Preferred Conformation of Selected ACE Inhibitors for Interaction with ACE Active Site

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Theoretical methods were used to study structural properties of most common angiotensin-converting enzyme inhibitors (ACEIs): captopril, enalapril, perindopril, ramipril, benazepril, trandolapril, and cilazapril. In the first step, the active metabolites of ACEIs were modeled and all atoms were parametrized by extended MM2 parametrization set. Next, thorough conformational analysis was performed on all rotatable bonds, except those of 3-phenylpropyl or butyl fragment, which were set to low-energy (all-*trans*) extended arrangement. The values of dihedral angles were varied over the range of 360° in 15° increments and at each step MM2 energy of the rotamer was calculated. Valid low-energy rotamers were saved in a database file; those with intramolecular contact or those with high-energy strain were discarded. Optimal values of dihedral angles were derived from conformational maps and applied to the modeled structure. Several families of low-energy rotamers were identified. For each family, the best representative was chosen and fully optimized with the AM1 method. The lowest-energy conformations were compared to each other and a common pharmacophore was calculated. In addition, structures of ACEIs available in Cambridge Crystallographic Database were taken as a starting point for AM1 geometry optimization. The resulting relaxed structures were compared to those found in conformational search.

Angiotensin-converting enzyme inhibitors (ACEIs) are well-known drugs used especially in treatment of essential hypertension and congestive heart failure, but their importance is still increasing [1]. ACEIs help to reduce pathologically increased blood pressure by blocking the Renin-Angiotensin System (RAS), which plays the main role in the blood pressure regulation. The first enzyme of RAS – renin converts angiotensinogen (α_2-microglobulin) into an inactive decapeptide angiotensin I. The second enzyme – dipeptidyl carboxypeptidase (ACE, 3.4.1.15) cuts off a dipeptide from C-terminus of angiotensin I and the active octapeptide angiotensin II is formed. Angiotensin II is thought to be one of the most powerful vasoconstrictors in human body. It stimulates release of aldosterone, noradrenalin, and endothelin. Their excessive amounts can cause the damage to cardiovascular system. ACE is also responsible for the degradation of vasoprotective factors, *e.g.* bradykinin. ACEIs reversibly inhibit ACE by binding into its active site and thus the formation of angiotensin II in blood and tissues is decreased [1]. The structure of ACEIs is similar to the C-terminal sequence of ACE's natural substrate – angiotensin I (Phe—His—Leu). The long-lasting inhibition is achieved by replacing the scissile amide group of angiotensin I (between Phe and His) with a resistant one, *e.g.* methylene group. Moreover, ACEIs coordinate the Zn^{2+}cation in the active site of the ACE with carboxyl or thio functional group instead of amide carbonyl group (Formula 1).

