Journal of Computer-Aided Molecular Design, 1 (1987) 3–16
ESCOM
J-CAMD 001

A unique geometry of the active site of angiotensin-converting enzyme consistent with structure-activity studies

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Received 8 December 1986
Revised 5 January 1987
Accepted 8 January 1987

Key words: Pharmacophore; Metallopeptidase; Captopril; Molecular mechanics; Systematic search

SUMMARY

Previous structure-activity studies of captopril and related active angiotensin-converting enzyme (ACE) inhibitors have led to the conclusion that the basic structural requirements for inhibition of ACE involve (a) a terminal carboxyl group; (b) an amido carbonyl group; and (c) different types of effective zinc (Zn) ligand functional groups. Such structural requirements common to a set of compounds acting at the same receptor have been used to define a pharmacophoric pattern of atoms or groups of atoms mutually oriented in space that is necessary for ACE inhibition from a stereochemical point of view. A unique pharmacophore model (within the resolution of approximately 0.15 Å) was observed using a method for systematic search of the conformational hyperspace available to the 28 structurally different molecules under study. The method does not assume a common molecular framework, and, therefore, allows comparison of different compounds that is independent of their absolute orientation.

Consequently, by placing the carboxyl binding group, the binding site for amido carbonyl, and the Zn atom site in positions determined by ideal binding geometry with the inhibitors' functional groups, it was possible to clearly specify a geometry for the active site of ACE.

INTRODUCTION

The most important guiding principle in the design of active and specific enzyme inhibitors is the identification of potential binding interactions that can occur at the active site, irrespective of whether the structure of the enzyme is known. In the majority of cases, the crystal structures of target enzymes are not known and design of inhibitors has to rely on models based on structures of related enzymes, or on an active analogue approach [1, 2].

In contrast to carboxypeptidase A [3], carboxypeptidase B [4], and thermolysin [5], which are

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0920-654X/87/$ 3.50

zinc (Zn) metallopeptidases whose structures have been determined by X-ray crystallography, the crystal structure of angiotensin-converting enzyme (ACE), also a metallopeptidase, has not yet been determined. However, it appears to be similar in many ways to carboxypeptidase A [6]. ACE catalyzes the hydrolytic removal of carboxy-terminal dipeptide residues from the decapeptide, angiotensin I, to produce the potent vasoconstrictor octapeptide, angiotensin II. Inhibitors of ACE have an antihypertensive effect that may be mediated by two different mechanisms: (1) inhibition of angiotensin II formation or (2) potentiation of bradykinin [7], as ACE also hydrolyzes bradykinin.

The first ACE inhibitor that found widespread experimental use was teprotide, a synthetic nonapeptide identical in sequence to one of the natural constituents of the venom from the Brazilian viper, *Brothrops jararaca* [8]. In 1977, Ondetti and his collaborators developed a new class of potent and specific ACE inhibitors, designing the active structures based on their hypothetical, two-dimensional model of the ACE active site [2, 9–11]. Captopril [1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline], the most potent member of this new class, has been shown to be an orally effective antihypertensive agent [12] that possesses clinical usefulness [8, 10, 13]. This development stimulated the search for related compounds with improved pharmacological profiles that also probed the hypothetical active site model of the enzyme proposed by Ondetti and Cushman [9, 10, 13–26]. Extensive structure-activity studies have been performed in an attempt to characterize the active site of ACE [2, 7, 9, 13–31]. The observed results led to the conclusion that the basic structural requirements for ACE inhibition involve (a) a terminal carboxyl group for ionic binding with a positively charged residue on the enzyme; (b) a carbonyl group, preferably of an amidic nature, for hydrogen bonding to the unspecified active site residue X-H, and, finally; (c) a functional group that is a particularly effective Zn ligand [2, 9, 10, 11, 14, 17–21, 27, 32]. Further studies have also identified several types of Zn-binding functional groups: carboxylic acid, hydroxamic acid, and sulfhydryl [11, 14, 25, 32]. A more recent development has been the introduction of phosphorus-containing Zn-binding functions [19, 24, 30]. Some studies have shown that hydrophobicity plays an important role in the binding of inhibitors to the active site of ACE [21], as the presence of hydrophobic substituents on an ACE inhibitor generally increases its potency [14, 25, 27, 28].

There is also evidence that inhibitor potency is very sensitive to stereoisomerism. Previous structure-activity studies have shown that the most active compounds have an absolute stereochemical requirement for the *S* configuration at the chiral center adjacent to the terminal carboxyl group [16, 18, 21–27, 32]. There is also evidence of a preference for the three-dimensional arrangement associated with the *S* configuration at other chiral centers [21, 23, 27, 32].

Such structural requirements common to a set of compounds acting at the same receptor may be used to define a pharmacophoric pattern of atoms or groups of atoms mutually oriented in space, which are necessary for inhibition from a stereochemical point of view. The comparison of the three-dimensional characteristics of structurally related ACE inhibitor molecules is relatively easy, as one only has to list a set of common geometrical parameters such as torsional rotations. This rather static approach fails when one considers a series of structurally non-homogeneous compounds that interact at the same receptor. The matter becomes even more complex when the molecules under consideration possess considerable conformational flexibility. Some investigations have been made to define the three-dimensional relationship between the three most important pharmacophoric groups of ACE inhibitors, but structurally similar compounds were considered [2, 7, 16, 17, 18, 22, 28] in each case.

