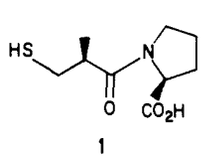
# Angiotensin Converting Enzyme Inhibitors. (Mercaptoaroyl)amino Acids

Paul R. Menard,† John T. Suh,*† Howard Jones,† Bernard Loev,† Edward S. Neiss,† Joyce Wilde,† Alfred Schwab,‡ and William S. Mann‡

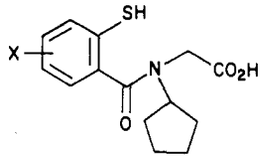
*Departments of Medicinal Chemistry, Biochemistry, and Pharmacology, Revlon Health Care Group, Tuckahoe, New York 10707.* *Received May 10, 1984*

A series of (mercaptoaroyl)amino acids and related compounds was synthesized and tested for ability to inhibit angiotensin converting enzyme (ACE). The most active compound was *N*-(3-chloro-2-mercaptobenzoyl)-*N*-cyclopentylglycine, having an in vitro $I_{50}$ = 0.28 μM. Substitution of the aromatic 3-position by small polar groups enhanced ACE inhibitory activity, whereas bulky groups diminished it. Alteration of the β relationship between the mercaptan and amide carbonyl or masking of the thiol by acylation reduced activity. Replacement of the thiol by nitro, hydroxy, or carboxy gave compounds lacking ACE inhibitory activity.

Captopril[1,2] (1) is representative of a class of potent, orally active inhibitors of angiotensin converting enzyme (ACE). As part of a program on the development of specific and potent ACE inhibitors, we wished to study the effect of bridging positions 2 and 3 of the mercaptopropionyl side chain of 1 with an aromatic system. Since previous work here has shown that the proline terminus of 1 could be replaced by N-substituted glycine with retention of ACE inhibitory activity,[3] a series of *N*-(2-mercaptobenzoyl)-*N*-cyclopentylglycines (2) and related compounds was prepared.



1

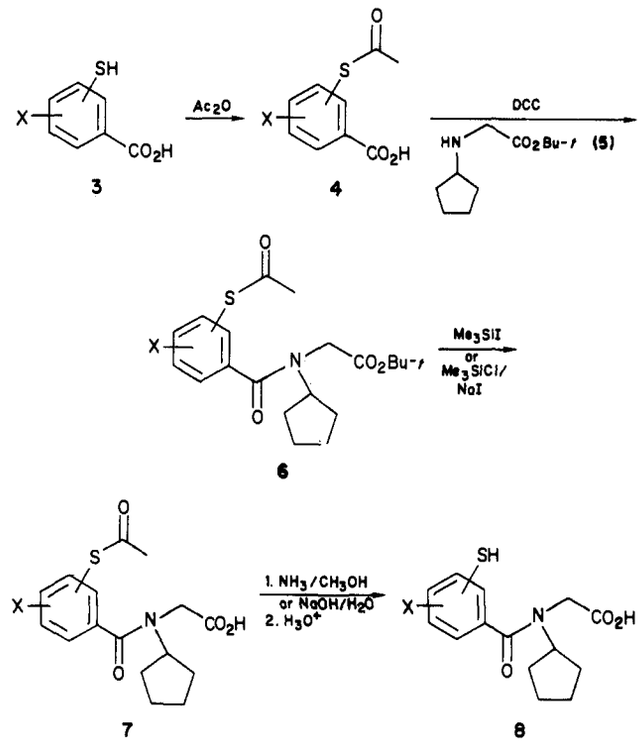


2

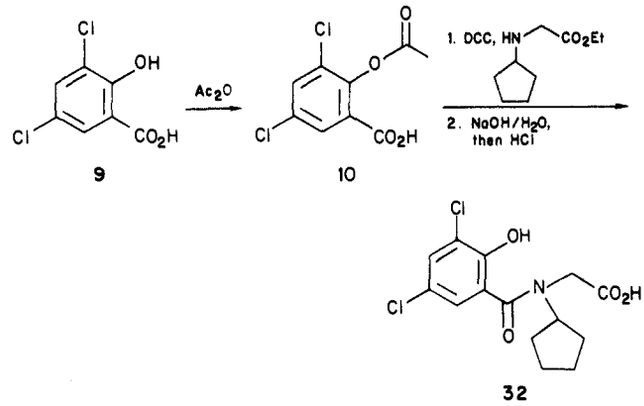
All sulfur-containing ACE inhibitors reported to date[1,2,4-9] contain the mercaptan functionality bonded to an aliphatic carbon. In compounds 2, however, the thiol is bound to an aromatic ring. Since the chemical properties and reactivity of alkyl and aryl thiols are known to differ,[10] the pharmacological profiles of compounds representative of these classes might also differ. In particular, the clinical side effects[11,12] thought due to the aliphatic mercaptan might be reduced or eliminated. In this paper we report the synthesis and preliminary pharmacology of a new class of orally active (mercaptoaroyl)amino acid ACE inhibitors, the most potent of which is *N*-(3-chloro-2-mercaptobenzoyl)-*N*-cyclopentylglycine (17) ($I_{50}$ = 0.28 μM for in vitro ACE inhibition).