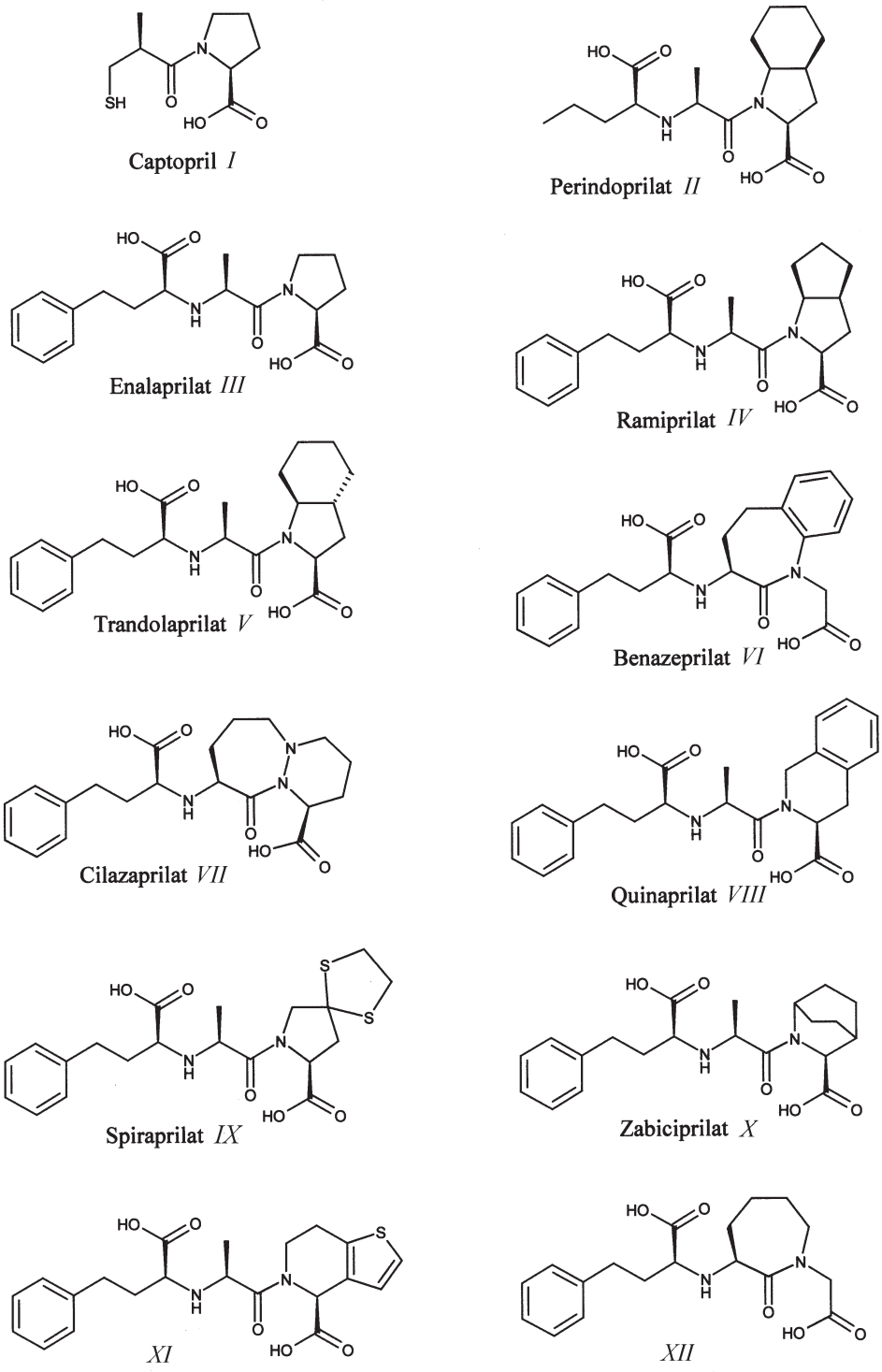
In the past several theoretical and experimental studies of ACEIs were carried out in order to elucidate the molecular mechanism of action and to describe the spatial arrangement of functional groups (amino residues) present in ACE's active site. In these studies the compounds were first synthesized and then analyzed for ACE inhibiting potency [2—6]. ACE is a highly specific enzyme and we think the new structures should be designed on the basis of structural and chemical parameters derived from currently used compounds. Some data derived from conformational analyses [7—9] and X-ray crystallographic studies [10—13] were previously published. The crystal conformations of ACEIs show high variability and it is not clear, which conformation best fits into the active site. Crystallographic data of some metallopeptidases are available, but may not necessarily be valid for ACE [14]. That is why we tried to identify all low-energy rotamers of selected ACEIs and among them the common most favourable one that could be responsible for their inhibiting effect. The main objec-

tive of this study was to compare AM1-optimized conformations of most common ACEIs identified in conformational search to the AM1-optimized X-ray structures of ACEIs (X-ray crystal structure was taken as a starting point for geometry optimization). According to data obtained a four-centre pharmacophore was derived.

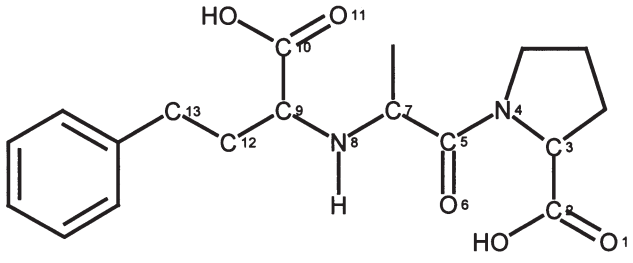
THEORETICAL

Conformational Analysis

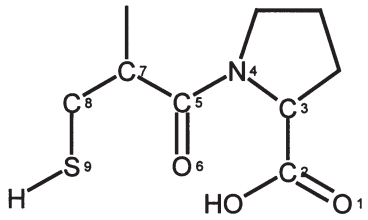
2D structural formulae of active metabolites of ACEIs *I*—*VII* (Formula 1) were converted to 3D models. Chiral centres in the models of ACEIs were set to