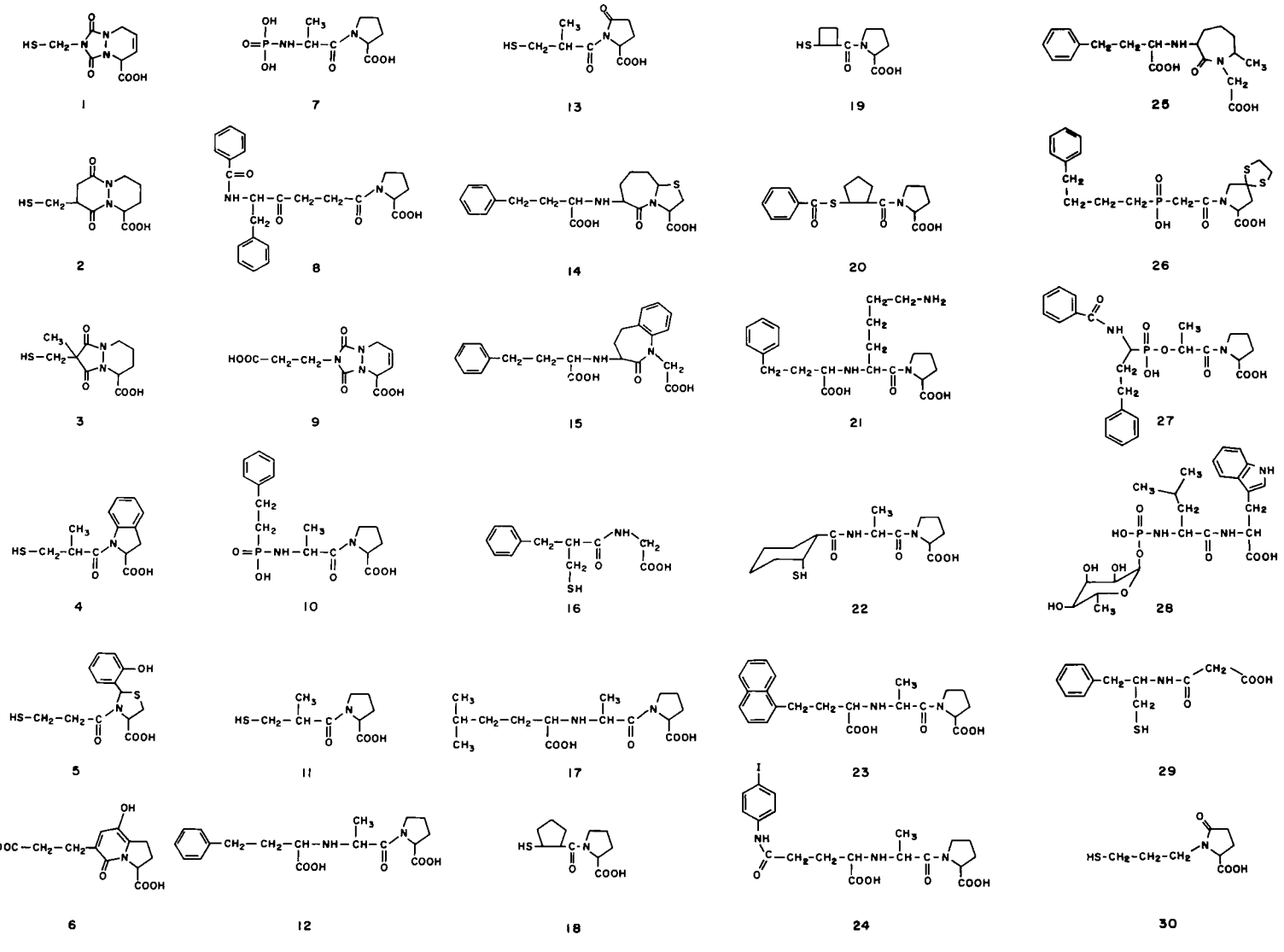

Fig. 1. Twenty-eight analyzed potent ACE inhibitors (1–28) and two inactive molecules.

In order to verify our approach to pharmacophore model validation, we analyzed 28 structurally different (Fig. 1) potent ACE inhibitors (Table 1) and two inactive molecules (Nos. 29 and 30 in Fig. 1) that possessed all three active pharmacophore groups.

METHODS

Using molecular modeling, we tried to find the unique geometrical orientation of the amide carbonyl, the carboxyl group, and the Zn ligand — the three common and most important functional groups of all active ACE inhibitors.

The approximate locations of the enzyme Zn atom, -NH-, and $-NH_3^+$ groups were marked as dummy atoms, and were placed in positions determined by ideal bonding geometry with the

