1,2-Cyclomethylenecarboxylic Monoamide Hydroxamic Derivatives. A Novel Class of Non-Amino Acid Angiotensin Converting Enzyme Inhibitors

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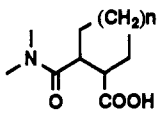
A series of monoamidic derivatives of *cis*- and *trans*-1,2-cyclohexanedicarboxylic and 1,2-cyclopentanedicarboxylic acids bearing either a carboxylic, sulfhydrylic, or hydroxamic group in the side chain were synthesized and evaluated in vitro for their inhibitory activity against angiotensin converting enzyme. The compounds were designed as potential ACE inhibitors of novel structure, assuming that a monoamidic residue of an 1,2-cyclomethylenedicarboxylic acid could be an alternative structure to the acylproline moiety, the carboxyl-terminal portion common to various ACE inhibitors. The most active compounds were found in the hydroxamic derivatives of cyclohexane series; within this series of derivatives a marked increase of potency was caused by alkylation of the amidic nitrogen with a methyl or ethyl group. Therefore enantiomers of the selected hydroxamic derivatives of *cis*- and *trans*-1,2-cyclohexanedicarboxylic acid were prepared by two different chiral synthetic routes and evaluated in vitro for their ACE inhibitor potencies. The active enantiomers both of the cis series (**21a**, **21c**) and trans series (**16b**, **16d**) were found to have all *R* configuration at the C-2 and *R* or *S* configuration at the C-1, while in the classical ACE inhibitors *S* configuration at the terminal carboxylate (corresponding to the C-1 of our compounds) is strictly required for activity. The most potent compound of the series was (1*S*,2*R*)-*cis*-2[[[2-(hydroxyamino)-2-oxoethyl]methylamino]carbonyl]cyclohexanecarboxylic acid (**21a**) with an IC_{50} value of 7.0 nM compared with the value of 3.0 nM for captopril. Further **21a** was shown to be highly selective and competitive ACE inhibitor. These results indicate that this non-amino acid structure of inhibitors meets the ACE active site requirements for the binding. The binding compatibility of the most active compounds with a model of ACE active site was evaluated by molecular modeling techniques.

The clinical success of angiotensin converting enzyme (ACE) inhibitors in hypertension and in congestive heart failure is well established.[1-3] Several potential new clinical applications of these agents have been under investigation in the last years,[4] and there is convincing evidence coming out to support novel indications for ACE inhibitors such as diabetic nephropathy,[5,6] myocardial infarction,[7,8] cardiac hypertrophy,[9] vascular proliferation,[10] and cognitive disorders.[11] Thus the search for ACE inhibitors of novel structure and biological profile is still an appealing objective.

The basic structure of ACE inhibitors is a dipeptide or acylamino acid derivative bound to a Zn-ligand functional group (sulfhydryl, carboxyl, or phosphoryl). These groups, according to a frequently used classification, chemically mark out the three corresponding classes of ACE inhibitors, while an acylamino acid moiety in the carboxyl terminal portion[12] is a feature common to all ACE inhibitors of each class.

In this research we made the hypothesis that a monoamidic residue of an 1,2-cyclomethylenedicarboxylic acid (1) could be an alternative structure to the acylproline moiety 2 which is the carboxyl-terminal portion of various ACE inhibitors. The structure 1 seems indeed able both to mimick the distance between C=O and COOH groups[13]



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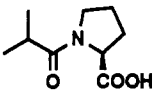

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and produce the same rigidity[16] of the system **2**, which are two important geometrical parameters for the inhibition of ACE.

Several chemically novel potential ACE inhibitors were designed starting from the original moiety **1**, as shown in the Chart I. A cyclomethylene-2-carboxylic acid residue instead of an amino acid residue is the common feature of this new structure of ACE inhibitors. Sulfhydrylic, carboxylic, and hydroxamic groups were planned to be tested as Zn-ligand functional groups in this structure. The general formula **3** further includes chemical modifications of substituents on the amidic nitrogen, and cis or trans isomers of the cyclomethylene ring. We report here the synthesis and in vitro ACE inhibitory activity of a series of compounds of general formula **3**.

Chemistry

The carboxylic, sulfhydrylic, and hydroxamic compounds listed in Table I, II, and III were prepared by the general synthetic method outlined in Scheme I. Condensation of the Zn-ligand amino derivatives **4** with commercially available or synthetically obtainable 1,2-

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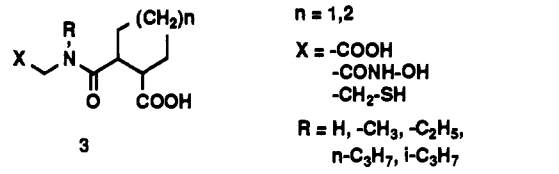
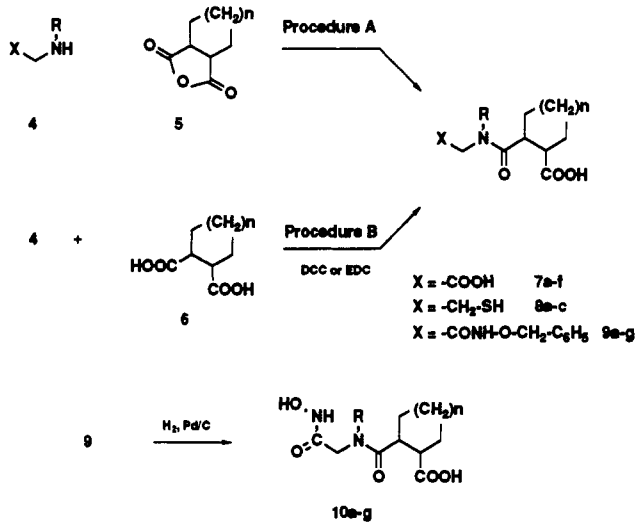
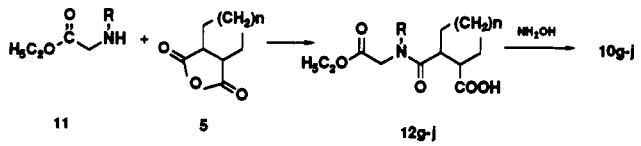
Chart I



Scheme I



Scheme II

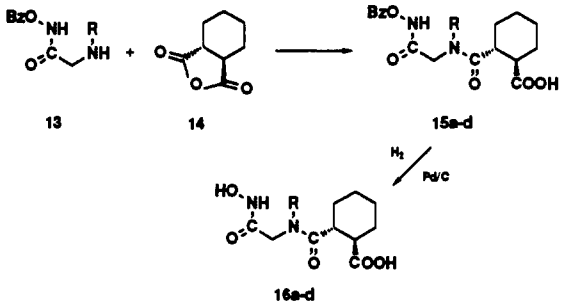


cyclomethylenedicarboxylic anhydrides **5** (procedure A) or with the corresponding acids **6** in presence of DCC or EDC (procedure B: not applicable when X in **4** is COOH) gave directly the final carboxylic derivatives **7** and sulfhydrylic derivatives **8**, or the precursors of the hydroxamic derivatives **9**. Hydrogenolysis of **9** to remove the benzyl protecting group afforded the desired hydroxamic derivatives **10**. Alternatively in some cases hydroxamic derivatives **10** were obtained by condensation of amino acid ethyl esters **11** with the same anhydrides **5** followed by treatment of the intermediate **12** with hydroxylamine, as depicted in Scheme II.