Formula 1



$\phi 1$ = d(O1C2C3N4)
$\phi 2$ = d(C2C3N4C5)
$\phi 3$ = d(C3N4C5O6)
$\phi 4$ = d(N4C5C7N8)
$\phi 5$ = d(C5C7N8C9)
$\phi 6$ = d(C7N8C9C10)
$\phi 7$ = d(N8C9C10O11)
$\phi 8$ = d(N8C9C12C13)



$\phi 1$ = d(O1C2C3N4)
$\phi 2$ = d(C2C3N4C5)
$\phi 3$ = d(C3N4C5O6)
$\phi 4$ = d(N4C5C7C8)
$\phi 5$ = d(C5C7C8S9)

Formula 2

the correct configuration, which must mimic the configuration of natural substrate of ACE – angiotensin I (the so-called all-*S* configuration) [12, 15]. All atoms were parametrized with MM2 extended parametrization set. Atom and dihedral angle numbering is described in Formula 2.

The amide bond (dihedral angle $\phi 3$ was set to *trans* as this is more stable arrangement than *cis* [10—12]. Moreover, in both *VI* and *VII*, the amide bond is fixed in *trans* arrangement in a seven-membered ring. It has the character of a double bond and barrier to rotation is high when compared to other analyzed bonds. The amide group maintains almost ideal planarity and we assumed that no further investigation was needed.

The rotatable bonds in the hydrophobic part of ACEIs (either 3-phenylpropyl or butyl), which mimic the Phe residue of angiotensin I, were set to the most stable extended (all-*trans*) conformation. These bonds were therefore excluded from conformational analysis.

Searched dihedral angles were assigned random starting values and varied over the range of 360° in 15° increment. The energy of each generated rotamer was calculated by the MM2 method. Valid low-energy rotamers were saved in a database file, those with intramolecular contact or those with energy above 42 kJ mol^{-1} over the global minimum, were discarded.

The molecule of *I* has only three rotatable bonds. All three dihedral angles $\phi 1$, $\phi 4$, and $\phi 5$ were searched in a single analysis, range 360° in 5° increment.

The molecules of *II*—*VII* are more complex and contain more rotatable bonds. We assumed that the position of C-terminal carboxyl group could not sterically affect the conformation of the chain behind the amide bond (C5—C7—N8—C9...). We could split the conformational analysis to two parts and perform a more detailed analysis. In the first part, the most favourable position of C-terminal carboxyl was sought by varying only one dihedral angle $\phi 1$ in 1° increment (for *VI* two dihedral angles – $\phi 1$ and $\phi 2$ in 5° increment). The best rotamer was used in the second part, when five $\phi 4$, $\phi 5$, $\phi 6$, $\phi 7$, and $\phi 8$ dihedral angles were varied over the range of 360° in 15° increment (four angles $\phi 5$, $\phi 6$, $\phi 7$, and $\phi 8$ for *VI* and *VII*).

Conformational maps were constructed according to database of saved rotamers. Optimal values of dihedral angles were derived from these maps and applied to the modeled molecule. For some dihedral angles multiple minima were found and we constructed models with all possible combinations. All constructed structures were AM1-optimized, but structural data of the most stable (the lowest-energy) conformations are presented only. These structures could be the gas-phase global minima.

Optimization of Structures from Crystallographic Database

Structures of *I*—*IV* and *VII*—*XII* were retrieved from Cambridge Crystallographic Database and then adjusted for optimization – hydrogen atoms were added according to valence, neutral (total charge = 0) dicarboxy-active metabolites were prepared as starting point for AM1 optimization. The structural data of final optimized structures are summarized in Table 1.

Molecular modeling studies were carried out by means of the Chem-X program [16].