TABLE 1
INHIBITORS OF ANGIOTENSIN-CONVERTING ENZYME. THE NUMBER OF INHIBITOR CORRESPONDS TO THAT IN FIGURE 1

Inhibitor no.	$IC_{(50)}$[a]	Ref.	OMAP no. used as constraint	No. of OMAP points	No. of valid conformers
1	7.0×10^{-7}	(12)	No	2786	10561
2	3.8×10^{-8}	(18)	Yes, No. 1	293	764
3	1.0×10^{-6}	(18)	Yes, No. 2	153	968
4	3.7×10^{-9}	(28)	Yes, No. 3	108	282
5	6.0×10^{-9}	(28)	Yes, No. 4	103	776
6	$\sim 1.0 \times 10^{-8}$	(31)	Yes, No. 5	101	5343
7	1.4×10^{-9}[b]	(24)	Yes, No. 6	99	1236
8	3.2×10^{-9}	(28)	Yes, No. 7	91	1259
9	1.0×10^{-9}	(31)	Yes, No. 8	91	9919
10	7.0×10^{-9}	(24)	Yes, No. 9	77	237
11	2.3×10^{-8}	(9)	Yes, No. 10	71	281
12	1.2×10^{-9}	(32)	Yes, No. 11	65	3209
13	9.0×10^{-9}	(28)	Yes, No. 12	64	218
14	6.0×10^{-10}	(22)	Yes, No. 13	46	295
15	2.8×10^{-9}	(31)	Yes, No. 14	45	3799
16	$\sim 1.0 \times 10^{-7}$	(38)	Yes, No. 15	43	314
17	2.6×10^{-9}	(31)	Yes, No. 16	43	4573
18	$\sim 4.0 \times 10^{-8}$	(20)	Yes, No. 17	16	22
19	$\sim 5.0 \times 10^{-8}$	(20)	Yes, No. 18	14	20
20	$\sim 2.0 \times 10^{-8}$	(20)	Yes, No. 19	7	9
21	1.2×10^{-9}	(31)	Yes, No. 20	7	249
22	3.0×10^{-9}	(31)	Yes, No. 21	7	29
23	1.1×10^{-9}	(31)	Yes, No. 22	7	250
24	2.3×10^{-10}	(31)	Yes, No. 23	5	201
25	3.0×10^{-9}	(31)	Yes, No. 24	5	35
26	4.0×10^{-9}	(31)	Yes, No. 25	4	24
27	1.2×10^{-8}	(31)	Yes, No. 26	4	11
28	1.6×10^{-9}	(31)	Yes, No. 27	2	9

drugs' functional groups [18, 28, 33]. The location of the Zn atom has been established using geometrical parameters taken from X-ray crystallographic data of Zn complexes with appropriate small organic molecules [33]; an NH enzyme group hydrogen bonded to the amide carbonyl has been located in accordance with standard hydrogen bonding geometry; and the protonated nitrogen of an arginine-guanidinium ion for electrostatic interaction with the terminal carboxylate has been placed approximately 2.8 Å from either carboxylate oxygen atoms.

We investigated such conformationally restricted inhibitors as bicyclic molecules with more than one heteroatom, in which the binding groups have been fixed in various geometrical arrangements [13, 17], and 7-membered lactams, as well as some conformationally flexible compounds with different Zn-binding functions. Accepting the suggestion from the literature [16, 18, 21, 22, 23, 25, 32], all 28 molecules were constructed with the *S* configuration at the chiral center adjacent to the terminal carboxyl group and with *S* or *R* configuration at other chiral centers in accordance

Inhibitor no.	$IC_{(50)}$[a]	OMAP no. used as constraint	No. of OMAP points	No. of valid conformers
1	7.0×10^{-7}	Yes, No. 28	2	47
2	3.8×10^{-8}	Yes, No. 28	2	15
3	1.0×10^{-6}	Yes, No. 28	2	15
4	3.7×10^{-9}	Yes, No. 28	2	9
5	6.0×10^{-9}	Yes, No. 28	2	17
6	$\sim 1.0 \times 10^{-8}$	Yes, No. 28	2	194
7	1.4×10^{-9}[b]	Yes, No. 28	2	22
8	3.2×10^{-9}	Yes, No. 28	2	46
9	1.0×10^{-9}	Yes, No. 28	2	562
10	7.0×10^{-9}	Yes, No. 28	2	6
11	2.3×10^{-8}	Yes, No. 28	2	17
12	1.2×10^{-9}	Yes, No. 28	2	74
13	9.0×10^{-9}	Yes, No. 28	2	8
14	6.0×10^{-10}	Yes, No. 28	2	69
15	2.8×10^{-9}	Yes, No. 28	2	160
16	$\sim 1.0 \times 10^{-7}$	Yes, No. 28	2	7
17	2.6×10^{-9}	Yes, No. 28	2	137
18	$\sim 4.0 \times 10^{-8}$	Yes, No. 28	2	4
19	$\sim 5.0 \times 10^{-8}$	Yes, No. 28	2	4
20	$\sim 2.0 \times 10^{-8}$	Yes, No. 28	2	4
21	1.2×10^{-9}	Yes, No. 28	2	59
22	3.0×10^{-9}	Yes, No. 28	2	5
23	1.1×10^{-9}	Yes, No. 28	2	53
24	2.3×10^{-10}	Yes, No. 28	2	49
25	3.0×10^{-9}	Yes, No. 28	2	74
26	4.0×10^{-9}	Yes, No. 28	2	17
27	1.2×10^{-8}	Yes, No. 28	2	9

a = Molar concentration for 50% inhibition.
b = K_i value.

