mM substrate in a total volume of 2.5 mL. At various times up to 30 min, aliquots (0.3 mL) were removed and quenched with MeOH (4.7 mL). After centrifugation (5000g, 10 min), the samples were diluted (1:1) with 0.01 M KH_2PO_4 and 20 μL of the resulting solution was injected directly onto the HPLC column for analysis.

$K_{M(app)}$ and V_{max} Determinations. The $K_{M(app)}$–V_{max} for the hydrolysis of *p*-NP-glc and *p*-NP-gal were determined with use of pooled cecal homogenates (200 mL) as described above. A range of substrate concentrations (56–1000 μM, final volume 2.25 mL), spanning their apparent K_M, was used for each reaction. The amount of cecal homogenate used was 0.04 mL. Reaction mixtures were incubated, in duplicate at 37 °C in a shaking water bath, and the reaction was stopped by addition of 0.2 N NaOH (0.25 mL) after 15 min. Release of *p*-nitrophenol was measured spectrophotometrically at 403 nm. Eadie–Hofstee plots were used to determine the $K_{M(app)}$ (μM) and V_{max} (μmol min^{-1} g^{-1}) of both reactions. The wet weight (g), measured immediately after removal and pooling, was used throughout.

The $K_{M(app)}$ and V_{max} were also measured for the hydrolysis of glycoside prodrugs **1**, **2**, **5**, **7**, and **9–12**. Again, cecal contents from four rats were pooled, weighed, diluted (100 mL, 0.01 μM phosphate buffer, pH 7.0), and homogenized. A range of substrate concentrations (0.5–48 μM, final volume 2.5 mL) spanning the apparent K_M was used for each reaction. The amount of cecal homogenate used was 0.8 mL. Reactions were run, in duplicate, at 37 °C in a shaking water bath. After 15 min, the reactions were stopped by removing aliquots (0.3 mL) and quenching them with MeOH (4.7 mL). Following centrifugation (5000g, 10 min), the samples were diluted (1:1) with 0.01 M KH_2PO_4 and 20 μL of the resulting solution was injected directly onto the HPLC column for analysis. Eadie–Hofstee plots were used to determine the $K_{M(app)}$ and V_{max}.

Determination of Apparent Partition Coefficients. The partitioning of prodrugs and free steroids between 1-octanol and an aqueous phase (0.01 M phosphate buffer, pH 7.0) were determined at 37 °C. Both octanol and buffer were saturated with the relevant aqueous or organic phase before use. Equal volumes (1.0 mL) of both phases were used and agitated for 30 min. The initial concentration of glycoside was 10 mM, dissolved in the aqueous phase. The initial concentration of steroid was 10 mM dissolved in the organic phase. The amount of glycoside and free steroid in the aqueous phase at equilibrium was measured spectrophotometrically at 239 nm for the dexamethasone and fludrocortisone compounds and 242 nm for the prednisolone and hydrocortisone compounds. The concentration of glycoside or free steroid in the octanol phase was determined by difference.

Note Added in Proof: After this manuscript was accepted, the authors learned of an earlier publication describing the synthesis of steroid glycoside prodrugs for release in the synovial fluid of arthritis victims (Hirschmann, R., Strachan, R. G.; Buchschacher, P.; Sarett, L. H.; Steelman, S. L.; Silber, R. *J. Am. Chem. Soc.* **1964**, *86*, 3903).

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Registry No. 1, 88158-43-4; 2, 88158-44-5; 3, 50-02-2; 4, 50-24-8; 5, 92901-21-8; 6, 50-23-7; 7, 92901-22-9; 8, 127-31-1; 9, 92901-23-0; 10, 92901-24-1; 11, 92901-25-2; 12, 92901-26-3; 13, 92901-27-4; 16, 92901-28-5; 17, 92901-29-6; 18, 92901-30-9; 19, 92901-31-0; 20, 92937-53-6; 21, 92901-32-1; 22, 92901-33-2; 23, 572-09-8; 24, 3068-32-4; 25, 14227-66-8; β-D-glucosidase, 9001-22-3; β-D-galactosidase, 9031-11-2.

Angiotensin-Converting Enzyme Inhibitors. New Orally Active Antihypertensive (Mercaptoalkanoyl)- and [(Acylthio)alkanoyl]glycine Derivatives[1]

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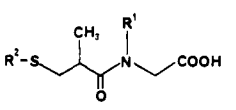
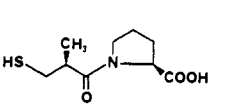
A variety of N-substituted (mercaptoalkanoyl)- and [(acylthio)alkanoyl]glycine derivatives was synthesized and their ability in inhibiting the activity of angiotensin-converting enzyme (ACE) was examined in vitro and in vivo. The acylthio derivatives prepared are assumed to act as prodrugs since they are much less active than the corresponding free SH compounds in vitro and can be expected to act in vivo only after conversion to the free sulfhydryl compounds. A number of these compounds are potent ACE inhibitors that lowered blood pressure in Na-deficient, conscious spontaneously hypertensive rats (SHR), a high renin model. One of the most active members of the series was (*S*)-*N*-cyclopentyl-*N*-[3-[(2,2-dimethyl-1-oxopropyl)thio]-2-methyl-1-oxopropyl]glycine (REV 3659-(S), pivopril). Structure–activity relationships are discussed.

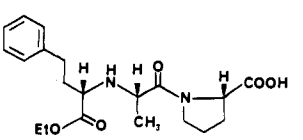
The renin–angiotensin–aldosterone system is an important humoral mechanism involved in the regulation of blood pressure[2-4] and renal function.[5] In particular, the development of antihypertensive drugs that act selectively by inhibiting angiotensin-converting enzyme[6,7] (ACE) has received much attention in recent years. Recently orally active ACE inhibitors have been reported to show promising clinical antihypertensive properties.[8-14] We now report the design and synthesis[15] of an orally active novel series of substituted (mercaptoalkanoyl)glycines of generic formula 1. Unlike the known inhibitors such as captopril (**2**)[6a,b] and enalapril (**3**),[7e] which embody a C-terminal proline, this series of compounds contains exclusively the nonchiral amino acid glycine.



