only the greatest number of active compounds but also the lowest number of poorly active and inactive compounds. Thus, in this point of the descriptor space we have the highest 'concentration' of conformations of active compounds together with the lowest of those inactive. This cluster represents the best solution and can be defined as the 'active' cluster.

Some interesting features can be achieved by a graphic comparison of the conformations included in the active cluster. Each compound presents two different dispositions of the lateral chain; these dispositions are quite symmetrical with respect to the least-square plane defined by the atoms X, Y, L and M of the pharmacophore, atom A being, respectively above and below such a plane. For each of the two dispositions, the lactonic fragment can adopt different orientations. Figure 6 shows, as an example, the minimum energy conformations of compound **1** present in the cluster.

For each compound relative energies and main geometrical parameters of the two lowest energy conformations (one for each disposition) are reported in Table 4. In Fig. 7 the superposition of these conformations is depicted.

The two symmetrical dispositions of the lateral chain involve the same d_1, d_4 and d_5 distance values; only the presence of a chiral partner, such as the enzyme active site, can differentiate between them.

The average τ values of the active conformations ($\pm$ 52°, SD = 14°) agree with those indicated by Roth et al. [13], although these authors do not explicitly report the possibility that, due to the symmetrical disposition of the two-carbon chain, τ can assume both positive and negative values. The mean values of the l_1 to l_3 distances averaged on all conformations present in the active cluster are 4.30 $\pm$ 0.01, 1.53 $\pm$ 0.10 and 7.96 $\pm$ 0.14, respectively, in agreement with reported values [13]. Nevertheless, we want to stress that our model is able to individualize not only an upper but also a lower limit for the selected distances.

For compounds **1**, **2** and **4** we find minimum energy conformations characterized by τ values in the range 110°–130°, as reported by Sit et al. [14]. However, the cluster containing these conformations never includes conformations of compound **5**: the presence of a *t*-butyl substituent ortho to the chain makes such conformations sterically inaccessible in this compound. Thus this cluster does not fulfill the adopted criterion for an active cluster.

The main geometrical features of the pharmacophore are summarized in Fig. 8.

The centroid active cluster represents a geometrical model for the activity; nevertheless, it should be noted that the active compound **3a** is not present in this cluster while the poorly active

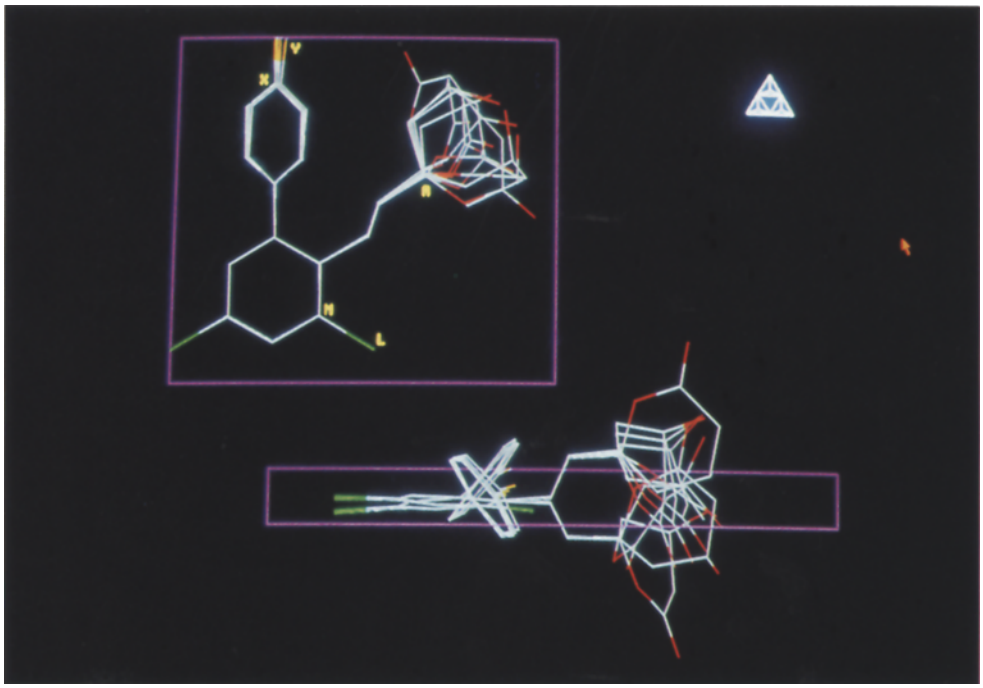

Fig. 6. Perspective views of the minimum energy conformations of compound **1** present in the cluster (hydrogens not displayed). The least-square plane passing through M, L, X and Y atoms is depicted.