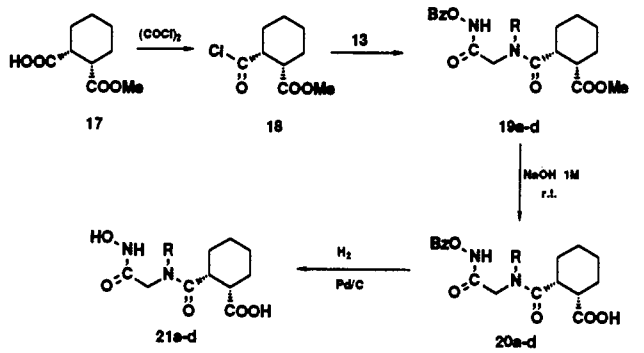
Cis and trans isomers of the products **7**, **8**, **9**, and **10** were obtained through synthetic routes I and II, from the corresponding cis and trans compounds (**5** or **6**). Further, starting from achiral amino derivatives **4** synthetic routes of Schemes I and II led to final racemic compounds **7**, **8**, and **10**.

The (+)- and (−)-enantiomers of selected trans- and cis-substituted cyclohexane hydroxamic acid compounds were prepared by the chiral synthesis outlined in Schemes III and IV. Condensation of 2-(alkylamino)-*N*-(benzyloxy)-acetamide **13** with (1*R*,2*R*)-*trans*-1,2-cyclohexanedicarboxylic anhydride (**14**), prepared[20] from corresponding commercially available acid, followed by catalytic hydrogenolysis of the precursors **15** gave the (1*R*,2*R*)-trans enantiomers represented by formula **16**, as depicted in Scheme III. Analogously (1*S*,2*S*)-*trans*-1,2-cyclohexanedicarboxylic anhydride gave the final (1*S*,2*S*)-trans enantiomers. The intermediates **15** assessed for enantiomeric purity by chiral HPLC showed ee > 99%.

Scheme III



Scheme IV

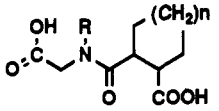


(1*R*,2*S*)-*cis*-2-(methoxycarbonyl)cyclohexanecarboxylic acid (**17**), prepared from the *meso*-dimethyl ester according to a previously described enzyme-catalyzed hydrolysis procedure,[23] was converted into the corresponding acid chloride **18** with oxalyl chloride. Condensation of **18** with **13** afforded the amido derivatives **19** which were then saponified under mild conditions followed by catalytic hydrogenolysis of the precursors **20** to give the (1*S*,2*R*)-cis enantiomers, represented by formula **21**, as outlined in Scheme IV. HPLC analysis of the crude intermediates **20** showed an ee = 97% and a trace amount of contaminant trans isomers which indicated negligible racemization and epimerization during the synthesis steps of Scheme IV. Crystallization from acetone gave the pure (1*S*,2*R*)-cis enantiomers **20**, ee > 99%. (1*S*,2*R*)-cis-2-(methoxycarbonyl)cyclohexanecarboxylic acid, prepared by resolution of its racemic modification via diastereomer salts,[22] analogously afforded through synthetic route IV the (1*R*,2*S*)-cis enantiomers. Condensation of the appropriate Boc-*N*-alkylglycines with *O*-benzylhydroxylamine in presence of DCC followed by acid hydrolysis to remove the protecting group afforded the 2-(alkylamino)-*N*-(benzyloxy)acetamides **13** used as starting materials in Schemes I, III, and IV.

Results and Discussion

ACE inhibition was evaluated in vitro against rat serum ACE using hippurylglycylglycine as substrate,[23] and the potencies of the tested compounds were expressed as IC_{50} values (Tables I–III). Inhibition of rat ACE by a selected compound (**21a**) was found to be competitive: when substrate dependence of the reaction rate was tested in the presence of increasing concentrations of the inhibitor, apparent K_m increased, but V_{max} values remained virtually constant, as shown in Table IV. K_i for this compound, obtained from the expression $K_i = [I]/(K_{mI}/K_m - 1)$, where K_{mI} is the apparent K_m in the presence of the concentration [I] of inhibitor was 2.7 ± 0.2 nM. Moreover, ACE inhibitory activity of these compounds were highly se-

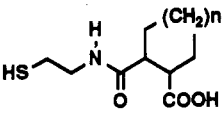
Table I. Physicochemical Properties and in Vitro ACE Inhibitory Activity of Carboxylic Derivatives



compd[a]	R	n	formula	MW	mp, °C	isomer[b]	IC_{50} (μM)
7a	H	2	$C_{10}H_{15}NO_5$	229.22	142–144	cis	>150
7b	CH_3	2	$C_{11}H_{17}NO_5$	243.25	156–159	cis	>150
7c	C_2H_5	1	$C_{11}H_{17}NO_5$	243.25	115–116	cis	>150
7d	C_2H_5	2	$C_{12}H_{19}NO_5$	257.27	110	cis	75
7e	C_2H_5	2	$C_{12}H_{19}NO_5$	257.27	146–148	trans	97
7f	n-C_3H_7	2	$C_{13}H_{21}NO_5$	271.30	103–105	cis	>150
captopril							0.003
succinyl-L-proline							624[c]
2-D-methylsuccinyl-L-proline							52[c]
captopril							0.023[c]

[a] Elemental analyses are within ±0.4 of theoretical values unless otherwise noted. [b] Unless specifically designated, all compounds are racemic mixture. [c] IC_{50} (μM) values reported by Ondetti, M. A. et al. (ref 24): the difference between the captopril (μM) value in our test and value reported by Ondetti has to be considered to compare the two series of IC_{50} values.

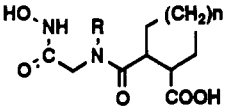
Table II. Physicochemical Properties of and Vitro ACE Inhibitory Activity of Sulfhydrylic Derivatives



compd[a]	n	formula	MW	mp, °C	isomer[b]	IC_{50} (μM)
8a	1	$C_9H_{15}NO_3S$	217.27	110–112	cis	11
8b	2	$C_{10}H_{17}NO_3S$	231.30	102–109	cis	10
8c	2	$C_{10}H_{17}NO_3S$[c]	231.30	149–152	trans	>150
cis-2-(3-mercapto-1-oxopropyl)-cyclohexanecarboxylic acid						1.4[d]
trans-2-(3-mercapto-1-oxopropyl)-cyclohexanecarboxylic acid						1.7[d]

[a,b] See Table I. [c] C: calcd 51.92, found 52.34; H: calcd 7.40, found 6.85; S: calcd 13.86, found 13.42. [d] Reference 14.