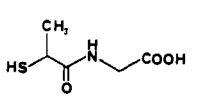
1



2 (captopril)



3 (enalapril)



4 (tiopronin)

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‡ Department of Biochemistry.
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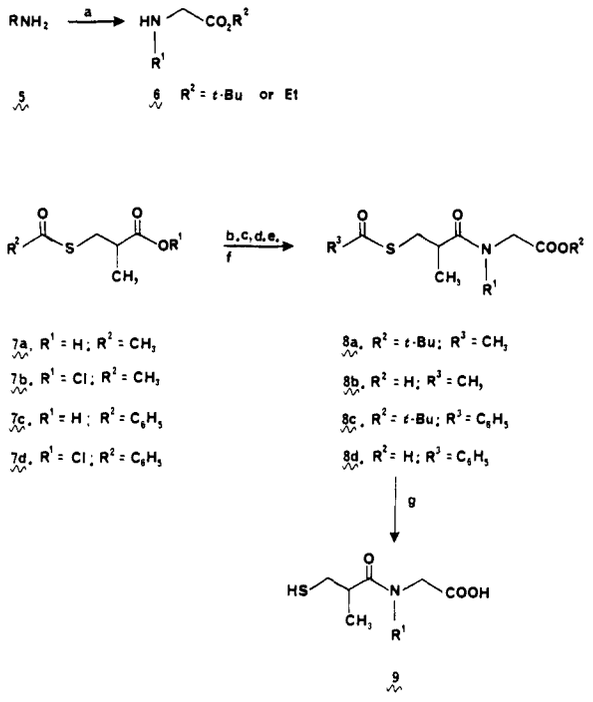
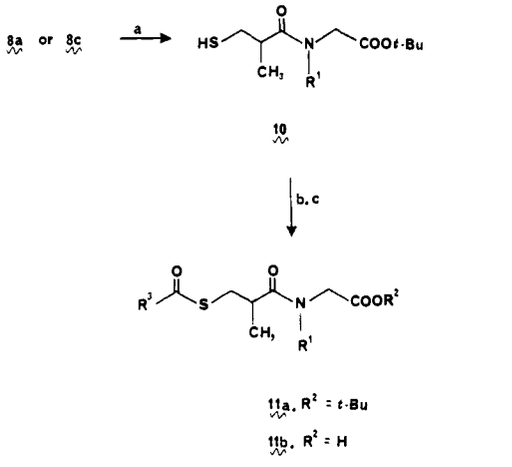
During the course of our investigation we observed that *N*-(2-mercaptopropionyl)glycine (**4**; tiopronin)[16] is a moderately active inhibitor (IC_{50} = 1.9 μM) of rabbit lung ACE in vitro, but the inhibitory activity is diminished in serum or in the presence of other peptidases. This is presumably due to the instability of the unsubstituted amide of tiopronin (**4**) to undergo cleavage by other hydrolytic enzymes. With this hypothesis in mind, a series of potent ACE inhibitory compounds was designed and synthesized in which the amide nitrogen was substituted by various alkyl and aromatic functionality. The compounds of interest are exemplified by the generic formula **1**. Our study differs from the design and synthesis of ACE inhibitors by Ondetti and co-workers, who reported that C-terminal proline was the amino acid that provided the maximum ACE inhibitory activities.[6a,b,17]

Scheme I. Synthesis of N-Substituted (3-Mercapto-2-methylpropanoyl)glycines[a]



[a] Reagents: a, $BrCH_2CO_2R^2$; b, 7a-toluene-$SOCl_2$-pyridine or DMF to give 7b or 7c-CH_2Cl_2-$SOCl_2$-DMF to give 7d; c, 7a-6-CH_2Cl_2-DCC or 7b-6-CH_2Cl_2-Et_3N to give 8a; d, 7d-6-CH_2Cl_2-Et_3N to give 8c; e, 8a-TFA-anisole or 8a-$(CH_3)_3SiI$-CH_2Cl_2 to give 8b; f, 8c-TFA-anisole to give 8d; g, 8b or 8d-anhydrous NH_3-CH_3OH.

Scheme II. Synthesis of Hindered Thio Esters of N-Substituted (3-Mercapto-2-methylpropanoyl)glycines[a]



[a] Reagents: a, 8a or 8c-anhydrous NH_3-CH_3OH; b, R^3COCl-CH_2Cl_2-Et_3N to give 11a; c, 11a-$(CH_3)_3SiI$-CH_2Cl_2.

Chemistry. The compounds of Table I were conven-

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(11) Gavras, H.; et al. *Lancet* **1981**, 543.
(12) Gavras, H.; Brunner, H. R.; et al. *N. Engl. J. Med.* **1978**, *298*, 991.
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(15) (a) Suh, J. T.; Skiles, J. W.; Williams, B. E.; Schwab, A. U.S. Patent 4 256 761, 1981. (b) Suh, J. T.; Skiles, J. W.; Williams, B. E.; Schwab, A. U.S. Patent 4 304 771, 1981.
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(17) (a) Cushman, D. W.; Ondetti, M. A. *Prog. Med. Chem.* **1980**, *17*, 41. (b) Cushman, D. W.; et al. *Experientia* **1973**, *29*, 1032. (c) Cushman, D. W.; Cheung, S. H.; Sabo, E. F.; Ondetti, M. A. *Prog. Cardiovasc. Dis.* **1978**, *21*, 176.