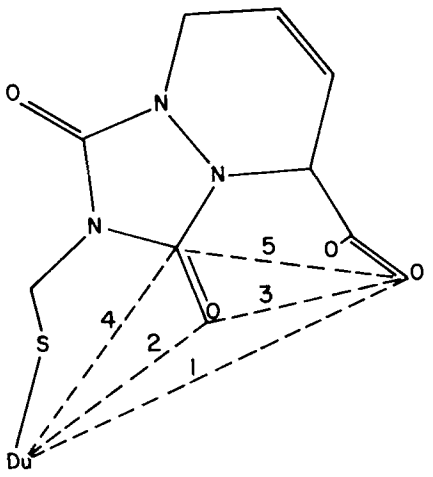

Fig. 2. Defining distances between the most important groups of the active site hypothesis derived from the structure-activity data.

with structure-activity data [9–32] and minimized [34]. The torsion angles were defined by clockwise rotation around the appropriate bonds according to the convention of Klyne and Prelog [35] using rotation intervals of 10°. Using the systematic SEARCH [36, 37] function within the molecular modeling program SYBYL [34], each of the 28 molecules was placed in the conformation that best overlapped its functional groups to the corresponding groups of the other molecules.

Using systematic SEARCH [36, 37] with orientation map calculations [36], it is possible to determine the common orientations of the predicted important active site groups. In order to include the direction of carbonyl function, orientation maps were generated by defining five distances between the three most important points of the active site hypothesis derived from the structure-activity data (Fig. 2) [14, 15, 21, 24, 27]. Those five distances were between: (1) the assumed position of the Zn atom in the enzyme and one of the oxygen atoms in the carboxyl group of the ligand because either of the oxygen atoms in the carboxyl group can serve to interact electrostatically with the opposite charge in the enzyme site; (2) the Zn atom and the amide carbonyl oxygen; (3) the amide carbonyl oxygen and one carboxyl oxygen; (4) the enzyme Zn atom and the ligand amide carbonyl carbon; and (5) the ligand amide carbonyl carbon and one terminal carboxyl oxygen. The position of the Zn atom was chosen as a reference point because of the variety of Zn-binding functions in the different structures. The different types of Zn-binding groups form Zn complexes that differ geometrically from each other, precluding the existence of a unique common position for all of the Zn-binding functions with respect to the other pharmacophoric groups.

RESULTS AND DISCUSSION

Given the geometrical constraints on the binding of the ligand to enzyme described above, we searched for all conformations of each ligand that possessed relative orientations of the active site groups common to all ligands. Using the pairwise distances between groups as coordinates, and

transforming each conformation into this relative distance space, i.e., orientation space, one can compare different compounds independently of their absolute orientations. A distance constraint is specified by the minimum and maximum allowed distances between any atoms belonging to different aggregates.

Since our assumption about the active site includes a common geometry of binding of any active compounds, the point corresponding to this pattern must be occupied in each orientation map generated for each analogue. This fact allows the use of the range of orientation space of molecule Mi (Fig. 3A) as a set of distance constraints in the systematic search performed for the molecule Mi + 1, $1 \leqslant i \leqslant n$, where n is the total number of molecules under consideration (Fig. 3B). The result is the final orientation map that displays a common set of points representing the common active site patterns belonging to all examined analogues (Fig. 3C).

Looking at all valid conformations of any active compound studied (Table 1), two examples of which are shown in Figs. 4 and 5, we can make the following observations after superposition utilizing the common amide bond for fitting.

First, the positions of two of the functional groups of the inhibitor are very restricted, while the third has a little more orientational freedom. Usually, this orientational freedom is connected with the position of the Zn ion in the enzyme structure (Figs. 4 and 5), or more rarely with the position of carboxyl oxygen in the ligand structure (Fig. 4).

Secondly, the amide carbonyl group in many of the compounds may adopt two alternative orientations relative to the carboxyl group. This is confirmed by several X-ray analyses which have shown that in the most active compounds the amide carbonyl is oriented either towards (*trans* amido bond) or away from (*cis* amido bond) the carboxylic acid function [15, 17, 18, 28, 29]. In reality, it has not been clearly determined whether the *trans* conformation, which is the most commonly observed in crystalline structures, is also the preferred one in solution [15, 29]. However, several experimental studies have indicated that the isomer with the *trans* amido bond is the biologically more active form [12, 17, 18, 22, 28, 29]. Furthermore, conformational analysis studies have shown that in most cases the *trans* amido bond isomer is the most energetically favorable [13, 18, 28, 29].

Our results, which show that the amido bond is planar (180° ± 10°) and in the *trans* configuration in the active conformations of all examined inhibitors, are in agreement with the above observations. Consequently, the amide carbonyl and carboxyl acid function are in gauche conformation with the amide carbonyl directed towards the carboxylic acid group [12, 17, 18].

Moreover, in all examined inhibitors, the position of the amide carbonyl group is severely restricted by necessity of maintaining the planarity of the amide bond as well as by the distance constraints to the other two important groups. In consequence of this, the position of the proposed hydrogen-bonding site in the enzyme is restricted to lie on a circle whose center lies along the C=O axis. The locus of the hydrogen bond donor group can be further defined by performing potential energy calculations between the inhibitors and a hypothetical NH group placed at various points around the circle in the optimal geometry for hydrogen binding to the carbonyl group [18, 28, 33]. When this was performed with 10° increments for all molecules, the only acceptable position for the hydrogen donor group is found when the torsion angle N–C=O... X lies in the range 0° ± 60°. This result is partially in accordance with the results of Andrews et al. [28], which suggested an N–C=O... X torsion angle of either 120° ± 40° or 300° ± 20°. However, the results of Andrews et al. were based on only a few inhibitors, all of which were at least as flexible as cap-