Table III. Physicochemical Properties and in Vitro ACE Inhibitory Activity of Hydroxamic Derivatives



compd[a]	R	n	formula	MW	mp, °C	isomer[b]	IC_{50} (μM)
10a	H	2	$C_{10}H_{16}N_2O_5$	244.23	133–135	cis	3.0
10b	H	2	$C_{10}H_{16}N_2O_5$	244.23	162–165	trans	1.5
10c	CH_3	1	$C_{10}H_{16}N_2O_5$	244.23	132–135	trans	1.4
10d	CH_3	2	$C_{11}H_{18}N_2O_5$	258.25	140.141	cis	0.012
10e	CH_3	2	$C_{11}H_{18}N_2O_5$	258.25	162–164	trans	0.045
10f	C_2H_5	2	$C_{12}H_{20}N_2O_5$	272.28	76–77	cis	0.057
10g	C_2H_5	2	$C_{12}H_{20}N_2O_5$	272.28	147–148	trans	0.018
10h	n-C_3H_7	2	$C_{13}H_{22}N_2O_5$	286.31	84–86	cis	0.40
10i	n-C_3H_7	2	$C_{13}H_{22}N_2O_5$	286.31	132–133	trans	0.15
10j	i-C_3H_7	2	$C_{13}H_{22}N_2O_5$	286.31	136	trans	0.50
21a	CH_3	2	$C_{11}H_{18}N_2O_5$	258.25	128–130	1*S*,2*R*-cis	0.007
21b	CH_3	2	$C_{11}H_{16}N_2O_5$	258.25	129–131	1*R*,2*S*-cis[c]	1.8
16a	CH_3	2	$C_{11}H_{18}N_2O_5$	258.25	139–141	1*S*,2*S*-trans[d]	23.5
16b	CH_3	2	$C_{11}H_{18}N_2O_5$	258.25	138–140	1*R*,2*R*-trans[e]	0.024
21c	C_2H_5	2	$C_{12}H_{20}N_2O_5$	272.28	glass	1*S*,2*R*-cis[f]	0.028
21d	C_2H_5	2	$C_{12}H_{20}N_2O_5$	272.28	glass	1*R*,2*S*-cis[g]	2.9
16c	C_2H_5	2	$C_{12}H_{20}N_2O_5$	272.28	141	1*S*,2*S*-trans[h]	2.9
16d	C_2H_5	2	$C_{12}H_{20}N_2O_5$	272.28	138–140	1*R*,2*R*-trans	0.014
captopril							0.003
1-[4-(hydroxyamino)-4-oxo-2-methyl-1-oxobutyl]-L-proline							0.6[i]

[a,b] See Table I. [c] $[\alpha]^{20}_D$ −26.1° (c = 1, EtOH). [d] $[\alpha]^{20}_D$ −21.5° (c = 1, EtOH). [e] $[\alpha]^{20}_D$ +22.5° (c = 1, EtOH). [f] $[\alpha]^{20}_D$ +25.8° (c = 2, EtOH). [g] $[\alpha]^{20}_D$ −25.3° (c = 2, EtOH). [h] $[\alpha]^{20}_D$ −10.1° (c = 2, EtOH). [i] K_i μM: reference 17.

lective. Most of the carboxylic, sulfhydrylic, and racemic hydroxamic compounds were tested for the in vitro inhibitory activity on exo- and endopeptidases, namely endopeptidase 24.11, brain enkephalin-degrading aminopeptidases, carboxypeptidase B, trypsin, and chymotrypsin: in particular, compounds **10d–g**, with ACE IC_{50} values in the nM order, caused much less than 50% inhibition of those enzymes when tested up to 0.1 mM.

The synthesis intermediates of hydroxamic compounds depicted in the Schemes I, III, and IV, namely the benzyl hydroxamate derivatives **9**, **15**, and **20** (Table V) were found to be all inactive in the in vitro assay for ACE inhibition (IC_{50} > 150 μM).

It is evident from the consistently higher potency of the hydroxamic derivatives as compared to the corresponding carboxylic and sulfhydrylic analogues, that the Zn-ligand group plays a dominant role on affinity of these new structures. The hydroxamic group brings fairly high

Table IV. Effects of Compound 21a on K_m and V_{max} for the Hydrolysis of Hyppurylglycylglycine by Rat Serum ACE (Means ± Standard Errors Are Reported)

21a (nM)	K_m (mM)	V_{max} (μmol/min per mL)
0	21 ± 1.5	1.31 ± 0.03
3	43 ± 1.2	1.25 ± 0.02
6	62 ± 1.3	1.16 ± 0.01
12	129 ± 15	1.36 ± 0.10

affinity for ACE in the structure 3 compounds, and the comparison with the analogous hydroxamic derivatives of the proline series (Table III) clearly indicates that this Zn-ligand group works better in the non-amino acid structure 3.

The carboxylic compounds 7a–f have a low affinity even if compared with the weakly active corresponding analogues of the proline series,[24] as shown in Table I. The low affinity of sulfhydrylic compounds 8a–c is possibly due to the length of the chain between the sulfhydryl and the carbonyl group, assuming that the structural requirements for affinity of captopril apply to the sulfhydrylic compounds of formula 3. Mercapto keto acids[25] described by M. Condon et al.[14] having the same interval of atoms as captopril between the sulfhydryl and carbonyl group show higher ACE inhibition than our sulfhydrylic derivatives (Table II).

Within the hydroxamic compounds series, marked effects on potency were observed among the amidic nitrogen variations. Substitution of the amidic hydrogen with methyl or ethyl groups increased the affinity up to 250 times (10d versus 10a). The similar α-methyl substitution of the acyl portion increases slightly the affinity in captopril (about 10 times)[24] and markedly in enalapril (about 200 times).[26]

The cis or trans configuration in our cyclohexane derivatives of structure 3 is not critical for the affinity. However, only one enantiomer of each pair shows marked activity. The active enantiomers both of the cis series and trans series were found to have all *R*-configuration at the C-2, while they have *S*-configuration at C-1 in the cis series (21a, 21c) and C-1 *R*-configuration in the trans series (16b, 16d). These results indicate that only *R*-configuration at the cyclohexane C-2 is a stereochemical feature required for the activity in these structure, while in the classical ACE inhibitors *S*-configuration at the C-α to the carboxylate of the terminal amino acid (corresponding to the C-1 in our compounds) is strictly required for activity.[27]

Molecular Modeling

In order to investigate the 3D relationship of these new structures with the classic ACE inhibitors, molecular modeling techniques were used to provide a preliminary evaluation of the binding compatibility of our most active hydroxamic compounds with the Mayer–Marshall[31] active site of ACE. The (1*S*,2*R*)-cis enantiomer 21a and (1*R*,2*R*)-trans enantiomer 16b were constructed from the SYBYL[32] fragment database and each was superimposed with MULTIFIT in SYBYL using the TRIPOS force field. MULTIFIT performs a flexible fit of pair-wise atoms between molecules while optimizing their geometries. The zinc interaction was represented as a dummy atom bonded to the hydroxamic carbonyl in the geometry defined for carboxylate carbonyls by Mayer et al.[31] The molecules were fit so that the important pharmacophoric groups (COOH, amide-N, CO, and Zn_{dummy}) were paired with the corresponding groups of captopril. (Note that the amide-N of the cyclomethylene inhibitors corresponds to the Cα of the Ala residue in captopril.) These fits resulted in energetically accessible conformations[33] having a root mean square (rms) distance between pairwise atoms of 0.2 Å.