Table 1. Dihedral Angles of the Most Stable AM1-Optimized Structures of ACEIs, when Crystal Structures were Taken as a Starting Point for Optimization

Compound	Dihedral angle ϕ/°							
	ϕ1	ϕ2	ϕ3	ϕ4	ϕ5	ϕ6	ϕ7	ϕ8
I	−31.9	−69.1	−4.4	118.3	–	–	–	–
II	−34.6	−80.7	−3.2	138.5	−64.5	−81.4	−101.5	−169.4
III	−19.4	−89.2	−0.5	146.3	−59.1	−70.8	60.8	50.3
IV	−16.4	−83.7	−2.4	142.5	−59.4	−71.5	61.0	50.2
VII	−14.4	−80.3	−15.8	169.6	−69.0	−82.8	69.4	−63.7
VIII	−8.3	−100.2	−5.0	157.1	−71.1	−89.2	−111.5	−81.1
IX	−27.4	−66.7	−8.6	140.6	−77.2	−94.2	−66.5	−78.2
X	−21.3	−88.6	12.2	159.6	−60.1	−71.3	62.6	49.7
XI	−45.1	−77.2	28.5	122.9	−77.8	−114.1	−47.8	64.9
XII	4.5	−87.2	−2.7	145.5	−65.5	−76.9	55.1	51.7

Table 2. Dihedral Angles of the Most Stable AM1-Optimized Structures of ACEIs, when Low-Energy Conformations were Taken as a Starting Point for Optimization

Compound	Dihedral angle ϕ/°							
	ϕ1	ϕ2	ϕ3	ϕ4	ϕ5	ϕ6	ϕ7	ϕ8
I	−31.7	−67.1	−1.6	130.7*	−62.1*	–	–	–
II	−34.0	−81.3	−2.9	140.3	−61.2	−72.7	71.1	−68.2
III	−19.2	−82.7	−0.4	146.2	−59.9	−71.1	70.2	−67.0
IV	−16.3	−84.2	−2.4	142.8	−60.0	−72.0	70.5	−65.8
V	−31.9	−96.5	13.4	148.9	−58.9	−71.9	72.8	−65.4
VI	10.7	−85.7	−8.8	155.7	−72.3	−90.1	70.9	−80.9
VII	−15.1	−80.4	−15.5	169.5	−69.3	−82.6	69.0	−62.8
Mean value	**−19.6**	**−82.6**	**−2.6**	**150.6**	**−63.6**	**−76.7**	**70.8**	**−68.4**
$\Delta_{\text{max-min}}$	**44.7**	**29.4**	**28.9**	**29.2**	**13.4**	**19.0**	**3.8**	**18.1**

*Different binding mode, not included in mean value calculation.

RESULTS AND DISCUSSION

The values of dihedral angles of most favourable conformations (AM1-optimized), when rotamers from conformational search were taken as a starting point, are summarized in Table 2. The mean values of searched dihedral angles, the difference of the highest and the lowest measured value ($\Delta_{\text{max-min}}$), and distances between pharmacophoric groups are calculated.

Dihedral angle ϕ1 – the values are similar for almost all optimized structures.

The C-terminal carboxyl of *VI* is not sterically restricted and that is why its minimum is slightly different from other ACEIs. It can rotate almost freely around C2—C3 and adjust its position for the best interaction. On the other hand, the saturated ring systems condensed to proline subunit (*II*, *IV*—*VII*) can restrict the free rotation of neighbouring carboxyl group. As the charge—charge (—$COO^- \cdots {}^+NH_3$—) interactions have long range and do not require specific spatial arrangement of interacting groups, we think that the precise position of C-terminal carboxyl of ACEIs is not essential for tight binding, *i.e.* it can vary in reasonable range. Values observed after optimization of crystal structures show similarity as well.

Dihedral angle ϕ2 – the values are very similar as the four atoms are fixed in the proline ring system. *VI* is the exception to this rule, but in its conformational minimum it has the dihedral angle very similar to other ACEIs. No substantial differences are found between fused-ring ACEIs and other ACEIs. Small differences are found for optimized crystal structures.

Dihedral angle ϕ3 – the amide bond – is very similar for all molecules. It is set to *trans* arrangement and after optimization it remains near the ideal value (the mean value −2.6°). For optimized crystal structures no exception to *trans* arrangement is found.

Dihedral angles ϕ4, ϕ5, and ϕ6 – only one energetically favourable arrangement of these three consecutive dihedral angles was found in conformational search. In this part of molecule there is no substantial difference between semi-rigid fused-ring and non-restricted ACEIs. The same is true about the optimized crystal structures.