**Chemistry.** (Mercaptoaroyl)amino acids and *S*-acetates 7 and 8 were prepared by the general procedure shown in Scheme I. Starting mercaptobenzoic acids 3 were either obtained commercially or made by treatment of appropriately substituted aminobenzoic acids with nitrous acid, followed by reaction of the diazonium salt with sodium polysulfide and reduction of the product so formed to the thiol using zinc and acetic acid[13] or sodium dithionate. If not commercially available, substituted anthranilic acids were prepared from the corresponding amines by using the isatin procedure[14] followed by hydrogen peroxide oxidation.[15] (Acetylthio)benzoic acids 4 were conveniently prepared by treatment of 3 with acetic anhydride at reflux or under Schotten–Baumann conditions. *tert*-Butyl esters 6 were generally used without further purification in the next step. Deprotection of the acid using trimethylsilyl iodide (TMSI) generated in situ from trimethylsilyl chloride and sodium iodide in acetonitrile[16] was more economical than using commercial TMSI and gave equally good results. Most *S*-acetates 7 were amorphous solids and were purified by column chromatography or HPLC. Pi-

**Scheme I**



**Scheme II**



† Department of Medicinal Chemistry.
‡ Departments of Biochemistry and Pharmacology.

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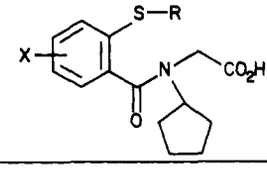
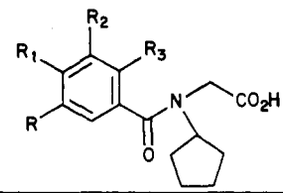
**Table I.** Physical Data and in Vitro ACE Inhibitory Activity of *N*-[2-(Acetylthio)benzoyl]-*N*-cyclopentylglycines and *N*-(2-Mercaptobenzoyl)-*N*-cyclopentylglycines



| compd | X | R | procedure | yield, % | mp, °C | formula[a] | $IC_{50}$,[b] M × $10^{-6}$ |
|---|---|---|---|---|---|---|---|
| **12** | H | H | F | 100 | 146.5–148 | $C_{14}H_{17}NO_3S$ | 4.8 |
| **13** | H | $COCH_3$ | C, D | 26 | glass | $C_{16}H_{19}NO_4S$[c] | 9.1 |
| **14** | H | $COC(CH_3)_3$ | C, E | 44 | 155.5–157.5 | $C_{19}H_{25}NO_4S$ | >100 |
| **15** | 3-F | H | G | 40 | 149–151 | $C_{14}H_{16}FNO_3S$ | 0.55 |
| **16** | 3-F | $COCH_3$ | C, E | 39 | glass | $C_{16}H_{18}FNO_4S$ | 0.60 |
| **17** | 3-Cl | H | F | 92 | 176–178[d] | $C_{14}H_{16}ClNO_3S$ | 0.28 |
| **18** | 3-$CF_3$ | H | G | 100 | 144–146 | $C_{15}H_{16}F_3NO_3S$ | 0.68 |
| **19** | 3-$OCH_3$ | H | G | 96 | 136–138 | $C_{15}H_{19}NO_4S$ | 0.38 |
| **20** | 3-$CH_3$ | H | F | 100 | 117–119 | $C_{15}H_{19}NO_3S$ | 4.6 |
| **21** | 3-$CH_3$ | $COCH_3$ | C, E | 36 | 122–124 | $C_{17}H_{21}NO_4S$ | 2.2 |
| **22** | 3-$CH(CH_3)_2$ | H | G | 100 | glass | $C_{17}H_{23}NO_3S$ | 120 |
| **23** | 3-$CH(CH_3)_2$ | $COCH_3$ | C, E | 37 | glass | $C_{19}H_{25}NO_4S$ | >100 |
| **24** | 4-Cl | H | F | 96 | 74–77 | $C_{14}H_{16}ClNO_3S$ | >100 |
| **25** | 5-Cl | H | C, E[e] | 12 | 161–163 | $C_{14}H_{16}ClNO_3S$[f] | 80 |
| **26** | 3,5-$Cl_2$ | H | C,[g] G | 10 | 130–131 | $C_{14}H_{15}Cl_2NO_3S$ | 4.1 |
| captopril | | | | | | | 0.027 |

[a] All compounds gave satisfactory C, H, N analyses unless otherwise indicated. [b] Molar concentration required for 50% inhibition of ACE; see Experimental Section. [c] C: calcd, 59.79; found, 59.03. [d] Lit.[22] mp 160–163.5 °C. [e] Isolated during workup of procedure E. [f] N: calcd, 4.46; found, 3.93. [g] Ethyl *N*-cyclopentylglycinate used.