While the three most important pharmacophoric groups (COOH, CO, and CONHOH) of both 21a (Figure 1) and 16b (Figure 2) are oriented in a manner similar to the corresponding groups of captopril, the superimpositions indicate that the methyl substituent of the amidic nitrogens and the cyclohexane rings of 21a and 16b show a spatial arrangement that is clearly different from the analogous moieties of captopril. Notably, the cyclohexane ring is in a conformation that is approximately perpendicular to the plane of the proline ring, which represents space not occupied by the acyl amino acid or dipeptide ACE inhibitors. This conformation is probably reflective of the "local minima" problem and should be further explored with a systematic search of the cyclohexane rings. Although structure–activity data indicate a strong preference for the *S*-configuration at the Cα to the C-terminal carboxylate,[27] the (1*R*,2*R*)-trans enantiomer 16b matches the geometry in this region. These preliminary results

Table V. Physicochemical Properties of Benzyl Hydroxamate and Ethyl Carboxylate Intermediates[c]

compd[a]	R	*n*	formula	MW	isomer[b]	mp, °C
9a	H	2	$C_{17}H_{22}N_2O_5$	334.35	cis	141–143
9b	H	2	$C_{17}H_{22}N_2O_5$	334.35	trans	140–143
9c	CH_3	1	$C_{17}H_{22}N_2O_5$	334.35	trans	114–118
9d	CH_3	2	$C_{18}H_{24}N_2O_5$	348.37	cis	86–90
9e	CH_3	2	$C_{18}H_{24}N_2O_5$	348.37	trans	149–152
9f	C_2H_5	2	$C_{19}H_{26}N_2O_5$	362.40	cis	146–147
9g	C_2H_5	2	$C_{19}H_{26}N_2O_5$	362.40	trans	135–136
20a	CH_3	2	$C_{18}H_{24}N_2O_5$	348.37	1*S*,2*R*-cis	120–125
20b	CH_3	2	$C_{18}H_{24}N_2O_5$	348.37	1*R*,2*S*-cis	115–120
15a	CH_3	2	$C_{18}H_{24}N_2O_5$	348.37	1*S*,2*S*-trans	130–131
15b	CH_3	2	$C_{18}H_{24}N_2O_5$	348.37	1*R*,2*R*-trans	130–131
20c	C_2H_5	2	$C_{19}H_{26}N_2O_5$	362.40	1*S*,2*R*-cis	96–98
20d	C_2H_5	2	$C_{19}H_{26}N_2O_5$	362.40	1*R*,2*S*-cis	glass
15c	C_2H_5	2	$C_{19}H_{26}N_2O_5$	362.40	1*S*,2*R*-trans	glass
15d	C_2H_5	2	$C_{19}H_{26}N_2O_5$	362.40	1*R*,2*R*-trans	98
19a[d]	CH_3	2	$C_{19}H_{26}N_2O_5$	362.40	1*S*,2*R*-cis	85
12g	C_2H_5	2	$C_{14}H_{23}NO_5$	285.32	trans	78–79
12h	*n*-C_3H_7	2	$C_{15}H_{25}NO_5$	299.34	cis	79–80
12i	*n*-C_3H_7	2	$C_{15}H_{25}NO_5$	299.34	trans	94–96
12j	*i*-C_3H_7	2	$C_{15}H_{25}NO_5$	299.34	trans	130–132

[a,b] See Table I. [c] All these compounds were found to be inactive in the in vitro ACE inhibition test. [d] The analogues 19b–d were obtained as oily products and directly utilized in the next step without further purification.

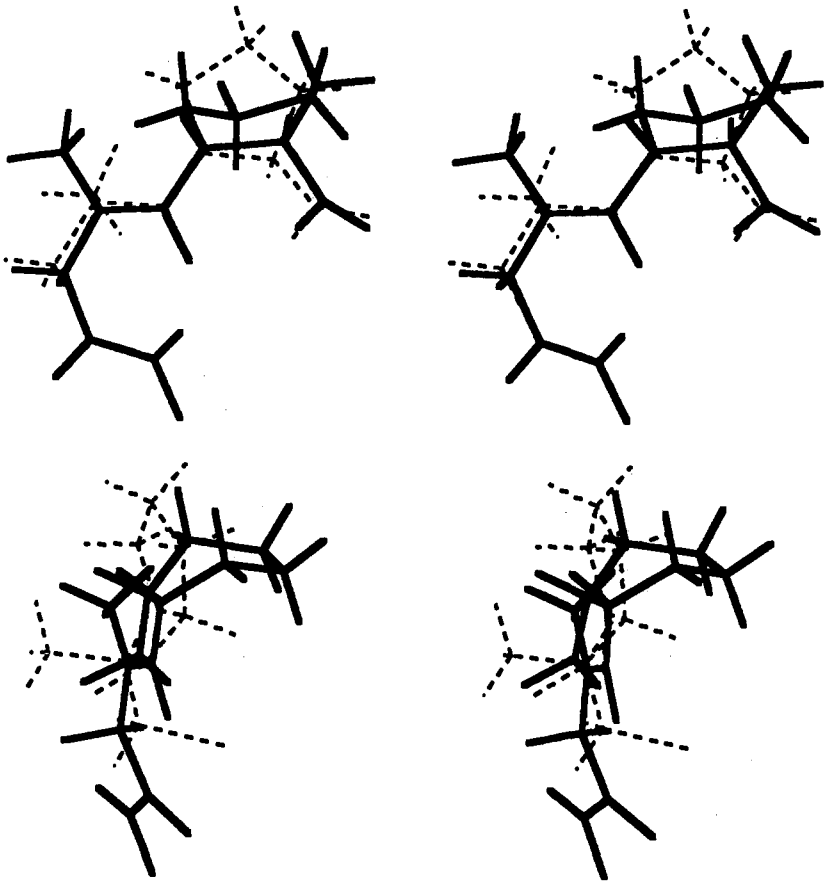


Figure 1. Orthogonal stereoviews of the (1*S*,2*R*)-*cis*-2-[[[2-(hydroxyamino)-2-oxoethyl]methylamino]carbonyl]cyclohexanecarboxylic acid (**21a**) (solid lines) superimposed on captopril (dashed lines).

indicate that the three pharmacophoric groups of both the (1*S*,2*R*)-cis enantiomer **21a** and (1*R*,2*R*)-trans enantiomer **16b** can match the geometry of the Mayer active site of ACE. The compatibility of the aliphatic portions of our structures in the hydrophobic pockets of the hypothetical ACE active site model[34] deserves investigation of a wider set of compounds. Such investigations should result in improvements in the topography of the ACE active site.

Conclusion

Selected compounds of this novel class of non-amino acid angiotensin converting enzyme inhibitors are highly potent, competitive, and selective inhibitors of ACE. The combination of three molecular characteristics (an unusual Zn-ligand group, a shifted N-alkylated amide function, and a 1,2-cyclohexanedicarboxylic acid moiety) leads to a non-amino acid structure which meets the ACE active site requirements for the binding as effectively as the amino acid structures of classical ACE inhibitors. The pharmacotoxicological study of the selected (1*S*,2*R*)-*cis*-[[[2-(hydroxyamino)-2-oxoethyl]methylamino]carbonyl]-cyclohexanecarboxylic acid (**21a**) showed that this compound is a potent ACE inhibitor in vitro and in vivo in different animal species and experimental models[35] and is characterized by an extremely low toxicity. Clinical trials are in progress.

Experimental Section

Except where otherwise stated, the following procedures were adopted. Melting points were determined on a Reichert Thermovar hot stage apparatus and are uncorrected. Elemental analyses were performed by Microan.Lab. of Istituto Chimica Farmaceutica Università di Pisa. Analytical results are indicated by the elements' symbols and are within ±0.4% of theoretical values. IR spectra were recorded on a FT-IR Bruker IFS25. NMR spectra were obtained by using a Bruker AC-200E NMR spectrometer. All chemical shifts were reported in ppm from TMS. Mass spectra were obtained by using a Hewlett-Packard MS 5988 spectrometer with thermospray ionization and are reported in atomic mass units. NMR and mass spectral data are reported also in supplementary material. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Analytical thin-layer-chromatography (TLC) was carried out on Merck precoated silica gel 60F-254. Flash chromatography was performed using 230–400 mesh Kieselgel 60 (E.Merck). Analytical HPLC was performed on a Waters 600E, by using a Nucleosil 5 C18 Column (250 × 4.6 mm) at 0.8-mL/min flow rate, 214-nm detection, and CH_3CN/H_3PO_4 0.1% as mobile phase. The enantiomeric excess (ee) was determined by chiral HPLC methods using Ciclobond I column and MeOH/$AcONH_4$ 0.02 M, pH 4.6 (90:10) at 1.0 mL/min flow rate. MeOH was distilled to eliminate iron traces.