iently prepared as illustrated in Scheme I in an analogous manner to that reported by Cushman and Ondetti[6b] in which 3-(acetylthio)-2-methylpropionic acid (**7a**) was reacted with naturally occurring α-amino acids with use of dicyclohexylcarbodiimide (DCC) as the amide-generating reagent. In our study, non-naturally-occurring N-substituted glycines **6** were utilized. The appropriately substituted glycine esters **6** were prepared by treatment of known primary amines **5** with either *tert*-butyl bromoacetate or ethyl bromoacetate in a polar solvent such as ethanol or acetonitrile. The glycine esters **6** were normally obtained as oils which were used directly and were characterized by NMR, MS, and TLC analysis. In a manner similar to that previously described,[15,18] 3-(acetylthio)-2-methylpropionic acid (**7a**) was prepared by the addition of thiolacetic acid to methacrylic acid in a Michael fashion. The corresponding acid chloride **7b**[15] was prepared conveniently in toluene in the presence of thionyl chloride with a few added drops of pyridine or DMF as initiator. The appropriately substituted amino acid esters **6** were condensed with **7a** in CH_2Cl_2 or Et_2O with DCC as the amide-generating reagent to give **8a**. Alternatively the amides **8a** were also prepared with use of the acid chloride **7b** under standard Schotten–Baumann acylating conditions. In general, the crude amides **8a** were converted directly to the free carboxylic acids **8b** without further purification. In those instances in which **8a** were purified, the general method was high-performance LC using the solvent system of AcOEt/*n*-C_6H_{14} (5:95). The *tert*-butyl esters **8a** were deprotected with either trimethylsilyl iodide ($(CH_3)_3SiI$) in CH_2Cl_2 or by means of trifluoroacetic acid (TFA) in anisole, both at room temperature. In the case of the ethyl esters **8a**, treatment with ethanolic KOH gave directly the mercapto acids **9**. In general, the pure acids **8b** were obtained by high-performance LC over silica gel with the solvent system of *n*-C_6H_{14}/AcOEt/AcOH (60:40:1) as eluent. All acids **8b** were fully characterized by NMR, MS, and elemental analysis. In the case where the acids **8b** are liquids or low melting, the elemental analyses were generally performed on the corresponding dicyclohexylamine (DCHA) or benzathine salts. The free mercaptans **9** were generated from the thio esters **8b** by treatment with anhydrous NH_3 in CH_3OH followed by ion-exchange chromatography (AG-50W-X2, Bio-Rad Laboratories) using CH_3OH as the eluting solvent. The mercaptans **9** were fully characterized by means of NMR, MS, and combustion microanalysis.

In a few selected cases, hindered thio esters **11b**, such as neopentylcarbonyl and pivaloyl, were prepared in order to increase in vivo plasma stability and to decrease nucleophilic displacement of the thio ester carbonyl. These hindered esters were prepared as outlined in Scheme II. The thio esters **8a** were treated with anhydrous NH_3 in CH_3OH to give the mercaptans **10**. Alternatively, optically active amides **8c** were conveniently prepared by conversion of commercially available D-(−)-3-(benzoylthio)isobutyric acid (**7c**) to its corresponding acid chloride **7d** by means of $SOCl_2$ followed by treatment with the appropriately substituted glycine ester **6**. The thiobenzoyl ester **8c** was treated with anhydrous ammonia in CH_3OH to give the optically active thiol **10**. After purification the mercaptans **10** were treated with the appropriate acid chloride under standard Schotten–Baumann acylating conditions to give the hindered thio esters **11a**. The *tert*-butyl esters **11a** were deesterified in CH_2Cl_2 at room temperature by treatment with $(CH_3)_3SiI$ to afford the acids **11b**.

The mercapto acids **9** and the corresponding thio esters **8b** and **11b** which were synthesized and evaluated for ACE inhibition are listed in Table I. Of the over 400 variants of structure **1** prepared, we report hereto approximately 70 representative alkanoylglycines in which the glycine nitrogen is alkylated with various substituents including alkyl, cycloalkyl, bicycloalkyl, aryl, alkynyl, and heterocyclic groups.

Results and Discussion

The compounds presented in Table I represent an important novel class of N-substituted glycines that are very potent and specific competitive inhibitors of ACE in vitro and in vivo. This series of compounds has demonstrated potential as therapeutic agents for hypertension[14] and congestive heart failure. The in vitro IC_{50} values of the most active mercaptans, **17**, **21**, **23**, **25**, **27**, **29**, **37**, **57**, **59**, **63**, and **68**, are in the range of 0.0050–0.035 μM. These values are similar to the IC_{50} obtained in our laboratories for captopril (**2**), IC_{50} = 0.017 μM.

In order to increase the in vitro potency of tiopronin (**4**),[16] which is an (α-mercaptoalkanoyl)glycine, we proceeded to systematically design a series of (β-mercaptoalkanoyl)glycines. It was previously noted by Cushman and Ondetti that (β-mercaptoalkanoyl)prolines are much more potent inhibitors of ACE than their α counterparts.[6b] Upon preparation and evaluation of the glycine analogue **12** in vitro, an IC_{50} of 0.21 μM was obtained. This is to be compared with an IC_{50} of 1.9 μM for tiopronin (**4**). Upon proceeding to substitute the nitrogen of **12** by various alkyl functionalities, **13–15**, **39**, and **40**, the ACE inhibitory IC_{50} values proceeded to decrease from 0.21 μM for **12** to 0.072 μM for the isopropyl analogue **15** and to 0.055 μM for the thio ether **40**. The isopropyl analogue **15** appeared promising and gave us the incentive to prepare the cyclopropyl analogue **17**. The IC_{50} of **17** (0.030 μM) relative to that of **15** (0.072 μM) decreased by a factor of 2–3. With this encouraging result, a series of N-substituted monocycloalkyl analogues **17**, **21**, **23a**, **25**, and **27** was prepared in which the ring varied from cyclopropyl to cycloheptyl. In this series the maximum activity appeared to reside in the cyclobutyl **21** and cyclopentyl **23a** ring systems. The next logical course of action to follow in our systematic design was to prepare a series of *N*-bicycloalkyl-substituted analogues: **29–36**. Suprisingly it was found that the *exo*-norbornyl thio ester **30** was a potent inhibitor of purified rabbit lung ACE having an average IC_{50} of 0.020 μM over many different experiments. This is to be compared with an IC_{50} of 0.032 μM for the thiol **29**. This result was unlike the other analogues of our series in which the acetyl thio esters were a factor of 10 or more less potent than their respective mercaptans when tested in purified rabbit lung ACE.