**Table II.** Physical Data and in Vitro ACE Inhibitory Activity of Aroylamino Acids



| compd | R | $R_1$ | $R_2$ | $R_3$ | procedure | yield, % | mp, °C | formula[a] | $IC_{50}$,[b] M × $10^{-6}$ |
|---|---|---|---|---|---|---|---|---|---|
| **27** | H | H | SH | H | G | 51 | glass | $C_{14}H_{17}NO_3S$ | 25 |
| **28** | H | H | $SCOCH_3$ | H | C, E | 35 | glass | $C_{16}H_{19}NO_4S$ | 28 |
| **29** | H | SH | H | H | G | 43 | glass | $C_{14}H_{17}NO_3S$ | 21 |
| **30** | H | $SCOCH_3$ | H | H | C, E | 57 | glass | $C_{16}H_{19}NO_4S$ | 21 |
| **31** | Cl | H | Cl | $OCOCH_3$[c] | *d* | *d* | 144.5–146.5 | $C_{18}H_{21}Cl_2NO_5$ | 100 |
| **32** | Cl | H | Cl | OH | *d* | *d* | 156.0–157.5 | $C_{14}H_{15}Cl_2NO_4$ | 100 |
| **33** | H | H | H | $CO_2H$ | *d* | *d* | glass | $C_{15}H_{17}NO_5$[e] | 100 |
| **34** | H | H | H | $NO_2$ | *d* | *d* | 175.5–177.5 | $C_{14}H_{16}N_2O_5$ | 100 |

[a] All compounds gave satisfactory C, H, N analyses unless otherwise indicated. [b] Molar concentration required for 50% inhibition of ACE; see Experimental Section. [c] Ethyl ester. [d] See Experimental Section. [e] Monohydrate.

valate ester **14** (Table I) was prepared by acylating 2-mercaptobenzoic acid with pivaloyl chloride and carrying the resulting product through Scheme I as for an *S*-acetate.

The acetoxy and hydroxy derivatives **31** and **32** (Table II) were made as shown in Scheme II. Carboxy compound **33** was prepared by treatment of phthalic anhydride with ethyl *N*-cyclopentylglycinate (**11**) followed by basic hydrolysis. Nitro analogue **34** was obtained by treating **11** with 2-nitrobenzoyl chloride and hydrolyzing the product with aqueous sodium hydroxide.

## Results and Discussion

Bridging of the 2- and 3-positions of the 3-mercaptopropionyl side chain of **1** and concurrent replacement of proline with *N*-cyclopentylglycine result in a series of novel compounds wherein the thiol moiety is bonded to an aromatic rather than aliphatic carbon. These molecules contain no asymmetric centers, obviating the need for resolution. As shown in Table I, in vitro ACE inhibitory activity, though less than that observed with **1**, is signif-

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Table III. In Vivo[a] ACE Inhibitory Activity of Aroylamino Acids

| no. | dose, mg/kg po | no. of rats | mean decrease, % | mean duration |
|---|---|---|---|---|
| 12 | 1.5 | 4 | 34 | 35 min |
| 13 | 1.5 | 2 | 25 | 0 |
| 14 | 1.5 | 2 | 19 | 0 |
| 15 | 10 | 2 | 84 | 105 min |
| 16 | 10 | 2 | 82 | 90 min |
| 17 | 10 | 5 | 85 | >4 h |
| | $ED_{50}$ = 300 μg/kg | | | |
| 18 | 10 | 2 | 31 | 20 min |
| 19 | 10 | 5 | 92 | 4 h |
| | $ED_{50}$ = 800 μg/kg | | | |
| 20 | 10 | 2 | 77 | 50 min |
| 21 | 1.5 | 2 | 35 | 20 min |
| 22 | 10 | 2 | 23 | 0 |
| 25 | 10 | 2 | 22 | 0 |
| 27 | 10 | 2 | 13 | 0 |
| 34 | 10 | 1 | 25 | 0 |

[a] See Experimental Section.

icant, with 17 having an $I_{50}$ of 0.28 μM.

Sulfur-containing ACE inhibitors have been proposed to function by chelation of the thiol with zinc cation in ACE.[1,2] Since the acidity of substituted thiophenols is known to vary with the electronic character of the substituent,[17,18] the chelating ability of the thiol might also be substituent dependent. To investigate this point, a number of (mercaptoaroyl)amino acids (**15**, **17**, **18**, **19**, and **20**) containing electron-donating or withdrawing groups were prepared. The 3-position of the aromatic ring was chosen for initial studies in order to maximize electronic effects on the thiol. Due to close proximity of the 3-substituent and thiol, steric effects may have some importance and will be discussed later.