8 and the inactive **9** are. This finding purely states that compound **3a** has a different spatial disposition of the atoms defining the pharmacophore with respect to the other active compounds, while compounds **8** and **9** have the same.

Thus, it seems that geometrical similarity is not a sufficient criterion to rationalize the activity/inactivity of these compounds and other properties have to be included in the model for a more realistic description of the recognition process.

For these reasons the MEP distributions of compounds **1–11** in the common plane containing the lipophilic fragment are calculated. In this plane some general features can be highlighted (Fig. 5).

In all compounds the lower zone of the maps is characterized by a negative region associated with the presence of the pharmacophore polar group (a fluorine atom or a carbonyl moiety). This zone is necessary but not sufficient to elicit activity and can be associated with a secondary binding site of the enzyme.

The upper left zone of the maps does not present common characteristics. It assumes positive or negative values without any relationships with the activity of compounds. Thus no specific electronic properties in the portion of the molecule associated with this zone are requested.

The upper right zone seems to be the most discriminant with respect to activity. In fact all the active compounds show positive MEP values in this zone while all the poorly active and inactive compounds show negative values. An exception to this general behavior is found for compounds

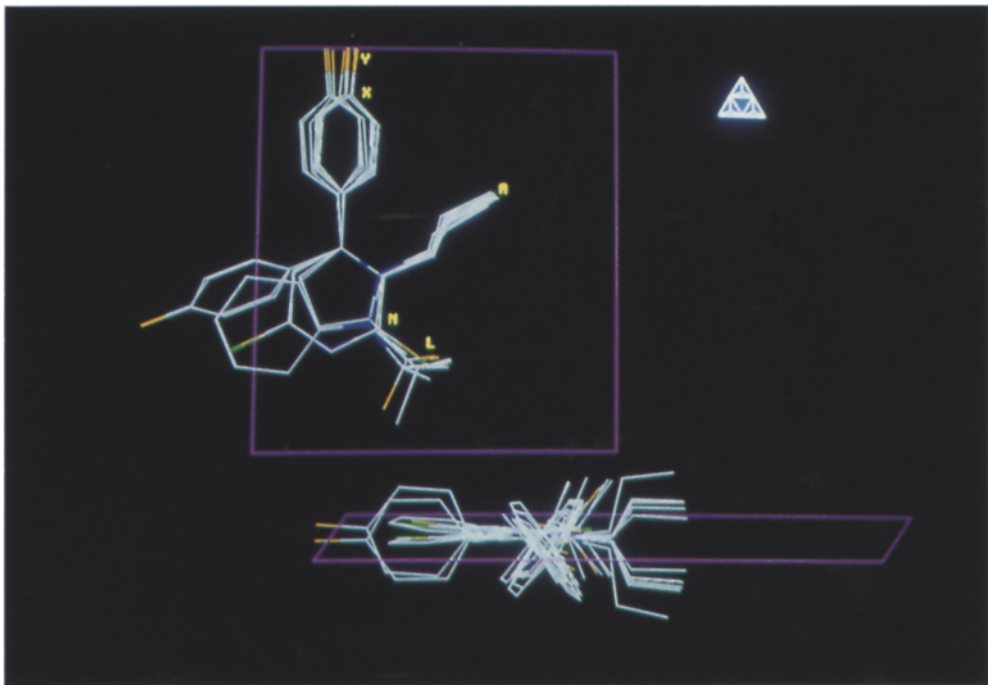

Fig. 7. Perspective views of conformations reported in Table 4 (hydrogens and lactonic fragment not displayed).

6 (poorly active) and **11** (inactive) which present MEP distributions similar to those of the active compounds.

The poorly active and inactive compounds **7** and **10** are not present in the active cluster. Moreover, they show MEP distribution characteristics different from those of the active compounds and do not need further discussion.

The particular behavior of compounds **6** and **11** can be explained taking into account the following considerations. The 5-membered ring condensed to the biphenyl moiety in compound **6** causes a constraint on the lactonic fragment conformational behavior; furthermore the MEP minimum associated with the fluorine atom has a different orientation with respect to the active compounds. These findings can explain why compound **6** is only poorly active.