A series of heterocycloalkyl derivatives, **42**, **44**, **46**, **48**, and **50**, was prepared that exhibited little or no substantial increases in inhibitory potency over the unsubstituted glycine analogue **12** or any of the other substituted analogues. The thienyl derivative **46** had the greatest potency in this series (IC_{50} = 0.055 μM).

A series of *N*-aryl derivatives, **53**, **55**, **57**, **59**, **61**, **63**, **65**, **66**, **68**, **70**, and **72**, was prepared and evaluated. This series was very fruitful in producing the most active member of the compounds prepared by us. The in vitro IC_{50} values of this series ranged from a low of 0.30 μM for the *N*-phenyl analogue **53** to 0.0050 μM for the *p*-tolyl analogue **59**. The *p*-tolyl derivative **59** exhibited the maximum in vitro potency of all of the inhibitors of generic formula **1** prepared by us.

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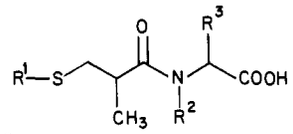
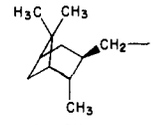
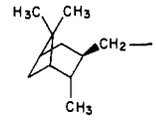
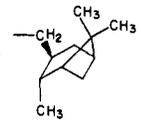
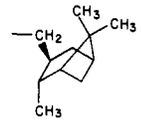
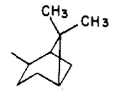
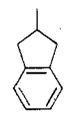
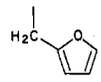
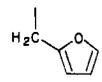
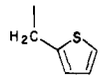
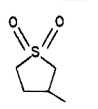
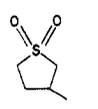
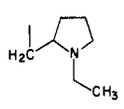
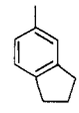
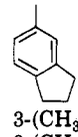
Table I. N-Substituted Mercaptopropanoylglycines and Inhibition of ACE in Vitro



compd[a]	R^1	R^2	R^3	mp,[b] °C	yield,[c] %	procedure[d]	formula[e]	remarks	IC_{50},[f] μM
12	H	H	H	115–117	92	I	$C_6H_{11}NO_3S$		0.21
13	H	CH_3	H	71–73	90	I	$C_7H_{13}NO_3S$		0.13
14	H	C_2H_5	H	131–132	93	I	$C_8H_{15}NO_3S$	DCHA[g]	0.075
15	H	$(CH_3)_2CH$	H	159–160	95	I	$C_9H_{17}NO_3S$	DCHA[g]	0.072
16	CH_3CO	$(CH_3)_2CH$	H	104–105	72	B, D, F	$C_{11}H_{19}NO_4S$		5.9
17	H	c-C_3H_5	H	89–91	84	I	$C_9H_{15}NO_3S$		0.030
18	CH_3CO	c-C_3H_5	H	68–70	61	A, D, F	$C_{11}H_{17}NO_4S$	DCHA[g]	0.54
19	H	c-C_3H_5	CH_3	129–130.5	72	I	$C_{10}H_{17}NO_3S$	DCHA[g]	0.079
20	CH_3CO	c-C_3H_5	CH_3	83–85	42	B, D, F	$C_{12}H_{19}NO_4S$		0.22
21	H	c-C_4H_7	H	liquid	82	I	$C_{10}H_{17}NO_3S$		0.018
22	CH_3CO	c-C_4H_7	H	162.5–164.5	72	B, E, G	$C_{12}H_{19}NO_4S$	DCHA[g]	0.22
23a (*R* + *S*)	H	c-C_5H_9	H	173–176[h]	87	I	$C_{11}H_{18}NO_3S$	calcium salt	0.018[i]
23b (*S*)[j]	H	c-C_5H_9	H	186–188	89 (82)	B, E, G, I (M, N)	$C_{11}H_{18}NO_3S$	calcium salt	0.016
24	CH_3CO	c-C_5H_9	H	172–174	75	B, E, G	$C_{13}H_{21}NO_4S$	DCHA[g]	0.082
25	H	c-C_6H_{11}	H	158–160[k]	92	I	$C_{12}H_{21}NO_3S$	DCHA[g]	0.035[l]
26	CH_3CO	c-C_6H_{11}	H	142–144	83	B, E, G	$C_{14}H_{23}NO_4S$	DCHA[g]	0.27
27	H	c-C_7H_{13}	H	oil[m]	88	I	$C_{13}H_{23}NO_3S$		0.031[n]
28	CH_3CO	c-C_7H_{13}	H	116–117	86	B, E, G	$C_{12}H_{25}NO_4S$	DCHA[g]	0.088
29[o]	H		H	120–122	96	I	$C_{13}H_{21}NO_3S$	DCHA[g]	0.032
30[o]	CH_3CO		H	125–126[p]	86	B, D, F	$C_{15}H_{23}NO_4S$	DCHA[g]	0.020
31[q]	CH_3CO		H	116	21	B, D, F	$C_{15}H_{23}NO_4S$	DCHA[g]	0.052
32	H		H	glass	87	I	$C_{17}H_{29}NO_3S$		0.44
33	CH_3CO		H	117	77	C, D, F	$C_{19}H_{31}NO_4S$		0.085
34	H		H	glass	84	I	$C_{17}H_{29}NO_3S$		0.16
35	CH_3CO		H	120	84	C, D, F	$C_{19}H_{31}NO_4S$		0.14
36	CH_3CO		H	134–138	63	C, D, F	$C_{17}H_{27}NO_4$	DCHA[g]	0.045
37	H		H	186–188[r]	95	I	$C_{15}H_{19}NO_3S$	DCHA[g]	0.031[s]
38	CH_3CO		H	149–150	75	B, E, G	$C_{17}H_{21}NO_4S$	DCHA[g]	0.34
39	H	$CH_3OCH_2CH_2$	H	129–132	90	B, E, G	$C_9H_{17}NO_4S$	DCHA[g]	0.095
40	H	$CH_3S(CH_2)_3$	H	122–128	91	I	$C_{10}H_{19}NO_3S_2$	DCHA[g]	0.055
41	CH_3CO	$CH_3S(CH_2)_3$	H	120–121	82	B, E, G	$C_{12}H_{21}NO_4S_2$	DCHA[g]	0.90
42	H		H	128–130	64	I	$C_{11}H_{19}NO_4S$	DCHA[g]	0.13
43	CH_3CO		H	138–140	80	B, D, F	$C_{13}H_{21}NO_5S$	DCHA[g]	1.9