***cis*-2-[[[2-Hydroxy-2-oxoethyl]methylamino]carbonyl]-cyclohexanecarboxylic Acid (7b)** (Scheme I, procedure A). An amount of 5.0 g (32.5 mmol) of *cis*-1,2-cyclohexanedicarboxylic anhydride and 8.0 mL of 4 N NaOH were added portionwise to a stirred solution of 2.9 g (32.5 mmol) of sarcosine in 33.0 mL of 1 N NaOH at a rate of addition to maintain the reaction mixture at pH 10 and at a temperature of 5 °C. Stirring was continued at room temperature for 2 h. The mixture cooled at 5 °C was acidified at pH 1 with 10% HCl. The solid that separated was isolated by filtration and washed thoroughly with cold water to afford 7.0 g of title compound, mp 142–156 °C. Recrystallization from EtOH afforded 3.2 g (40.5%) of **7b** as white crystals: mp 156–9 °C; ^{1}H NMR (DMSO-d_6 + $CDCl_3$) δ 1.10–2.30 (m, 8 H, CH_2 cyclohexane); 2.40 (m, 1 H, C*H*(CO) cyclohexane); 2.76, 3.04 (2 s, 3 H, NCH_3); 3.29 (m, 1 H, C*H*COOH cyclohexane); 3.62–4.29 (2 dd, 2 H, $N(CH_3)CH_2CO$); 10.90 (br s, COO*H*) (doubling of

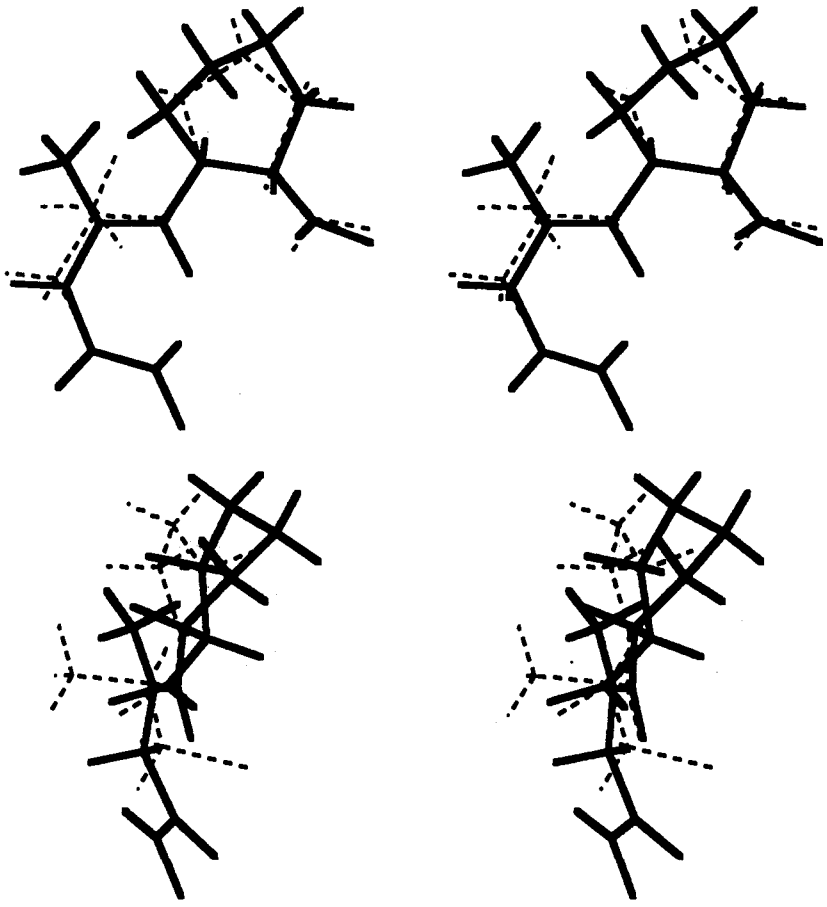


Figure 2. Orthogonal stereoviews of the (1*R*,2*R*)-*trans*-2-[[[2-(hydroxyamino)-2-oxoethyl]methylamino]carbonyl]cyclohexanecarboxylic acid (**16b**) (solid lines) superimposed on captopril (dashed lines).

resonances is due to *Z*- and *E*-amide conformers); MS *m/e* 244 ($M^+ + 1$). Anal. ($C_{11}H_{17}NO_5$) C, H, N.

***cis*-2-[[(2-Mercaptoethyl)amino]carbonyl]cyclopentanecarboxylic Acid (8a) (Scheme I, procedure A).** An amount of 2.0 g (14.3 mmol) of *cis*-1,2-cyclopentanedicarboxylic anhydride prepared according to Hawarth[36] and 7.3 mL of 2 N NaOH were added portionwise at 20 °C to a stirred solution of 1.8 g (15.6 mmol) of 2-aminoethanethiol hydrochloride in 7.3 mL of 2 N NaOH at a rate of addition to maintain the reaction mixture at pH 8. Stirring was continued at room temperature for 2 h. The mixture cooled at 5 °C was acidified at pH 1 with 10% HCl, and the precipitate was filtered to afford 1.6 g of a white solid. Recrystallization from ethyl acetate–hexane (2:1) gave 1.2 g (38.6%) of the title compound as white crystals: mp 110–112 °C; ^{1}H NMR (DMSO-d_6) δ 1.10–2.05 (m, 6 H, CH_2 cyclopentane); 2.35 (m, 2 H, CH_2SH); 2.70 (m, 1 H, *CH*CO cyclopentane); 2.85–3.45 (m, 3 H, $NHCH_2$ + *CH*COOH cyclopentane); 7.72 (t, N*H*); 11.55 (br s, COO*H*); MS *m/e* 218 ($M^+ + 1$). Anal. ($C_9H_{15}NO_3S$) C, H, N, S.

***cis*-2-[[[2-(Benzyloxyamino)-2-oxoethyl]amino]carbonyl]cyclohexanecarboxylic Acid (9a) (Scheme I, procedure A).** To a solution of 3.6 g (12.1 mmol) of 2-amino-*N*-(benzyloxy)-acetamide trifluoroacetate in H_2O (90 mL) was added 3 mL of 4 N NaOH. The resulting suspension was added under stirring at room temperature with 1.9 g (12.1 mmol) of *cis*-1,2-cyclohexanedicarboxylic anhydride (**5**) and then 3.0 mL of 4 N NaOH was dropped at a rate of addition to maintain the reaction mixture at pH 10. The reaction mixture was stirred at room temperature for additional 2 h. Following filtration, the solution was cooled at 0 °C and acidified at pH 1 with 10% HCl to give 2.7 g (66%) of the title compound in form of ivory crystals: mp 141–143 °C; ^{1}H NMR (DMSO-d_6) δ 1.10–2.05 (m, 8 H, CH_2 cyclohexane); 2.45 (m, 1 H, *CH*CO cyclohexane); 3.05 (m, 1 H, *CH*COOH cyclohexane); 3.50–4.00 (m, 2 H, $NHCH_2CO$); 4.75 (s, 2 H, $OCH_2C_6H_5$); 7.25 (m, 5 H, $OCH_2C_6H_5$); 7.75 (t, N*H*); 10.05 (s, N*H*OCH_2); 10.60 (br s, COO*H*); MS *m/e* 335 ($M^+ + 1$). Anal. ($C_{17}H_{22}N_2O_5$) C, H, N.