In vitro ACE inhibitory activity did not show a clear relationship to the nature of the substituent. Replacement of the 3-hydrogen of **12** with a number of polar groups led to significant increases in activity. Substituents having free electron pairs which, though more electronegative than hydrogen, can conjugatively donate electron density to the ring, gave an approximately tenfold increase in activity. Examples are the 3-fluoro (**15**), chloro (**17**), and methoxy (**19**) analogues of **12**. Inductively withdrawing trifluoromethyl (**18**) caused a similar increase in ACE inhibitory activity; in contrast, the relatively nonpolar and weakly inductively donating methyl (**20**) caused no change. These trends were generally confirmed by an in vivo ACE inhibition assay (Table III), showing **17** and **19** to be significantly more active than **12**. A possible explanation of these results may include, in addition to electronic effects, an electrostatic interaction between the polar 3-substituents and additional binding sites on ACE.

The latter possibility was studied by preparation of isomers of the highly potent ACE inhibitor **17**. The aromatic chlorine was moved consecutively from the 3- to the 5-position; the 3,5-dichloro compound **26** was also made. In vitro the 4- and 5-chloro isomers (**24** and **25**, respectively) were not only less active than **17** but also less active than unsubstituted **12**. That chlorine in the 5-position is detrimental to ACE inhibitory activity is shown by the reduced activity observed when the aromatic 5-hydrogen of **17** is replaced by chlorine. Since electronic influences on the thiol might not be expected to vary greatly when chlorine is moved from the 3-position to elsewhere on the aromatic ring, the large in vitro changes observed suggest a specific favorable interaction between the 3-chloro (or other polar) substituent and a binding site on ACE.

Due to lack of conformational flexibility of the aromatic ring, bulky substituents adjacent to the aryl mercaptan might sterically hinder binding of the thiol to the proposed zinc cation at the binding site of ACE. This was demonstrated by successively replacing the aromatic 3-hydrogen of **12** with the increasingly sterically demanding methyl and isopropyl groups (**20** and **22**, respectively). Compound **20** was similar in activity to **12** in vitro, with good in vivo potency. Compound **22**, however, showed greatly decreased in vitro activity ($I_{50}$ = 120 μM) and was essentially inactive in vivo at 10 mg/kg po.

As previously mentioned, work in this laboratory has shown that the proline terminus of **1** could be replaced by N-substituted glycine with retention of ACE inhibitory activity.[3] This observation was verified in the current series by the preparation of *N*-(2-mercaptobenzoyl)-L-proline (**35**), the proline analogue of **12**. Compound **35** was less active than **12** in vitro ($I_{50}$ of 23 vs. 4.8 μM, respectively) and in vivo caused less inhibition of the pressor response to angiotensin I at 10 mg/kg orally (mean decrease 22%, mean duration 24 min, two rats) than did **12** at 1.5 mg/kg orally (see Table III).

The free mercapto group is thought to be responsible for certain side effects, such as rashes and loss of taste, observed in the clinical use of captopril.[11,12] In an effort to develop an ACE inhibitor lacking this group, a number of (mercaptoaroyl)amino acid *S*-esters were prepared. *S*-Acetates generally showed in vitro activities similar to their corresponding free thiols (Tables II and III). The *S*-acetate **13** and pivalate **14** esters of **12** were both less active than **12** in vitro, with **14** being essentially inactive. In vivo studies revealed a similar trend. The pivalate ester should show the greater degree of steric retardation of hydrolysis to the mercaptan. These results suggest that a percentage of biological activity observed with *S*-esters may actually be due to free thiol generated under the assay conditions.

Compound **12** resembles captopril and other reported sulfur-containing ACE inhibitors in that the thiol is β to the amide carbonyl. Changes in this relationship might be expected to result in lower ACE inhibitory activity since the steric requirements necessary for optimum binding to the proposed active site on ACE would be violated. Isomers of **12** were made wherein the aryl mercaptan was moved to the 3- and 4-positions of the ring (**27** and **29**, respectively). Both **27** and **29** are in fact severalfold less active than **12** (Tables II and III).

Replacement of the mercaptan with the substituents acetoxy (**31**), hydroxy (**32**), carboxy (**33**), and nitro (**34**) gave compounds devoid of ACE inhibitory activity. Thus the thiol appears essential for this class of compounds to be ACE inhibitors.

Compounds **17** and **19**, being the most potent ACE inhibitors, were studied for antihypertensive activity in spontaneously hypertensive rats maintained on a low-sodium diet. Compound **17** at 60 mg/kg orally decreased arterial pressure a maximum of 25% and 26% in two rats with a duration of action of 3.5 and 9 h, respectively. Compound **19** at the same oral dose decreased arterial pressure a maximum of 25% and 26% also, with a duration of 5 and 13 h.

In summary, we have found that a number of *N*-(2-mercaptobenzoyl)-*N*-cyclopentylglycines possess in vitro and in vivo ACE inhibitory activity. Maximum inhibition

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is seen when the mercaptan is $\beta$ to the amide carbonyl. Alteration of this geometrical relationship or masking of the thiol by acylation reduces activity. Attempts to replace the thiol by other functional groups give compounds devoid of ACE inhibitory activity.