Instead of all other compounds, the Z configuration of the double bond in **11** constrains the lactonic fragment in a completely different region of the space dramatically changing the relative orientation of this fragment with respect to the other pharmacophore groups: this fact could suffice to explain the inactivity. Furthermore as the contribution of the lactone to the MEP distribution in the considered plane will be different, the similarity found for the model compound is meaningless.

Although present in the active cluster, compounds **8** and **9** have indeed different characteristics in their MEP distributions in this zone and thus can be differentiated from the active compounds.

Finally although not present in the active cluster, compound **3a** shows all the MEP distribution characteristics of the active compounds. Moreover the MEP minimum located in the lower zone of the map is deeper than the corresponding minima of the other active compounds (-70.4 kcal

TABLE 4
RELEVANT ENERGETIC AND GEOMETRICAL FEATURES OF THE TWO LOWEST ENERGY CONFORMATIONS (ONE FOR EACH DISPOSITION, SEE DISCUSSION) OF EACH COMPOUND PRESENT IN THE ACTIVE CLUSTER

Compound	Conf.	ΔE^a (kcal mol^{-1})	d_1	d_4	d_5	h[b]	τ^c (deg)
			(Å)				
1	1	0	5.88	5.05	6.53	1.46	53
	5	0.45	5.91	5.09	6.53	1.32	−56
2	1	0	6.30	5.35	6.45	0.53	33
	8	0.42	6.41	5.33	6.45	0.55	−32
4	1	0	5.89	5.00	6.66	1.37	43
	7	0.55	5.89	4.98	6.66	1.35	−44
5	1	0	5.99	5.15	6.59	1.09	65
	5	0.49	5.96	5.15	6.59	0.87	−68
8	2	1.92	6.04	5.07	6.24	1.26	−71
	6	2.12	6.28	5.06	6.26	1.66	72
9	1	0	5.92	4.93	6.52	1.52	47
	2	0.48	5.86	4.95	6.52	1.47	−47

[a] With respect to each global minimum.
[b] Distance of atom A from the least-square plane defined by atoms M, L, X, Y.
[c] For a definition of τ see Fig. 3.

mol^{-1} vs. −43.6 kcal mol^{-1} in compound **1**). The carbonyl moiety present in this compound allows a stronger interaction with the secondary binding site unlike compounds with a fluorine atom as polar group. This interaction can partially overcome the geometrical differences between the conformations of compound **3a** and the centroid of the active cluster.

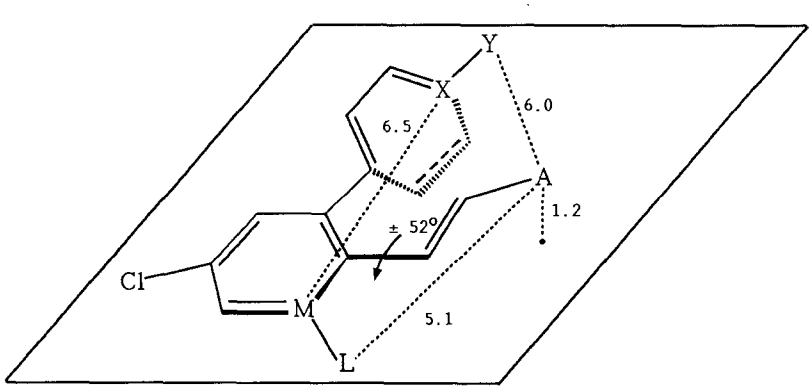

Fig. 8. Geometrical featues of the proposed HMG-CoA pharmacophore.

CONCLUSION

The case of HMG-CoA reductase inhibitors presents an interesting SAR problem because both the geometrical and electronic properties of these compounds have to be taken into account for a modeling of the activity.

The search for geometrical similarity, although not sufficient, is a necessary step in the SAR analysis, allowing the selection of a reasonable model for the 'binding conformation' of compounds. Our methodology is a good tool in this search: its strength lies in the fact that the choice of the geometrical descriptors is optimized by selecting variables carrying relevant and independent information on the system.

The search for electronic distribution similarity on the 'active' conformations can reveal further analogies between compounds useful for the rationalization of the requirements for the activity.

Finally, we want to point out that only some parts of the electronic distributions can be linked to the activity, as in this particular case, and care must be taken when analyzing and comparing the whole MEP distributions.

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