***trans*-2-[[[2-(Benzyloxyamino)-2-oxoethyl]methylamino]carbonyl]cyclopentanecarboxylic Acid (9c) (Scheme I, procedure B).** To a solution of 2.8 g (8.8 mmol) of 2-(methylamino)-*N*-(benzyloxy)acetamide in H_2O/*n*-BuOH (60 mL) were added 1.2 g (8.8 mmol) of K_2CO_3, a solution of 1.4 g (8.8 mmol) of *trans*-1,2-cyclopentanedicarboxylic acid in H_2O/Bu^{t}OH (60 mL), and then 1.6 g (8.8 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride. After stirring for 48 h, the solution was extracted with $CHCl_3$ (100 mL). The organic layer was extracted with 5% $NaHCO_3$ (60 mL) and the aqueous solution was washed with $CHCl_3$ (2 × 40 mL), acidified (pH 2) with 5% HCl, and immediately extracted with EtOAc (3 × 60 mL). The organic extracts were dried ($MgSO_4$) and concentrated in vacuo to give an oily residue. Crystallization from EtOH/Et_2O gave 0.7 g (23%) of the title compound as a white solid: mp 114–118 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.40–2.25 (m, 6 H, CH_2 cyclopentane); 2.45–3.10 (m, 2 H, C*H* cyclopentane); 3.15 (s, 3 H, NCH_3); 4.10–4.90 (m, 2 H, $N(CH_3)CH_2CO$); 4.65 (s, 2 H, $OCH_2C_6H_5$); 7.40 (m, 5 H, $OCH_2C_6H_5$); 10.50 (s, N*H*), 11.50 (br s, COO*H*). Anal. ($C_{17}H_{22}N_2O_5$) C, H, N.

***cis*-2-[[[2-(Hydroxyamino)-2-oxoethyl]amino]carbonyl]cyclohexanecarboxylic Acid (10a) (Scheme I).** An amount of 2.1 g (6.3 mmol) of intermediate **9a** dissolved in EtOH (135 mL) was hydrogenated over 10% Pd/C at room temperature and atmospheric pressure. The calculated amount of H_2 (150 mL) was absorbed in about 4 h. After filtration of the catalyst, the solution was evaporated to dryness and the residue (consisting of hygroscopic crystals) was crystallized from acetone to give 0.8 g (53%) of the title compound, as a white solid: mp 133–135 °C; ^{1}H NMR (DMSO-d_6) δ 1.20–2.35 (m, 8 H, CH_2 cyclohexane); 2.52 (m, 1 H, *CH*CO cyclohexane); 2.90 (m, 1 H, *CH*COOH cyclohexane); 3.80–4.10 (m, 2 H, $NHCH_2CO$); 7.70 (t, N*H*); 9.10 (br s, O*H*); 9.95 (s, N*H*OH); 11.50 (br s, COO*H*). Anal. ($C_{10}H_{16}N_2O_5$) C, H, N.

***trans*-2-[[[2-(Hydroxyamino)-2-oxoethyl]methylamino]carbonyl]cyclopentanecarboxylic Acid (10c) (Scheme I).** A solution of 0.6 g (1.9 mmol) of **9c** in MeOH (20 mL) was hydrogenated over 10% Pd/C (0.1 g) at room temperature and

atmospheric pressure. After the reaction was complete, the catalyst was removed by filtration and the solvent was evaporated in vacuo to give an oily residue. The residue was taken up with petroleum ether to afford 0.4 g of the title compound (88%) as a white solid: mp 132–135 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.30–2.20 (m, 6 H, CH_2 cyclopentane); 2.50–3.00 (m, 2 H, CH cyclopentane); 3.10 (s, 3 H, NCH_3); 3.90–4.20 (m, 2 H, $N(CH_3)CH_2$CO); 8.60 (br s, OH); 9.75, 10.40 (2 s, NH); 12.10 (br s, COOH); MS *m/e* 245 (M^+ + 1), 212 (base peak, M^+ – NHOH). Anal. ($C_{10}H_{16}N_2O_5$) C, H, N.

***trans*-2-[[[2-(Hydroxyamino)-2-oxoethyl]ethylamino]carbonyl]cyclohexanecarboxylic Acid (10g) (Scheme II).** A solution of 6 g (21.0 mmol) of the intermediate **12g** in MeOH (30 mL) was added under stirring at 5 °C with 2.8 g (70.0 mmol) of NaOH in MeOH (30 mL) and then with 1.6 g (23.1 mmol) of hydroxylamine hydrochloride. The resulting suspension was allowed to react under vigorous stirring at 15 °C for 4 h, and then the reaction mixture was evaporated to dryness under vacuum at room temperature to give 9.8 g of colorless residue. This residue was dissolved under stirring in H_2O (10 mL), and the solution was acidified up to pH 2 with 6 N HCl, saturated with NaCl, and extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated to dryness under vacuum to give 5.6 g of colorless crystalline residue. Crystallization from acetone (400 mL) gave 4.6 g (80%) of the title compound (**10g**) as colorless crystals: mp 147–148 °C; ^{1}H NMR (DMSO-d_6) δ 0.92, 1.15 (2 t, 3 H, $N(CH_2CH_3)$); 1.20–2.00 (m, 8 H, CH_2 cyclohexane); 2.50 (m, 1 H, CHCO cyclohexane); 2.68 (m, 1 H, CHCOOH cyclohexane); 3.04, 3.46 (2 m, 2 H, $N(CH_2CH_3)$; 3.46–4.25 (2 dd, 2 H, $N(CH_2CH_3)CH_2$CO); 8.80, 8.96 (2 br s, OH); 10.18, 10.64 (2 s, NH); 12.08 (br s, COOH) (doubling of resonances is due to *Z*- and *E*-amide conformers); MS *m/e* 273 (M^+ + 1), 229 (base peak, MH^+ – CO_2). Anal. ($C_{12}H_{20}N_2O_5$) C, H, N.

***trans*-2-[[[2-Ethoxy-2-oxoethyl]ethylamino]carbonyl]cyclohexanecarboxylic Acid (12g) (Scheme II).** A solution of 6.0 g (45.7 mmol) of ethyl (ethylamino)acetate and 6.0 mL (46.0 mmol) of NEt_3 in CH_2Cl_2 (130 mL) was added under stirring at 5 °C with 7.0 g (45.7 mmol) of *trans*-hexahydro-1,3-isobenzofurandione and the resulting solution was allowed to stand at room temperature for 20 h. The solution was washed with 5% HCl (40 mL) and twice with brine (40 mL), dried (Na_2SO_4), and evaporated to dryness under vacuum to give 13.0 g (99%) of the title compound as a white solid: mp 78–9 °C; ^{1}H NMR ($CDCl_3$) δ 0.96, 1.16 (2 t, 3 H, $N(CH_2CH_3)$; 1.25 (t, 3 H, $COOCH_2CH_3$); 1.20–2.05 (m, 8 H, CH_2 cyclohexane); 2.60 (m, 1 H, CHCO cyclohexane); 2.85 (m, 1 H, CHCOOH cyclohexane); 3.06–3.36 (2 m, 2 H, $N(CH_2CH_3)$; 4.10–4.50 (m, 4 H, $N(CH_2CH_3)CH_2$CO + $COOCH_2CH_3$); 9.95 (br s, COOH) (doubling of resonances is due to *Z*- and *E*-amide conformers). Anal. ($C_{14}H_{23}NO_5$) C, H, N.