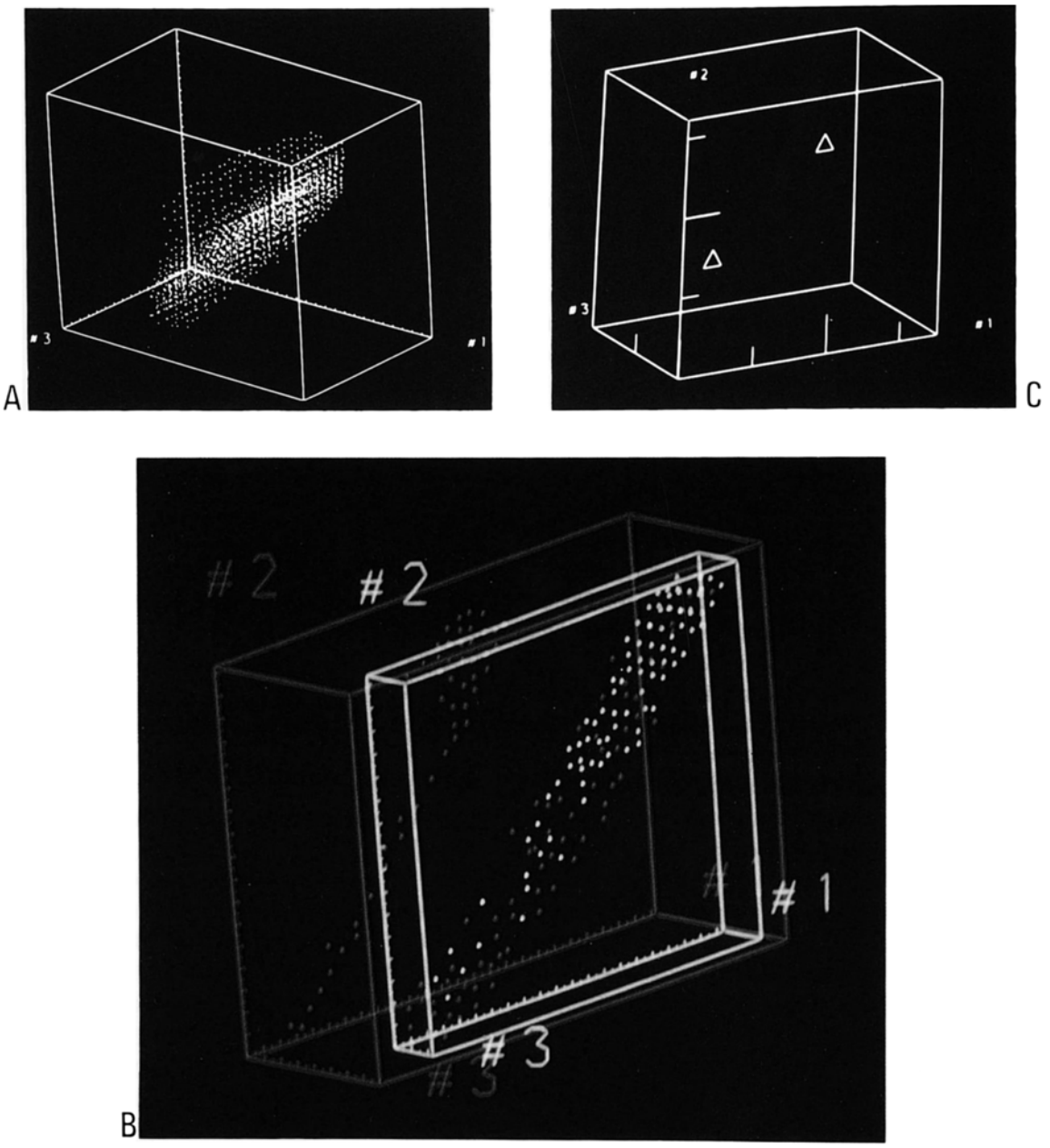

Fig. 3A. The first orientation map (OMAP) (molecule No. 1), generated without any constraints. The OMAP dimensions are 5.0 Å (axis 1) × 4.0 Å (axis 2) × 3.0 Å (axis 3).

3B. Orientation space of molecule Mi used as a set of distance constraints for the molecule Mi + 1. The larger OMAP has dimensions 5.0 Å (axis 1) × 3.5 Å (axis 2) × 1.6 Å (axis 3) and the smaller OMAP has dimensions 4.1 Å (axis 1) × 3.3 Å (axis 2) × 0.5 Å (axis 3).

3C. The final OMAP representing the common active site patterns belonging to all examined analogues. The final OMAP has dimensions 0.4 Å (axis 1) × 0.3 Å (axis 2) × 0.2 Å (axis 3).