compd[a]	R^1	R^2	R^3	mp,[b] °C	yield,[c] %	procedure[d]	formula[e]	remarks	IC_{50},[f] μM
44	H		H	150–153	80	I	$C_{11}H_{15}NO_4S$	DCHA[g]	0.17
45	CH_3CO		H	140–141	44	B, E, F	$C_{13}H_{17}NO_5S$	DCHA[g]	0.70
46	H		H	122–128	82	I	$C_{10}H_{19}NO_3S_2$	DCHA[g]	0.055
47	CH_3CO		H	149.5–150.5	49	B, E, G	$C_{13}H_{17}NO_4S_2$	DCHA[g]	0.75
48	H		H	38–40	90	I	$C_{10}H_{17}NO_5S_2$		0.28
49	CH_3CO		H	191–193	85	B, E, G	$C_{12}H_{19}NO_6S_2$	DCHA[g]	0.28
50	H		H	120–122	57	B, D, H	$C_{13}H_{24}N_2O_3S$	DCHA[g]	0.64
51	H	$CH{\equiv}CHCH_2$	H	164–166	91	I	$C_9H_{13}NO_3S$	DCHA[g]	0.27
52	CH_3CO	$CH{\equiv}CHCH_2$	H	154–156	62	B, E, G	$C_{11}H_{15}NO_4S$	DCHA[g]	4.5
53	H	C_6H_5	H	168–170[t]	90	I	$C_{14}H_{17}NO_4S$		0.30[u]
54	CH_3CO	C_6H_5	H	94–94.5	66	B, D, F	$C_{14}H_{17}NO_4S$		0.30
55	H	2-$(CH_3)C_6H_4$	H	97–101	89	I	$C_{13}H_{17}NO_3S$	Benz[v]	0.12
56	CH_3CO	2-$(CH_3)C_6H_4$	H	128–130	91	B, E, G	$C_{15}H_{19}NO_4S$	Benz[v]	0.55
57	H	3-$(CH_3)C_6H_4$	H	121–122	93	I	$C_{13}H_{17}NO_3S$	Benz[v]	0.019
58	CH_3CO	3-$(CH_3)C_6H_4$	H	104–105	87	B, E, G	$C_{15}H_{19}NO_4S$	Benz[v]	0.075
59	H	4-$(CH_3)C_6H_4$	H	134–137	95	I	$C_{13}H_{17}NO_3S$	Benz[v]	0.0050
60	CH_3CO	4-$(CH_3)C_6H_4$	H	146–148	84	B, E, G	$C_{15}H_{19}NO_4S$	Benz[v]	0.13
61	H	3,5-$(CH_3)_2C_6H_3$	H	125–126	96	I	$C_{14}H_{19}NO_3S$		0.044
62	CH_3CO	3,5-$(CH_3)_2C_6H_3$	H	89–92	90	B, E, G	$C_{16}H_{21}NO_4S$		0.044
63	H		H	164–167	92	I	$C_{15}H_{19}NO_3S$	Benz[v]	0.033
64	CH_3CO		H	117–118	82	B, E, G	$C_{17}H_{21}NO_4S$	Benz[v]	0.048
65	CH_3CO	3-$(CH_3O)C_6H_4$	H	103–105	80	B, E, G	$C_{15}H_{19}NO_5S$	Benz[v]	0.11
66	CH_3CO	3-$(CH_3S)C_6H_4$	H	110–112	72	B, E, G	$C_{15}H_{19}NO_4S_2$	Benz[v]	0.075
67	CH_3CO	3-FC_6H_4	H	oil	68	B, E, G	$C_{14}H_{16}FNO_4S$		0.051
68	H	4-FC_6H_4	H	155[w]	92	I	$C_{12}H_{14}FNO_3S$		0.023[x]
69	CH_3CO	4-FC_6H_4	H	oil	82	B, E, G	$C_{14}H_{16}FNO_4S$		0.60
70	H	4-$(n\text{-}C_4H_9)C_6H_4$	H	137–145[y]	88	I	$C_{16}H_{23}NO_3S$	Benz[v]	0.19[z]
71	CH_3CO	4-$(n\text{-}C_4H_9)C_6H_4$	H	151–153	66	B, E, G	$C_{18}H_{25}NO_4S$	Benz[v]	0.064
72	CH_3CO	4-$(i\text{-}C_3H_7)C_6H_4$	H	144	86	B, E, G	$C_{17}H_{23}NO_4S$	Benz[v]	0.060
73	$(CH_3)_3CCH_2CO$	c-C_5H_9	H	85–87	82	E, J, K, L	$C_{17}H_{29}NO_4S$		15
74a[aa]	$(CH_3)_3CCO$	c-C_5H_9	H	140–142	80	E, J, K, L	$C_{16}H_{27}NO_4S$		3.70
74b(*R*)[a,b]	$(CH_3)_3CCO$	c-C_5H_9	H	156	62	E, J, K, L	$C_{16}H_{27}NO_4S$		>100
74c(*S*)[ac] (pivopril)	$(CH_3)_3CCO$	c-C_5H_9	H	155–156	75.1	E, J, K, L	$C_{16}H_{27}NO_4S$		3.60
2 (captopril)									0.017[ad]
3 (enalapril)									8.0[ae]
4 (tiopronin)									1.9[af]