## Experimental Section

Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Analytical samples were homogeneous by TLC and had IR, $^1$H NMR, and mass spectra consistent with the assigned structures. IR spectra were taken on a Perkin-Elmer 298 infrared spectrophotometer. Proton NMR spectra were obtained on Varian EM-360 or EM-390 NMR spectrometers using tetramethylsilane as an internal standard. Mass spectra were obtained with a Varian MAT-112 reversed geometry mass spectrometer. Elemental analyses were performed on Perkin-Elmer 240-A, 240-B, and Control Equipment Corp. 240-XA elemental analyzers in this laboratory and are within ±0.4% of the theoretical values unless otherwise indicated.

(Mercaptoaroyl)amino acids **12–30** were prepared from appropriately substituted mercaptobenzoic acids by using similar methodology. The following examples will serve to illustrate the reaction conditions employed.

**(Acetylthio)benzoic Acids 4. Method A.** The thiobenzoic acid **3** (35 mmol), acetic anhydride (42 mmol, 1.2 equiv), and acetic acid (15 mL) were refluxed under $N_2$ for 15 min–4 h, until homogeneity was achieved. The solution was cooled to room temperature, poured into 2.5 N HCl (50 mL), and brought to 0 °C. Filtration provided the desired (acetylthio)benzoic acid.

**Method B.** The thiobenzoic acid **3** (0.046 mol) was dissolved in NaOH (0.138 mol)/$H_2O$ and cooled to 0 °C. Acetic anhydride (0.092 mol) was added in portions over 20 min with vigorous stirring; cold 1 N NaOH was added, as necessary, to prevent precipitation. The mixture was stirred 3 h, then acidified with concentrated HCl, and extracted with EtOAc. The extract was washed with brine, dried ($Na_2SO_4$), and concentrated. Recrystallization from an appropriate solvent gave the pure *S*-acetate.

By use of method A or B, the following compounds were prepared: 2-(acetylthio)benzoic acid, mp 127–129 °C (lit.[19] mp 125.5–126.0 °C); 2-(acetylthio)-3-chlorobenzoic acid, mp 109–111 °C; 2-(acetylthio)-3-(trifluoromethyl)benzoic acid, mp 147–148.5 °C; 2-(acetylthio)-3-methoxybenzoic acid, mp 131.5–133.5 °C; 2-(acetylthio)-3-methylbenzoic acid, mp 96–97 °C; 2-(acetylthio)-3-(2-propyl)benzoic acid, mp 92.5–94 °C; 4-(acetylthio)benzoic acid, mp 202–204 °C (lit.[20] mp 202.5–203.5 °C); 2-(acetylthio)-3-fluorobenzoic acid; 2-(acetylthio)-4-chlorobenzoic acid; 2-(acetylthio)-5-chlorobenzoic acid; 2-(acetylthio)-3,5-dichlorobenzoic acid; and 3-(acetylthio)benzoic acid (lit.[20] mp 152–153 °C).

***N*-[(Acetylthio)benzoyl]-*N*-cyclopentylglycine *tert*-Butyl Esters 6. Method C.** *N,N'*-Dicyclohexylcarbodiimide (DCC; 6.2 g, 30 mmol) in $CH_2Cl_2$ (50 mL) was added to a solution of the appropriately substituted (acetylthio)benzoic acid **4** (30 mmol) and *tert*-butyl *N*-cyclopentylglycinate (**5**) (6.0 g, 30 mmol) in $CH_2Cl_2$ (250 mL) at 0 °C. The mixture was brought to room temperature overnight and then filtered. The filtrate was washed successively with 1 N HCl, saturated aqueous $NaHCO_3$ and brine, then dried ($Na_2SO_4$), and concentrated in vacuo. The residue was dissolved in ether, filtered to remove remaining *N,N'*-dicyclohexylurea, and concentrated. The crude *tert*-butyl ester thus obtained was generally used without further purification in the next step.

***N*-[(Acetylthio)benzoyl]-*N*-cyclopentylglycines 7. Method D.** The crude *tert*-butyl ester was dissolved in $CCl_4$, under $N_2$, and trimethylsilyl iodide (1–2 equiv) was added. The solution was stirred 25 min and then quenched by addition of water. Additional $CCl_4$ was added, if needed, to dissolve the precipitate, and then the organic layer was separated, washed with water, aqueous sodium thiosulfate, and brine, and concentrated in vacuo. The residue was treated with aqueous $NaHCO_3$ and filtered. The filtrate was washed with EtOAc, acidified with concentrated HCl, and extracted with EtOAc. The organic layer was separated, dried ($Na_2SO_4$), and concentrated to an amorphous material. Purification was generally achieved by passage through a silica gel column using a solvent gradient from hexane to hexane/ethyl acetate/acetic acid, 50/50/3.