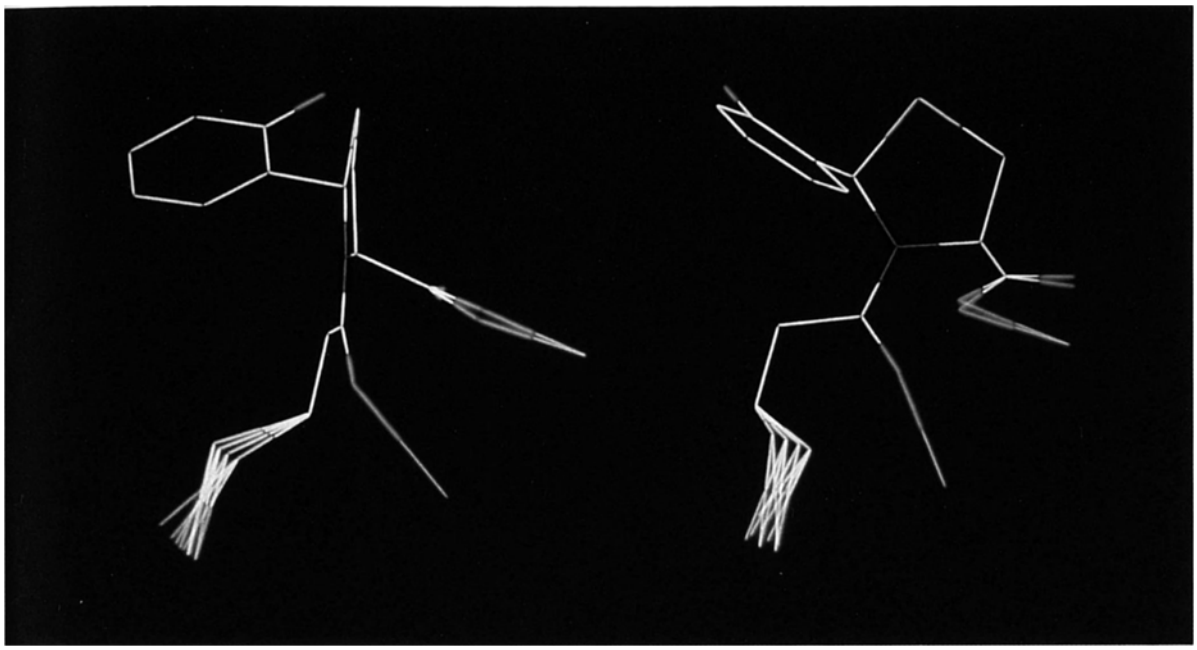

Fig. 4. The 17 valid conformations of molecule No. 5 (Fig. 1) belonging to the two final OMAP points (2 different views).

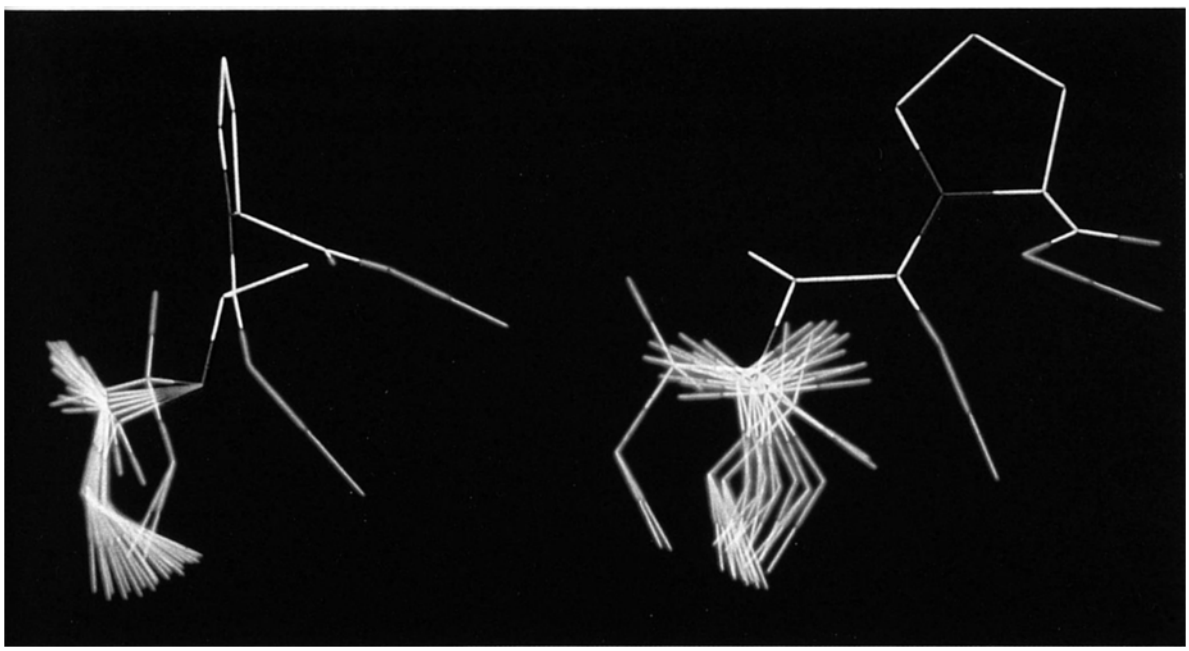

Fig. 5. The 22 valid conformations of molecule No. 7 (Fig. 1) belonging to the two final OMAP points (two different views).

topril. Thus, considering the fact that our results were derived from a much larger data set that contained several rigid and semi-rigid analogues, we suggest that the position for the hydrogen bonding site group on the enzyme is in that region where N–C=O... X torsion angle is around 300°, since the region around 120° is not energetically acceptable for all 28 examined chemically and structurally different ACE inhibitors.

Thirdly, the position of the terminal carboxyl group was oriented in all cases in an axial position with respect to the ring system. This was based on experimental observation that the carboxyl group has a strong preference for the axial orientation in bicyclic systems that possess two rigid planar amide bonds [12, 17, 18]. In such an orientation, the carboxyl group avoids the 1, 3 steric interaction with the amido carbonyl group.