[a] Except where indicated all compounds are racemic. [b] Uncorrected. [c] Yield refers to the last step in each synthetic sequence. [d] See Experimental Section. [e] All compounds had satisfactory C, H, and N microanalyses and were within 0.4% of theoretical values. All compounds exhibited IR, ^1H NMR, and MS spectra consistant with the assigned structures. [f] Concentration inhibiting 50% of the activity of rabbit lung ACE at pH 8.3 in 0.10 M potassium phosphate buffer containing 0.30 M NaCl with the substrate Hip-His-Leu at a concentration of 2 mM. [g] Dicyclohexylamine (DCHA) salt. [h] Literature[6n] mp (DCHA) 143–144 °C. [i] Literature[6n] IC_{50} = 0.007 μM. [j] Corresponds to *S* isomer, $[\alpha]_D$ −12.50° (*c* 1.0, $CHCl_3$). [k] Literature[6n] mp (DCHA) 160–162 °C. [l] Literature[6n] IC_{50} = 0.0075 μM. [m] Literature[6n] mp (DCHA) 143–145 °C. [n] Literature[6n] IC_{50} = 0.0071 μM. [o] Corresponds to exo isomer. [p] Calcium salt mp 157–161 °C. [q] Corresponds to endo isomer. [r] Literature[6n] mp (DCHA) 180–183 °C. [s] Literature[6n] IC_{50} = 0.012 μM. [t] Literature[6n] mp 170–171 °C. [u] Literature[6n] IC_{50} = 0.013 μM. [v] Benzathine salt, *N,N'*-dibenzylethylenediamine. [w] Literature[6n] mp 163–165 °C. [x] Literature[6n] IC_{50} = 0.011 μM. [y] Literature[6n] mp (DCHA) 124–126 °C. [z] Literature[6n] IC_{50} = 0.056 μM. [aa] Corresponds to a 1:1 mixture of the *R* and *S* isomers, REV 3659. [ab] Corresponds to *R* isomer, $[\alpha]_D$ +111.05° (*c* 1.0, $CHCl_3$). [ac] Corresponds to *S* isomer, $[\alpha]_D$ −104.64° (*c* 1.0, $CHCl_3$). Corresponds to REV 3659-(S). [ad] Literature[6b] IC_{50} = 0.023 μM. [ae] Literature[7e] IC_{50} = 1.2 μM. [af] Literature[6b] IC_{50} = 1.7 μM.

Table II. Angiotensin-Converting Enzyme (ACE) Inhibition of Selected Compounds in Conscious Normotensive Rats[a]

compd	ID_{50},[b] mg/kg,po	compd	ID_{50},[b] mg/kg,po
17	0.30	**30**	0.06
18	0.20	**37**	0.15
21	0.30	**41**	1.5
23a	0.15	**54**	0.2
24	0.15	**58**	0.15
25	0.10	**60**	0.15
27	0.05	**64**	1.5
28	0.06	**2** (captopril)	0.10[c]
29	0.10	**3** (enalapril)	0.08[d]

[a] See Experimental Section. [b] Dose (mg/kg, po) required to inhibit 50% of the angiotensin I induced vasopressor response in normotensive conscious rats. [c] Literature[23] ID_{50} = 0.015 mg/kg, iv. [d] Literature[7e] ID_{50} = 0.014 mg/kg, iv.

The very potent in vitro ACE inhibitory activities of the N-substituted alkanoylglycines (see Table I) were equally confirmed by oral administration in rats as shown in Table II. The oral ID_{50} values of 0.05–1.5 mg/kg were exhibited by the in vitro active thiols **17**, **21**, **23a**, **25**, **27**, **29**, and **37** as well as the thio esters **18**, **22**, **24**, **26**, **28**, **30**, **41**, **54**, **58**, **60**, and **64**. The compounds **23a** (ID_{50} = 0.15 mg/kg, po), **24** (ID_{50} = 0.15 mg/kg, po), and **28** (ID_{50} = 0.06 mg/kg, po) were among the most active and their oral activities are comparable to captopril (**2**) (ID_{50} = 0.10 mg/kg, po) when compared in our laboratories.

The most common side effects accompanying the clinical use of captopril are rashes and loss of taste, both of which usually clear on withdrawal or reduction of dose.[19] In the search for clinically useful potent and specific inhibitors of ACE lacking a free mercapto functionality, which has been reported to be associated with these common side effects, we selected the *N*-cyclopentyl compound **23a** for further development. A large number of analogues was prepared by this laboratory in which the free thiol of **23a** was acylated and alkylated by a wide range of functionality in order that relatively low levels of the free SH compound be generated in vivo while maintaining inhibition of ACE. Most of these prodrugs are much less active than the corresponding free SH compound in vitro and can be expected to act in vivo only after conversion to the free sulfhydryl compounds. Of the many analogues of **23a** prepared by us, the pivaloyl thio ester **74c** was selected for extensive preclinical and clinical development. The hindered pivaloyl thio ester **74a** was prepared from **8a** or **8c** according to Scheme II. The ability to inhibit the pressor response to angiotensin I for the pivaloyl thio ester **74a** and its corresponding free mercaptan **23a** in rats and dogs is presented in Table III.

A stereospecific synthesis of the *S* and *R* enantiomers of **23a** was efficiently achieved by the method described in Scheme I utilizing optically pure thio ester **7a** or **7c**.[20] As shown in Table III the pivaloyl thio ester **74c** with *S* configuration is considerably more potent both in vitro and in vivo in inhibiting ACE than the corresponding thio ester **74b** with *R* configuration but less potent than the free SH form **23a**.