2-(Methylamino)-*N*-(benzyloxy)acetamide Hydrochloride (13). To a stirred solution of 2.2 g (11.9 mmol) of BOC-sarcosine in $CHCl_3$ (10 mL) was added a solution of 1.9 g (11.9 mmol) of *O*-benzylhydroxylamine hydrochloride and 1.7 g (11.9 mmol) of triethylamine in $CHCl_3$ (15 mL). The suspension that formed was added dropwise (0.5 h) at 0 °C with a solution of 2.7 g (13 mmol) of 1,3-dicyclohexylcarbodiimide in $CHCl_3$ (10 mL), and the reaction mixture was allowed to stand at room temperature overnight. Following filtration, $CHCl_3$ was removed and the residue was taken up in EtOAc (35 mL). The organic solution was filtered, washed with 20% citric acid (2 × 10 mL), with 5% $NaHCO_3$ (2 × 10 mL), and with brine (3 × 10 mL), dried (Na_2SO_4), and evaporated under reduced pressure to give 2.9 g (83%) of 2-(BOC-methylamino)-*N*-(benzyloxy)acetamide as a white solid, mp 90–6 °C. An amount of 2.7 g (9 mmol) of this precursor was dissolved in EtOAc (20 mL), and dry HCl was bubbled through the stirred solution up to complete saturation. After being cooled 2 h at 0 °C, the solution was filtered to give 1.7 g (78.5%) of the title compound as white crystals: mp 185–90 °C; ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 3 H, NCH_3); 3.64 (s, 2 H, $N(CH_3)CH_2$CO); 4.87 (s, 2 H, $OCH_2C_6H_5$); 7.39 (m, 5 H, $OCH_2C_6H_5$); 9.26, 11.80 (2 br s, NH_2^+ + CONH). Anal. ($C_{10}H_{15}N_2O_2Cl$) C, H, N, Cl.

(1*R*,2*R*)-*trans*-2-[[[2-(Benzyloxyamino)-2-oxoethyl]ethylamino]carbonyl]cyclohexanecarboxylic Acid (15d) (Scheme III). A solution of 1.8 g (11.4 mmol) of (1*R*,2*R*)-*trans*-1,2-cyclohexanedicarboxylic anhydride, conveniently prepared from 2.1 g (12 mmol) of (1*R*,2*R*)-*trans*-1,2-cyclohexanedicarboxylic acid and 7.0 mL of acetic anhydride according to Polonski,[20] in CH_2Cl_2 (20 mL) was added at 0 °C with a solution of 2.9 g (12 mmol) of 2-(ethylamino)-*N*-(benzyloxy)acetamide hydrochloride and 3.9 mL (26 mmol) of triethylamine in CH_2Cl_2 (20 mL). The resulting solution was stirred at 5 °C for 2 h and then acidified with 5% HCl (40 mL). The organic layer that separated was dried ($MgSO_4$) and evaporated to dryness under vacuum to obtain 3.0 g of resinous residue. Chiral HPLC analysis showed an ee = 99%. Crystallization from Et_2O/CH_2Cl_2 (2:1) gave 2.0 g (45%) of expected compound as colorless crystals: mp 96–98 °C; $[\alpha]^{20}_D$ + 14.5° (*c* = 2, EtOH), ee > 99%; ^{1}H NMR (DMSO-d_6) δ 1.02, 1.24 (2 t, 3 H, NCH_2CH_3); 1.20–2.20 (m, 8 H, CH_2 cyclohexane); 2.50 (m, 1 H, CHCO cyclohexane); 2.70 (m, 1 H, CHCOOH cyclohexane); 3.24–3.40 (m, 2 H, $N(CH_2CH_3)$); 3.70–4.22 (2 dd, 2 H, $N(CH_2CH_3)CH_2$CO); 4.85, 4.94 (2 s, 2 H, $OCH_2C_6H_5$); 7.45 (m, 5 H, $OCH_2C_6H_5$); 9.40, 10.20 (2 s, NH); 10.80 (br s, COOH) (doubling of resonances is due to *Z*- and *E*-amide conformers). Anal. ($C_{19}H_{26}N_2O_5$) C, H, N.

(1*R*,2*R*)-*trans*-2-[[[2-(Hydroxyamino)-2-oxoethyl]ethylamino]carbonyl]cyclohexanecarboxylic Acid (16d) (Scheme III). A solution of 1.0 g (2.8 mmol) of **15d** in MeOH (10 mL) was hydrogenated over 10% Pd/C (0.1 g) at room temperature and atmospheric pressure. After the reaction was complete, the catalyst was removed by filtration, the solution was evaporated in vacuo, and the oily residue was dissolved in CH_2Cl_2 (about 6 mL). The solution was allowed to stand overnight at room temperature when a white precipitate began to form. The solid was filtered to give 0.6 g (84%) of the title compound as colorless crystals: mp 138.5–139 °C; $[\alpha]^{20}_D$ +10.7° (*c* = 2, EtOH); ^{1}H NMR (DMSO-d_6) δ 0.98, 1.21 (2 t, 3 H, $N(CH_2CH_3)$); 1.27–1.84 (m, 8 H, CH_2 cyclohexane); 2.58 (m, 1 H, CHCO cyclohexane); 2.70 (m, 1 H, CHCOOH cyclohexane); 3.10, 3.30 (2 m, 2 H, $N(CH_2CH_3)$); 3.42–4.20 (2 dd, 2 H, $N(CH_2CH_3)CH_2$CO); 6.88 (br s, OH); 9.85, 10.05 (2 s, NH); 11.50 (br s, COOH) (doubling of resonances is due to *Z*- and *E*-amide conformers). Anal. ($C_{12}H_{20}N_2O_5$) C, H, N.

(1*R*,2*S*)-*cis*-2-(Methoxycarbonyl)cyclohexanecarbonyl Chloride (18) (Scheme IV). To a stirred solution of 6 g (32 mmol) of (1*R*,2*S*)-*cis*-2-(methoxycarbonyl)cyclohexanecarboxylic acid (**17**), prepared according to Kobayashi,[21] in benzene (24 mL) was added dropwise a solution of 2.8 mL (33 mmol) of oxalyl chloride in benzene (12 mL) at room temperature: a gas evolution was observed. After the reaction was complete (2 h), benzene and all volatiles were completely removed in vacuo. An amount of 6.5 g (99%) of **18** were obtained as colorless oil and directly used for the next step: ^{1}H NMR ($CDCl_3$) δ 1.22–2.28 (m, 8 H, CH_2 cyclohexane); 2.82–3.48 (m, 2 H, CH-cyclohexane); 3.75 (s, 3 H, $COOCH_3$). Anal. ($C_9H_{13}O_3Cl$) C, H, Cl.