Although the axial orientation of the carboxyl group is reasonably well-defined, the results of previous investigations suggest that it can undergo relatively free rotation in the range from 0° to 180° [13, 17, 18, 28], since each of the oxygen atoms in the carboxyl group can serve to interact electrostatically with the opposite charge in the enzyme site.

Hence, identification of the precise location of the terminal carboxyl binding group is hampered by the lack of any significant conformational restriction on its rotation and the absence of any other groups which might sterically interfere with its placement. This means that the carboxyl binding group on the enzyme, which is probably an arginine guanidinium ion [2], could lie almost anywhere on a roughly hemispherical surface surrounding the carboxyl group, placing a protonated nitrogen approximately 2.8 Å from either or both carboxyl oxygen atoms [28]. Our investigations showed a restricted rotational freedom of the carboxyl group due to constrained distances between pharmacophoric groups common to all inhibitors under examination.

Since the position of the carboxyl group is more precisely defined in our study, the position of the positively charged enzyme group can be more specifically located. The results shown in Fig. 6 and summarized in Table 2 led us to the conclusion that the positively charged group site can ideally lie only in a very restricted area between both carboxyl oxygen atoms, because they are both qualitatively the same, with all possible locations of positively charged groups lying within 0.15 Å of each other.

Lastly, the orientation of the Zn-binding functions differs among the various inhibitors, probably because ligands can form both tetracoordinate and pentacoordinate complexes with Zn over a similar range of approach angles [24, 28, 30]. Also it is evident from Fig. 4 that the Zn ligand group may undergo relatively free rotation, but later results (Fig. 6) indicated only one small area for the position of the Zn ion in the enzyme common for all ACE inhibitors. All inhibitors studied

TABLE 2
FIVE DISTANCES IN THE FINAL ORIENTATION MAP COMMON FOR 28 EXAMINED ACE INHIBITORS

OMAP points	Distances (Å)[a]				
Number	1	2	3	4	5
1	8.5432	4.9381	3.8082	5.2785	4.0953
2	8.6946	5.0620	3.8082	5.3442	4.0953

[a]Distance number corresponds to that defined in Fig. 2.

were capable of adopting a conformation that positioned the Zn atom in a small and unique spherical locus of radius approximately 0.1 Å and, for steric reasons, in a *trans* position to the substituents on the carbon atom. The Roche group reported a similar observation in their study of a set of rigid captopril analogues [13, 17, 18]. They based their examinations on the assumption that the crystal form of captopril was the active conformation responsible for binding to the enzyme and that in vitro activities of conformationally restricted inhibitors are directly dependent on their ability to mimic the position of the thiol group in the captopril crystal structure. They focused their attention mainly on the locus of the thiol group by fitting the amide bonds to each other and largely ignoring the carboxyl groups. In order to reduce the number of possible solutions, potency arguments were combined with conformational energy calculations to predict a binding mode with the thiol group on the opposite side of the amide plane as the carboxyl group [12, 17, 18].

On the other hand, Andrews et al. [28] directed their attention to the captopril analogues with different Zn-binding functional groups. By changing torsional angles and calculating conformational energies they suggested a single low-energy conformation common throughout the series which could define the structural and conformational requirements for ACE inhibition. In their approach, they excluded a few possible alternative conformational solutions on the basis of very small energy differences. However, they did not examine any rigid or semirigid molecules whose conformational analysis could lead to different solutions. They suggested relatively few orientations for the Zn binding groups accessible to all of the examined molecules with the torsional angles $\psi \cong 165°$, $\varphi \cong 300°$, and $\tau \cong 60°$, but suggested the same relative orientations of the thiol and carboxyl groups.

In contrast to the methods described above, our approach is that of pharmacophore model validation [1,2] and the assessment of its uniqueness which is based on a method of the systematic search of the conformational hyperspace available to all the molecules considered [34, 36, 37]. This method is independent of the molecular framework and of the rotation of compounds, and thus allows easy comparison of different compounds that is independent of their absolute orientations. It is clear that our results, which were derived from chemically and structurally very different molecules, were obtained by a more comprehensive method, leading to a unique position of the Zn atom in the enzyme structure. Assuming a relatively rigid position of the Zn atom would allow some rotational freedom of the terminal carboxyl group from 130° to 150°, as can be seen in Fig. 6.

Since our examination did not include precise definition of the groups beyond the Zn-binding region, parts of the molecules are not shown in the figures and are not the concern of this paper, but form the basis of current studies.

Energy calculations were carried out for all valid conformations of all examined inhibitors using the MAXIMIN program [34, 38] without the calculation of electronic charge, due to ambiguities in handling electronic contributions, especially regarding a zinc ligand.