In order to obtain information on the potential stability of an ACE inhibitor in vivo, an aliquot of inhibitor solution (20 μM) in Me_2SO was diluted 20-fold with rat and human plasma incubated at 37 °C. At various times, 0.05-mL aliquots were removed and assayed immediately for thiol content. Thiol concentrations were determined at pH 7.4 according to a procedure described in the literature.[21] In vitro the thio ester **74a** was stable to hydrolysis by rat and human plasma (22 h, 37 °C) and rat gastric juice (24 h, 37 °C). These results tend to suggest that the sites of liberation of the active drug **23a** from the parent thio ester **74a** may be affected in a novel manner by the resistance to plasma and gastric juice enzymes. A detailed comparison of the thio ester **74a** with its possible metabolite **23a** is described elsewhere.[22a]

Table III. ACE Inhibitory Activities of *N*-Cyclopentylalkanoylglycines and Comparison with Captopril (**2**) and Enalapril (**3**) in Vitro and in Vivo

compd	IC_{50},[a] μM in vitro	ID_{50},[b] mg/kg, po, normotensive rats	ID_{50},[c] mg/kg, po, normotensive dogs
23a (*R* + *S*)	0.018	0.15	
23b (*S*)	0.018		
73	15	1.2	
74a(*R* + *S*)[d]	3.70	0.15	
74b(*R*)	100	25	
74c(*S*) (pivopril)	3.60	0.058	0.17
2 (captopril)	0.027[e]	0.10[f]	0.057
3 (enalapril)	8.0[h]	0.08[i]	0.10[j]

[a] Concentration inhibiting 50% of the activity of rabbit lung ACE at pH 8.3 in 0.10 M potassium phosphate buffer containing 0.30 M NaCl with the substrate Hip-His-Leu at a concentration of 2 mM. [b] Dose (mg/kg, po) required to inhibit 50% of the angiotensin I induced vasopressor response in normotensive conscious rats. [c] Dose (mg/kg, po) required to inhibit 50% of the angiotensin I induced vasopressor response in normotensive conscious dogs. [d] Corresponds to REV 3659. [e] Literature[6b] IC_{50} = 0.023 μM. [f] Literature[26] ID_{50} = 0.015 mg/kg, iv. [g] Literature[26] ID_{50} = 0.044 mg/kg, po. [h] Literature[7e] IC_{50} = 1.2 μM. [i] Literature[7e] ID_{50} = 0.014 mg/kg, iv. [j] Literature[7e] ID_{50} = 0.278 mg/kg, iv.

The new ACE inhibitor (*S*)-*N*-cyclopentyl-*N*-[3-[(2,2-dimethyl-1-oxopropyl)thio]-2-methyl-1-oxopropyl]glycine (**74c**) has been given the generic name of pivopril and corresponds to REV 3659-(S). This compound has shown promise both preclinically and clinically to be a potent and specific inhibitor of ACE while also being a antihypertensive. Herein we report some of pivopril's (**74c**) biological properties.

A comparison was made between pivopril (**74c**) and captopril (**2**) to determine their abilities to inhibit ACE in vivo, as judged by the inhibition of angiotensin I pressor responses in the conscious normotensive rat.[22b] Both pivopril and captopril inhibited angiotensin I pressor responses within 20 min after oral dosing in a dose-related fashion. Oral ID_{50} values for pivopril and captopril were 0.058 and 0.10 mg/kg, respectively. The intensity and duration of action for both pivopril and captopril were dose related and similar to each other except at 0.3 mg/kg. After intravenous administration pivopril had similar potency, and onset of action, as it did after oral administration. At 100 mg/kg, po, neither pivopril (**74c**) nor captopril (**2**) affected the pressor responses to angiotensin II or norepinephrine or lowered arterial pressure.

From the above study it is concluded that pivopril (**74c**) is a rapidly absorbed, orally effective inhibitor of angiotensin I pressor responses in the normotensive rat. Its

(19) (a) Atkinson, A. B.; Robertson, J. I. S. *Lancet II* **1979**, 836. (b) Parfrey, P. S.; Clement, M.; Vandenburg, M. J.; Wright, P. *Br. Med. J.* **1980**, *281*, 194. (c) Editorial *Lancet* **1980**, *2*, 129.

(20) (a) Optically pure *S* and *R* isomers of acetyl-β-mercaptoisobutyric acid (**7a**) were obtained from Chemical Dynamics Corp., South Plainfield, NJ 07080. (b) Optically pure (*S*)-3-(Benzoylthio)isobutyric acid was obtained from Chemical Dynamics Corp., South Plainfield, NJ 07080.

(21) Ellman, G.; Lysko, H. *Anal. Biochem.* **1979**, *93*, 98.

(22) (a) Schwab, C.; Weinryb, I.; Macerata, R.; Rogers, W.; Suh, J. T.; Khandwala, A. *Pharmacologist* **1983**, *25*, 241. (b) Wolf, P. S.; Mann, W. S.; Perone, M.; Suh, J. T.; Smith, R. D. *Pharmacologist* **1982**, *25*, 176.

mechanism of action is consistent with inhibition of ACE by its free SH hydrolysis product. On the basis of efficacy in inhibiting angiotensin I pressor responses, and onset of action, pivopril (**74c**) is approximately as active as captopril (**2**).

The effects of oral administration of pivopril (**74c**) and captopril (**2**), 1–100 mg/kg, on mean arterial pressure and heart rate were studied in spontaneously hypertensive rats (SHR) maintained on a sodium-deficient diet.[22b] Arterial pressures and heart rates were monitored via catheters from 11 groups of seven to nine freely roaming SHRs. Measurements were taken for 30 min before dosing and for 22 h thereafter. The SHRs were given either test compound at doses of 1, 3, 10, 30, or 100 mg/kg or equivalent volumes of the vehicle.

Both pivopril (**74c**) and captopril (**2**) caused dose-related decreases in arterial pressure over the entire dose range. At each dose, the peak effects of the two compounds were not significantly different. At 3 and 10 mg/kg, but not at other doses, the duration of captopril's antihypertensive effect was about 6 h longer than that of pivopril (**74c**). From 15 min after dosing to the completion of the experiment, heart rates tended to decrease in all groups. This decrease was least in rats treated with the 100 mg/kg doses of either compound. From 20 to 22 h after dosing, heart rates in rats treated with pivopril at 100 mg/kg were significantly higher than those in vehicle-treated rats. Heart rates in rats treated with captopril at 100 mg/kg were higher than those of the control animals at hours 10–22. Ten milligrams/kilogram, po, was the dose of pivopril (**74c**) or captopril (**2**) that decreased mean arterial pressure in the SHR by 25%.