**Method E.** The *tert*-butyl ester (50 mmol) was dissolved in $CH_3CN$ (65 mL) and NaI (11.2 g, 75 mmol) was added. The mixture was heated to 40–50 °C, trimethylsilyl chloride (8.1 g, 75 mmol) was added, and heating was continued for 30 min. The mixture was cooled and quenched with water. The organic layer was separated and treated as described in method D to yield the carboxylic acid.

***N*-(Mercaptobenzoyl)-*N*-cyclopentylglycines 8. Method F.** Ammonia gas was bubbled at a slow rate (for 70 min) through a solution of the *N*-[(acetylthio)benzoyl]-*N*-cyclopentylglycine **7** (15 mmol) in $CH_3OH$ (150 mL). Gas flow was stopped, the flask was sealed, and the solution was stirred 20 min and then concentrated in vacuo. The residue was partitioned between EtOAc (200 mL) and 5% aqueous sodium bisulfate (100 mL). The organic layer was separated and washed with 5% sodium bisulfate and brine, then dried ($Na_2SO_4$), and concentrated. The residue was purified by passage through silica gel using an appropriate solvent mixture (e.g., $C_6H_{14}$/EtOAc/HOAc, 50/50/3).

**Method G.** Acetate **7** (12 mmol) was dissolved in $H_2O$ (90 mL) containing NaOH (1.6 g, 40 mmol) and stirred for 2 h. The solution was acidified with concentrated HCl and extracted with EtOAc. The organic layer was removed, washed with water and brine, dried ($Na_2SO_4$), and concentrated. Passage through a silica gel column gave the pure compound.

***N*-(2-Mercaptobenzoyl)-L-proline (35).** DCC (6.2 g, 30 mmol) in $CH_2Cl_2$ (50 mL) was added to 2-(acetylthio)benzoic acid (5.9 g, 30 mmol) and L-proline methyl ester hydrochloride (5.0 g, 30 mmol) in $CH_2Cl_2$ (250 mL) at 0 °C. The mixture was stirred overnight and then filtered, washed with 1 N HCl and saturated aqueous $NaHCO_3$, dried ($Na_2SO_4$), and concentrated to a yellow oil (10.2 g). This material was dissolved in $CH_3OH$ (100 mL), and NaOH (3.6 g, 90 mmol) in water (10 mL) was added. The solution was stirred 1.5 h, and then methanol was removed in vacuo at <35 °C. The residue was acidified with concentrated HCl and extracted with EtOAc. Concentration of the extract gave a gum, which was then treated with aqueous $NaHCO_3$. Acidification of the aqueous layer with concentrated HCl gave a solid which was recrystallized from EtOAc and then $CHCl_3$ to give 1.7 g (23%) of **35**: mp 169.5–171 °C; $[\alpha]^{23}_D$ −140.4° (*c* 0.11, $CHCl_3$). Anal. ($C_{12}H_{13}NO_3S$) C, H, N.

**2-[(Trimethylacetyl)thio]benzoic Acid.** 2-Mercaptobenzoic acid (15 g, 0.10 mol) and trimethylacetyl chloride (15 g, 0.12 mol) were combined in toluene (150 mL) and refluxed for 2 h. The mixture was concentrated in vacuo to an oil which was partially solidified. This material was recrystallized from hexane to give 5.4 g (23%) of product, mp 98–102 °C.

**2-Acetoxy-3,5-dichlorobenzoic Acid (10).** A mixture of 3,5-dichloro-2-hydroxybenzoic acid (51.7 g, 0.25 mol), acetic anhydride (35.7 g, 0.35 mol), and a few drops of pyridine was heated to 80 °C over 30 min. Acetic acid (42 mL) was added and heating at 80–100 °C was continued for 1 h. The mixture was cooled and filtered to give 52 g (83%) of **10**.

**Ethyl *N*-(2-Acetoxy-3,5-dichlorobenzoyl)-*N*-cyclopentylglycinate (31).** DCC (16.9 g, 81.8 mmol) in $CH_2Cl_2$ (80 mL) was added to a mixture of **10** (20.4 g, 81.8 mmol) and ethyl *N*-cyclopentylglycinate (14.0 g, 81.8 mmol) in $CH_2Cl_2$ (400 mL) at −10 °C. The mixture was brought to room temperature overnight and filtered, washed successively with 1 N HCl, saturated $NaHCO_3$ and brine, and dried ($Na_2SO_4$). Concentration gave a gum which was recrystallized from EtOAc to give **31** (16.8 g, 51%), mp 144.5–146.5 °C.

***N*-(3,5-Dichloro-2-hydroxybenzoyl)-*N*-cyclopentylglycine (32).** A mixture of sodium hydroxide (5.0 g, 0.12 mol) and **31** (10.1 g, 0.025 mol) in water (250 mL) was refluxed 2 h, then cooled, and filtered. The filtrate was cooled to 0 °C and acidified with concentrated HCl. The mixture was extracted with EtOAc and the extract washed with brine, dried ($Na_2SO_4$), and concentrated to a white solid (8.0 g, 96%). An analytical sample was provided by recrystallization from EtOAc: mp 156.0–157.5 °C.