Dihedral angle ϕ7 – it describes the position of Zn-coordinating carboxyl group. Two minima are found

Table 3. Distances between Pharmacophoric Functional Groups of ACEIs, when Low-Energy Conformations were Taken as a Starting Point for Optimization

Compound	Distances between pharmacophoric groups/nm					
	$COOH_{C\text{-term}}$ —CO	$COOH_{C\text{-term}}$ —$COOH_{Zn\ lig.}$	$COOH_{Zn\ lig.}$ —CO	$COOH_{C\text{-term}}$ —Phenyl	CO —Phenyl	$COOH_{Zn\ lig.}$ —Phenyl
I	0.3120	–	–	–	–	–
II	0.3335	0.6833	0.4496	–	–	–
III	0.3349	0.6824	0.4388	0.9802	0.6605	0.6137
IV	0.3379	0.6825	0.4425	0.9800	0.6587	0.6148
V	0.3397	0.6805	0.4344	0.9647	0.6525	0.6144
VI	0.3349	0.7305	0.4424	0.8918	0.5886	0.6144
VII	0.3423	0.7458	0.4673	0.9042	0.5860	0.6181
Mean value	**0.3336**	**0.7008**	**0.4458**	**0.9442**	**0.6293**	**0.6151**
$\Delta_{max\text{-}min}$	**0.0303**	**0.0653**	**0.0329**	**0.0884**	**0.0745**	**0.0044**

in conformational search −57° and 136°. The difference between minima is ∼180° as the carboxylic group is almost symmetric, but in the form present in active site this functional group is probably negatively charged and completely symmetric. The conformational map shows very loose minima, which means that the carboxyl group can alter its position in a large range with very small energy changes. Here again, the charge—charge interaction is present (—$COO^- \cdots Zn^{2+}$—). The precise location of zinc atom is very hard to predict, as either or even both carboxyl oxygen atoms can coordinate the zinc atom (tetra- or pentacoordination). Further investigation of crystallographic data and/or precise *ab initio* calculations are needed.

Dihedral angle $\phi 8$ – in conformational search three minima are found for every ACEI: 58°, −75°, and −173°. After AM1 optimization, all lowest-energy conformers have the value of $\phi 8$ around −68°. Similar conformations are those of inhibitors found in active site of enzymes similar to ACE – thermolysin and carboxypeptidase A. On the other hand, the dihedral angle of the Phe residue in natural substrate angiotensin I is ∼180°. AM1-optimized crystal structures show very high conformational variability in this hydrophobic fragment. Only *VII*, *VIII*, and *IX* show higher similarity in $\phi 8$ with global minima found in conformational search. This gives us no clear picture about the active conformation of ACEIs. As this hydrophobic part of ACEIs is not conformationally restricted, the barrier to rotation of $\phi 8$ is low. We suppose that the ACEIs should adjust the shape of this part of molecule to one common conformation when interacting with the S_1 hydrophobic pocket of the active site. The solution, how to decide which position of the hydrophobic fragment is correct, could be the rational design and SAR study of rigid analogues, which could position the hydrophobic fragment exactly to the predicted region and effectively occupy the S_1 pocket.

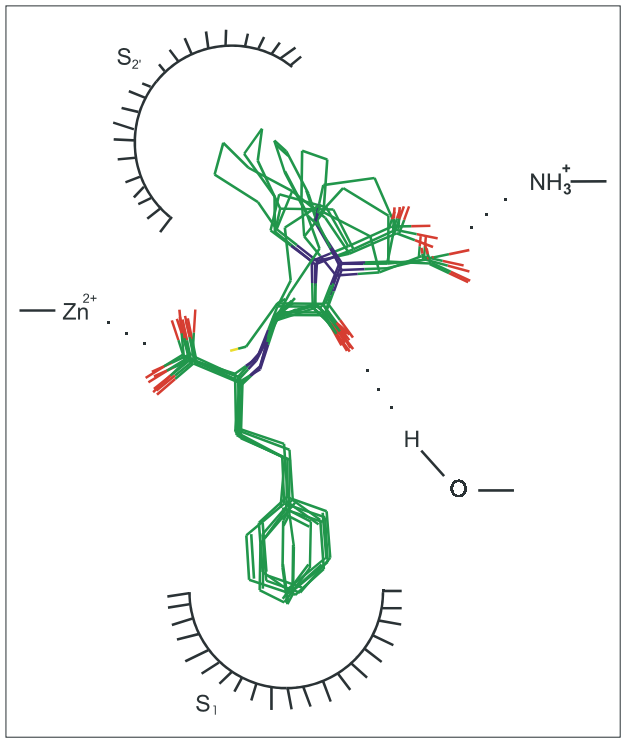


Fig. 1. Overlay of the lowest-energy (AM1-optimized) rotamers.