Comparison of the conformational profiles of various molecules may reveal those that overlap or intersect conformational space which are energetically acceptable. Sometimes interaction with a receptor perturbs the conformational energy surface of the drug. For this reason, one should consider not only conformers around the global minimum, but all conformers which are within a reasonable limit above the global minimum as potential candidates for the receptor-bound conformation [2, 5, 13, 18, 28, 29]. The credibility of our approach is supported by the observation

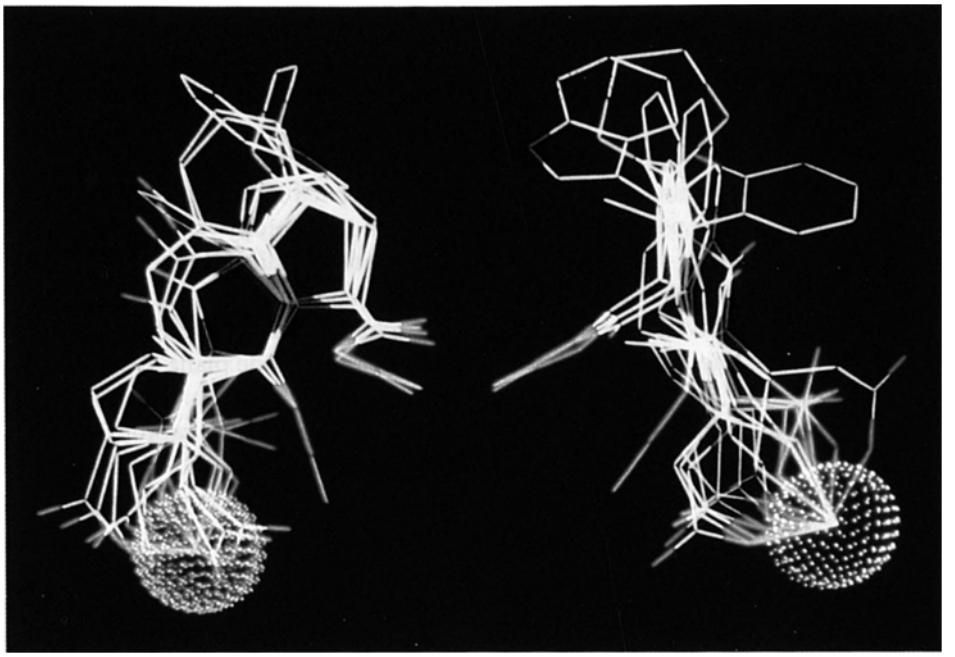

Fig. 6. One conformation of each of the 28 ACE inhibitors that fit the final OMAP. The dots represent the van der Waal's surface union of the locus of the Zn atom position in the enzyme structure (2 different views).

that all of the compounds map to the final orientation space with an excellent geometrical fit (Fig. 6) of the pharmacophore with acceptable energy values as shown in Table 3. The differences (ΔE kcal/mole) between the energy of the conformer of each inhibitor that best fits the final orientation space results (E_{con}) and the energies of each of the same conformers after minimization (E_{min}) range from approximately 0.3 to 3.9 kcal/mole. These small energy differences indicate that the pharmacophore topography is easily accessible to all compounds.

Further supporting the validity of our hypothetical pharmacophore model is its capability of correctly distinguishing between active and inactive analogues of thiorphan (see Fig. 1, active molecule No. 16 and inactive molecule No. 29) [39], as well as active and inactive analogues of captopril (see Fig. 1, inactive isomer molecule No. 30) [40]. The active analogue of captopril is not shown in Fig. 1 and is not discussed separately because the observed result showed the same behavior as captopril. The difference between the active and the inactive analogue is in the configuration of the amido bond. The *cis* amido isomer showed 2560 times less activity than the *trans* amido isomer whose activity is comparable to that of captopril [40]. The observed results also show that it is not possible to find any low energy conformation of the inactive *R*-thiorphan that fits to the common final orientation space of the active molecules. It is important to stress that the inactive analogues of thiorphan possess all three active pharmacophore groups, as is shown in Fig. 1, Nos. 29 and 30.

Such an observation leads to the conclusion that the present method is very useful for distinguishing active and inactive isomers. However, this does not necessarily mean that the method

TABLE 3
THE DIFFERENCES (ΔE) BETWEEN THE ENERGY OF THE CONFORMER OF EACH INHIBITOR THAT FITS THE FINAL OMAP RESULTS (E_{con}) AND THE ENERGY OF THE SAME CONFORMER AFTER MINIMIZATION (E_{min})[a]

Inhibitor no.	$\Delta E = E_{con} - E_{min}$	Inhibitor no.	$\Delta E = E_{con} - E_{min}$
1	2.374	15	1.465
2	1.042	16	1.739
3	2.360	17	0.700
4	0.662	18	1.239
5	1.000	19	3.903
6	3.574	20	1.003
7	0.985	21	1.590
8	3.074	22	0.275
9	3.739	23	0.557
10	1.259	24	0.753
11	1.295	25	1.625
12	0.870	26	1.994
13	0.964	27	2.320
14	2.211	28	0.737

[a]Energy is expressed in kcal/mole.

can be used as an excluding principle in the comparison of structurally and chemically different active and inactive compounds, as some inactive analogues may fit with a low energy conformation to the final orientation space of the active molecules, but for other reasons (which are not the subject of this paper) do not show high activity.

Furthermore, inspection of the data in Table 2 allows identification of a unique pharmacophore model at a resolution of approximately 0.15 Å. Actually, the observed data represent two possible pharmacophoric patterns belonging to all examined analogues.

Moreover, it is important to point out that the calculations for the pharmacophore model presented in this paper represent a significant improvement in the topographical and physicochemical specifications of the ACE active site over previous models [1, 13, 19, 28, 29], as well as providing a meaningful basis for comparison of the topography of structurally diverse molecules exhibiting the same biological action.

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