***N*-(2-Carboxybenzoyl)-*N*-cyclopentylglycine (33).** A mixture of phthalic anhydride (8.9 g, 60 mmol) and ethyl *N*-cyclopentylglycinate (12.3 g, 72 mmol) in $CHCl_3$ (350 mL) was

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stirred 1 h with occasional warming. The solution was washed with 1 N HCl and brine, then dried ($Na_2SO_4$), and concentrated. The residue was dissolved in saturated aqueous $NaHCO_3$ and the resulting solution was washed with hexane, acidified with concentrated HCl, and extracted with ether. The extract was washed with brine, dried ($Na_2SO_4$), and concentrated to an oil (17.3 g, 90%) which soon solidified: mp 146.5–148.5 °C (hexane/ethyl acetate). Anal. ($C_{17}H_{21}NO_5$) C, H, N.

A solution of the above compound (10.5 g, 33 mmol) and sodium hydroxide (5.3 g, 132 mmol) in water (100 mL) was heated in a water bath for 2 h, then cooled, and acidified with concentrated HCl. Filtration gave 7.6 g (79%) of an amorphous white solid. Analytically pure material was obtained by dissolution of the crude compound in EtOH/EtOAc (1:1) followed by filtration through Celite and concentration of the filtrate. The residue was dissolved in dilute NaOH and precipitated as an amorphous white solid with concentrated HCl.

***N*-(2-Nitrobenzoyl)-*N*-cyclopentylglycine (34).** 2-Nitrobenzoic acid (25.1 g, 0.15 mol) and thionyl chloride (26.8 g, 16.4 mL, 0.225 mol) were heated at 80–100 °C in an oil bath for 2 h. The mixture was concentrated in vacuo to give 2-nitrobenzoyl chloride as an oil in quantitative yield. This material (8.3 g, 45 mmol) was slowly added to a solution of ethyl *N*-cyclopentylglycinate (5.1 g, 30 mmol) and triethylamine (4.5 g, 45 mmol) in $CHCl_3$ (100 mL) at 0 °C. The mixture was stirred 2 h at 0–10 °C, then washed with 1 N HCl and saturated $NaHCO_3$, dried ($Na_2SO_4$), and concentrated. The residual oil was stirred with NaOH (4.8 g, 0.12 mol)/$H_2O$ (125 mL) for 3 h with gentle heating. The mixture was cooled and washed with EtOAc and then acidified to give a brown gum (6.8 g, 77%) which soon crystallized. Recrystallization from EtOAc provided light yellow crystals, mp 175.5–177.5 °C.

**In Vitro ACE Inhibitory Activity.** In vitro ACE inhibitory activity was determined in 0.1 M $KH_2PO_4$–0.3 M NaCl–2% dimethyl sulfoxide ($Me_2SO$) at pH 8.3 and 37 °C with hippuryl-L-histidyl-L-leucine (2 mM) as substrate.[23] A crude preparation of ACE was obtained by stirring 1 g of rabbit lung acetone powder (Pel-Freez) with 20 mL of cold 0.05 M $KH_2PO_4$–0.03 M *n*-octyl-β-D-glucopyranoside at pH 8.3 for 10 min. The suspension was centrifuged at 4 °C for 30 min at 30000*g* and the supernatant was dialyzed against 0.05 M $KH_2PO_4$, pH 8.3, before use. The inhibitory effect of test compounds (in $Me_2SO$) on ACE activity was determined by measuring enzyme activity in the presence and absence of each compound. Assays were initiated by adding enzyme to a buffered solution of substrate ± inhibitor. $IC_{50}$ values were based on one concentration–response curve for each compound.

**In Vivo ACE Inhibitory Activity.** Polyethylene catheters were implanted in the abdominal aortae and inferior vena cavae of normotensive male rats. At least 6 days later, the rats were restrained in plastic holders and the arterial catheters connected to transducers for continuous monitoring of pressure. Angiotensin I ($A_I$) and angiotensin II ($A_{II}$), 0.25 μg/kg, were injected via the venous catheters at 10-min intervals and the responses recorded. Following two doses of each agonist, the rats were given one dose of test compound, generally orally and suspended in a 0.5% gum tragacanth suspension. The $A_I$ injections were repeated every 10 min for at least 2 h except for occasional injections of $A_{II}$.

For each rat, the maximum inhibition of the $A_I$ pressor response following the test agent was determined as a percent of the mean of the two initial responses to $A_I$. The duration was measured as the time interval from dosing until the percent inhibition fell below 30%. For selected compounds a dose–response plot was drawn and $ED_{50}$'s calculated. These were the theoretical doses that caused a 50% inhibition of the pressor response to $A_I$.