Methyl (1*S*,2*R*)-*cis*-2-[[[2-(Benzyloxyamino)-2-oxoethyl]methylamino]carbonyl]cyclohexanecarboxylate (19a) (Scheme IV). To a stirred and cooled solution of 2.0 g (8.6 mmol) of 2-(methylamino)-*N*-(benzyloxy)acetamide hydrochloride (**13**) and 2.4 mL (17 mmol) of NEt_3 in CH_2Cl_2 (100 mL) was added dropwise a solution of 1.8 g (8.6 mmol) of **18** in CH_2Cl_2 (10 mL) at a rate of addition to maintain a reaction temperature between 0 and 5 °C. The reaction mixture was washed with 0.5% HCl (3 × 30 mL), H_2O (40 mL), 5% $NaHCO_3$ (3 × 30 mL), and H_2O (40 mL), and dried ($MgSO_4$). Following filtration the solvent was removed under reduced pressure and then in vacuo to give 2.8 g (90%) of the title compound as an oil. The oily product slowly solidified on standing at room temperature and an analytical sample was purified by recrystallizations from EtOAc, obtaining a crystalline compound: mp 85 °C; $[\alpha]^{20}_D$ = +24.15° (*c* = 1, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.44–2.45 (m, 8 H, cyclohexane); 2.55 (m, 1 H, CHCO cyclohexane); 2.79–3.08 (2 s, 3 H, NCH_3), 3.16–3.18 (m, 1 H, $CHCOOCH_3$ cyclohexane); 3.61 (s, 3 H, $COOCH_3$); 3.44–4.56 (2 dd, 2 H, $N(CH_3)CH_2$CO); 4.91, 4.92 (2 s, 2 H, $OCH_2C_6H_5$); 7.27–7.44 (m, 5 H, $OCH_2C_6H_5$); 8.49, 10.5 (2 s, N H); ^{13}C NMR ($CDCl_3$) δ 21.28–26.86 (CH_2 cyclohexane ring); 34.05, 37.72 (NCH_3); 37.73, 43.33 (CH cyclohexane ring); 50.48 (CH_2CONH); 51.63 ($COOCH_3$); 77.88 ($OCH_2C_6H_5$); 128.28, 128.79, 135.85 ($OCH_2C_6H_5$); 166.07 (CONHO); 174.97 (CON); 175.34 ($COOCH_3$) (doubling of resonances is due to *Z*- and *E*-amide conformers); MS *m/e* 363 (M^+ + 1). Anal. ($C_{19}H_{26}N_2O_5$) C, H, N.

(1*S*,2*R*)-*cis*-2-[[[2-(Benzyloxyamino)-2-oxoethyl]methylamino]carbonyl]cyclohexanecarboxylic Acid (20a) (Scheme IV). A solution of 2.0 g (5.7 mmol) of **19a** in MeOH (3.0 mL) was added to 1 N NaOH (40 mL), and the obtained mixture was stirred for 3–4 h. A clear solution resulted which was washed with $CHCl_3$ (3 × 20 mL), cooled at 0 °C, added to $CHCl_3$ (40 mL) and acidified (pH 2), under stirring, with 5% HCl. The organic extract was immediately separated, the aqueous solution was again extracted with $CHCl_3$ (2 × 40 mL), and the organic extracts were combined, washed with H_2O (40 mL), and dried ($MgSO_4$). Evaporation of the solvent in vacuo afforded 1.6 g of a white solid. Chiral HPLC analysis showed a 97.5% ee and the presence of 1% of contaminant trans isomers. Recrystallization from acetone (4 mL) gave 1.2 g (56%) of **20a** as white crystals: mp 120–5 °C; $[\alpha]^{20}_D$ = +21° (*c* = 1, EtOH), ee >99%; ^{1}H NMR ($CDCl_3$) δ 1.23–2.45 (m, 8 H, CH_2 cyclohexane); 2.55–2.75 (m, 1 H, *CH*CO cyclohexane); 2.79, 3.02 (2 s, 3 H, NCH_3); 3.02–3.11 (m, 1 H, *CH*COOH cyclohexane); 3.57–4.24 (2 dd, 2 H, $N(CH_3)CH_2CO$), 4.86, 4.93 (2 s, 2 H, $OCH_2C_6H_5$); 7.27–7.37 (m, 5 H, $OCH_2C_6H_5$); 9.60, 10.30 (2 s, N*H*); 11.50 (br s, COO*H*); ^{13}C NMR ($CDCl_3$): δ 22.10–26.35 (CH_2 cyclohexane ring); 34.50, 37.05 (NCH_3); 38.01, 41.88 (*CH* cyclohexane ring); 50.29, 52.23 (CH_2-CONH); 77.88 ($OCH_2C_6H_5$); 128.41, 129.20, 135.25 ($OCH_2C_6H_5$); 166.39 (*C*ONHO); 175.73 (*C*ON); 177.81 (*C*OOH) (doubling of resonances is due to *Z*- and *E*-amide conformers); MS *m/e* 349 (M^+ + 1). Anal. ($C_{16}H_{24}N_2O_5$) C, H, N.

(1*S*,2*R*)-*cis*-2-[[[2-(Hydroxyamino)-2-oxoethyl]methylamino]carbonyl]cyclohexanecarboxylic Acid (21a; Scheme IV). A solution of 1.0 g (2.9 mmol) of **20a** in THF (30 mL) was hydrogenated over 10% Pd/C (0.1 g) at room temperature and atmospheric pressure. After the reaction was complete, a white crystalline precipitate resulted which was dissolved by adding 10 mL of distilled MeOH. The catalyst was removed by filtration and washed with distilled MeOH (2 × 5 mL). The solutions were combined and concentrated at reduced pressure without heating (below 25 °C) up to 4–5 mL and the residue was combined with Et_2O (20 mL). The precipitate that formed was filtered and dried in vacuo (P_2O_5) to give 0.7 g (93%) of **21a** as white crystals: mp 128–130 °C; $[\alpha]^{20}_D$ +26.9° (*c* = 1, EtOH); ^{1}H NMR (DMSO-d_6) δ 1.23–2.13 (m, 8 H, CH_2 cyclohexane); 2.39–2.50 (m, 1 H, *CH*CO cyclohexane); 2.73–3.03 (2 s, 3 H, NCH_3); 3.21–3.23 (m, 1 H, *CH*COOH cyclohexane); 3.55–4.08 (2 dd, 2 H, $N(CH_3)CH_2$-CO); 8.84 (br s, O*H*); 9.96, 10.30 (2 s, N*H*); 11.45 (br s, COO*H*); ^{13}C NMR (DMSO-d_6) δ 21.69–26.42 (CH_2 cyclohexane ring); 34.01, 36.64 (NCH_3); 37.34, 42.18 (*CH* cyclohexane ring); 48.20, 50.09 (CH_2CONH); 165.15, 165.56 (CONHOH); 174.97 (*C*ON); 175.35 (*C*OOH) (doubling of resonances is due to *Z*- and *E*-amide conformers); MS *m/e* 259 (M^+ + 1), 215 (base peak, $MH^+ - CO_2$). Anal. ($C_{11}H_{16}N_2O_5$) C, H, N.

In Vitro ACE Inhibition Assay. A volume of 10 μL of diluted rat serum (approximately 1 milliunit of ACE) was incubated, for 1 h at 37 °C, with 10 μL of inhibitor solution or vehicle and 50 μL of 30 mM hippurylglycilglycine (substrate) solution in 50 mM Hepes buffer, pH 8, containing 300 mM NaCl, 400 mM Na_2SO_4, and 0.7 mM *o*-methylhippuric acid (internal standard). Reaction was stopped by adding 50 μL of 0.5 N perchloric acid and 380 μL of distilled water. The mixture was extracted with 500 μL of ethyl acetate, and organic phase evaporated to dryness, and the residue dissolved in the mobile phase. Aliquots were then analyzed by reverse-phase HPLC with UV detection for the content of hippuric acid produced by the action of ACE on the substrate.

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Supplementary Material Available: A table on effects of selected carboxyl, sulfhydrylic, and hydroxamic compounds on exo- and endopeptidases and tables of proton NMR spectral data and elemental analyses (12 pages). Ordering information is given on any current masthead page.

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