CONCLUSION

In this study we were able to identify the lowest-energy conformation (gas-phase global minimum) for seven selected ACEIs. Spatial orientation of main pharmacophoric groups is almost identical for all these structures (Fig. 1) and the four-centre pharmacophore could be derived (Fig. 2, Table 3). When relaxing the geometry of crystal structure of selected ACEIs by the AM1 method we found that some relaxed structures are very similar to global minima found in conformational analysis (Fig. 3). The other structures showed

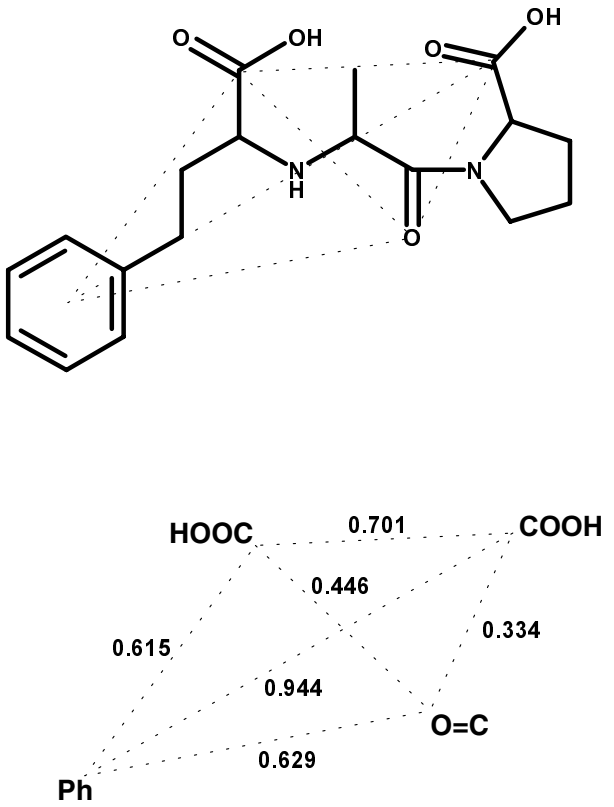


Fig. 2. Distances in nm between pharmacophoric functional groups.

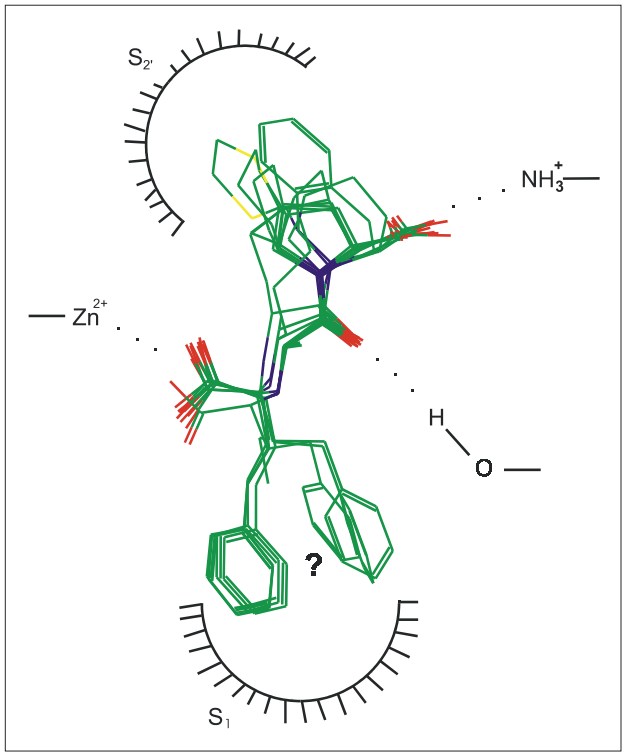


Fig. 3. The X-ray geometry of ACEIs found in CSD was relaxed by AM1 optimization. Terminal carboxyl, amide carboxyl, and coordinating carboxyl are overlayed.

high conformational similarity, only dihedral angle $\phi 8$ has completely different values, which correspond to other local minima (these local minima were identified in conformational search, too). The precise position of hydrophobic chain for the most favourable interaction remains unknown (3 possible arrangements). The design and SAR study of conformationally restricted compounds according to pharmacophore published is needed.

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