**Antihypertensive Activity.** Fourteen-week-old, male spontaneously hypertensive rats (Charles River Breeding Laboratories, Wilmington, MA), weighing 195–240 g, were used. They were maintained on a low-sodium diet (100 ppm, ICN Pharmaceuticals, Cleveland, OH) and distilled water for 2 weeks prior to use in order to elevate their plasma renin activities. Seven days prior to the experiments, polyethylene catheters were implanted in their abdominal aortae under ether anesthesia.[21] On the day of experimentation, the rats were harnessed and their catheters were attached to a recording system via swivels that allowed the animals to roam freely within individual cages while their arterial pressures were monitored. Two rats were given each test compound by gavage and postdosing arterial pressures were compared to the average arterial pressures during the half-hour just prior to dosing.

**Registry No.** **3** (2-SH), 147-93-3; **3** (2-SH, X = 3-Cl), 17839-51-9; **3** (2-SH, X = 3-$CF_3$), 83596-78-5; **3** (2-SH, X = 3-$OCH_3$), 83596-91-2; **3** (2-SH, X = 3-$CH_3$), 77149-11-2; **3** (2-SH, X = 3-$CH(CH_3)_2$), 92399-45-6; **3** (4-SH), 1074-36-8; **3** (3-SH), 4869-59-4; **4** (2-$SCOCH_3$), 55819-78-8; **4** (2-$SCOCH_3$, X = 3-Cl), 83596-87-6; **4** (2-$SCOCH_3$, X = 3-$CF_3$), 83596-79-6; **4** (2-$SCOCH_3$, X = 3-$OCH_3$), 83596-92-3; **4** (2-$SCOCH_3$, X = 3-$CH_3$), 83596-83-2; **4** (2-$SCOCH_3$, X = 3-$CH(CH_3)_2$), 92399-46-7; **4** (4-$SCOCH_3$), 24197-62-4; **4** (2-$SCOCH_3$, X = 3-F), 92399-47-8; **4** (2-$SCOCH_3$, X = 4-Cl), 92399-48-9; **4** (2-$SCOCH_3$, X = 5-Cl), 92399-49-0; **4** (2-$SCOCH_3$, X = 3,5-$Cl_2$), 92399-50-3; **4** (3-$SCOCH_3$), 90887-44-8; **5**, 78773-69-0; **6** (2-$SCOCH_3$), 83596-74-1; **6** (2-$SCOCH_3$, X = 3-F), 92399-51-4; **6** (2-$SCOCH_3$, X = 3-Cl), 83596-88-7; **6** (2-$SCOCH_3$, X = 3-$CF_3$), 92399-52-5; **6** (2-$SCOCH_3$, X = 3-$OCH_3$), 83596-93-4; **6** (2-$SCOCH_3$, X = 3-$CH_3$), 83596-84-3; **6** (2-$SCOCH_3$, X = 3-$CH(CH_3)_2$), 92399-53-6; **6** (2-$SCOCH_3$, X = 4-Cl), 92399-54-7; **6** (2-$SCOCH_3$, X = 5-Cl), 92399-55-8; **6** (2-$SCOCH_3$, X = 3,5-$Cl_2$), 92399-56-9; **6** (3-$SCOCH_3$), 92399-57-0; **6** (4-$SCOCH_3$), 92399-58-1; **7** (2-$SCOCH_3$, X = 3-Cl), 83596-89-8; **7** (2-$SCOCH_3$, X = 3-$CF_3$), 83596-81-0; **7** (2-$SCOCH_3$, X = 3-$OCH_3$), 83596-94-5; **7** (2-$SCOCH_3$, X = 4-Cl), 92399-59-2; **7** (2-$SCOCH_3$, X = 5-Cl), 92399-60-5; **7**(2-$SCOCH_3$, X = 3,5-$Cl_2$), 92399-61-6; **9**, 320-72-9; **10**, 54223-75-5; **12**, 83596-76-3; **13**, 83596-75-2; **14**, 92399-62-7; **15**, 92399-63-8; **16**, 92399-64-9; **17**, 83596-90-1; **18**, 83596-82-1; **19**, 83596-95-6; **20**, 83596-86-5; **21**, 83596-85-4; **22**, 92399-65-0; **23**, 92399-66-1; **24**, 92399-67-2; **25**, 92399-68-3; **26**, 92399-69-4; **27**, 92399-70-7; **28**, 92399-71-8; **29**, 92399-72-9; **30**, 92524-58-8; **31**, 92399-73-0; **32**, 92399-74-1; **33**, 92399-75-2; **34**, 92399-76-3; **35**, 70491-03-1; L-proline methyl ester hydrochloride, 2133-40-6; *N*-(2-acetylthiobenzoyl)-L-proline methyl ester, 92420-29-6; trimethylacetyl chloride, 3282-30-2; ethyl *N*-cyclopentylglycinate, 89479-61-8; phthalic anhydride, 85-44-9; 2-nitrobenzoic acid, 552-16-9; 2-nitrobenzoyl chloride, 610-14-0; 2-[(trimethylacetyl)thio]benzoic acid, 92399-77-4.

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