From this experiment it is concluded that pivopril (**74c**) is an orally effective antihypertensive agent in the sodium-deficient SHR at doses of 1 mg/kg and higher. The potency and duration of pivopril (**74c**) are similar to those of the reference ACE inhibitor, captopril (**2**).

A comparison was made of the effects of pivopril (**74c**) and captopril (**2**) on the pressor responses to angiotensin I in conscious dogs.[22b] Antagonism of the pressor response to angiotensin I, 0.25 μg/kg, iv, by oral administration of pivopril (**74c**) and captopril, 0.01–1 mg/kg, was studied in six conscious normotensive dogs. Captopril, pivopril, and the vehicle alone (0.3 M KH_2PO_4 buffer) were given to all six dogs at weekly intervals in a three-way crossover design.

Neither pivopril (**74c**) nor captopril (**2**) produced a significant effect at 0.01 mg/kg, but at doses of 0.03 mg/kg and larger, there was a dose-related inhibition of the pressor response to angiotensin I. From the regression analysis of the dose relationships, the doses that caused a 50% inhibition (ID_{50}) were 0.057 and 0.170 mg/kg for captopril (**2**) and pivopril (**74c**), respectively.

The new ACE inhibitor designated pivopril-(RS) (**74a**; REV 3659) was tested in 15 healthy male volunteers to study the ability of oral single doses of pivopril (5, 10, 20, 40, or 80 mg) to inhibit the pressor acitivity of exogenous angiotensin I and ACE activity.[23]

No clinically significant changes in the laboratory chemistries were observed after administration of pivopril-(RS) (**74a**). The results of this study indicate that **74a** is a potent ACE inhibitor, with no apparent adverse effects after single oral administration of doses up to 80 mg, po, to normal male subjects.

(23) (a) Burnier, M.; Turini, G. A.; Brunner, H. R.; Porchet, M.; Kruithof, D.; Vukovich, R. A.; Gavras, H. *Br. J. Clin. Pharmacol.* **1981**, *12*, 893. (b) Burnier, M.; Biollaz, J.; Brunner, H. R.; Turini, G. A.; Gavras, H. *Am. J. Cardiol.* **1982**, *49*, 1550.

Pivopril-(RS) (**74a**) was also investigated for safety and efficacy in mild to moderate hypertensive patients.[14] The oral administration of pivopril (10, 20, or 50 mg, tid) to 29 patients for 4 weeks reduced the supine diastolic blood pressure significantly. Pivopril (**74a**) exhibited no clinically significant changes in laboratory parameters.

In conclusion, N-substitution of *N*-(3-mercaptopropanoyl)glycines by alkyl, cycloalkyl, bicycloalkyl, aryl, alkynyl, and heterocyclic groups has resulted in an active series of inhibitors of ACE comparable in potency to the known mercapto inhibitor captopril (**2**). Unlike the known inhibitors of ACE which embody a C-terminal proline, this new series of N-substituted amides is constructed exclusively from the nonchiral amino acid glycine. Through structure–activity relationships this study has culminated in the design and development of pivopril (**74c**) as a potentially efficacious therapeutic agent for the treatment of cardiovascular diseases involving increased blood pressure or reduced vital organ perfusion due to excessive release of renin and angiotensin II.

Experimental Section

Chemistry. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Chemical microanalyses for carbon, hydrogen, and nitrogen were determined with a Perkin-Elmer Model 240 B or 240 XA elemental analyzer and are within ±0.4% of theoretical values. Solid samples were purified by recrystallization and dried in vacuo at appropriate temperatures. IR spectra were obtained with a Perkin-Elmer 589 or 298 spectrophotometer. Solid samples were taken in KBr pellets. Liquid samples were taken neat on NaCl salt plates. ^1H NMR spectra were determined with Varian EM-390 (90 MHz) or EM-360 (60 MHz) instruments using $CDCl_3$ as solvent and $(CH_3)_4Si$ as an internal standard. Low-resolution mass spectra were recorded with a Varian MAT 112 GC–MS equipped with an SS 100 data system at ionization potential of 70 eV. Optical rotations were determined at λ 589 (sodium D line) in $CHCl_3$ with a Perkin-Elmer 241 polarimeter. TLC separations were conducted with E. Merck silica gel 60 F-254 plates of 0.25-mm thickness and were visualized with UV, I_2, or sodium nitroprusside spray reagent (for detection of mercaptans and thio esters). Preparative high-performance LC separations were determined on a Waters Prep LC/System 500 instrument.

3-(Acetylthio)-2-methylpropionic Acid (7a).[15,18] Thiolacetic acid (1000 g, 13.2 mol) was placed in a 5-L round-bottom flask and cooled in an ice–water bath to approximately 5 °C. Methacrylic acid (610 g, 7.09 mol) was added dropwise with vigorous stirring. Cooling was continued for 15 min and then the reaction mixture was heated to a gentle reflux for 1 h. Stirring was continued at room temperature for 6 days. Excess thiolacetic acid was removed in vacuo and the residue was dissolved in $CHCl_3$ and washed four times with H_2O and dried over $MgSO_4$. Filtration and evaporation of the solvent yielded a yellowish-orange oil which was vacuum distilled at 110 °C (0.1 mmHg) to give **7a** initially as a yellow oil which slowly crystallized. Addition of Et_2O and filtration of the product afforded a pale yellow solid (890 g, 77.5%): mp 35–37 °C. Anal. ($C_6H_{10}O_3S$) C, H, N.

3-(Acetylthio)-2-methylpropionyl Chloride (7b).[15] To a solution of **7a** (6.3 g, 38.9 mmol) dissolved in toluene (50 mL) was added a few drops of pyridine. Thionyl chloride (10 mL) was added in one portion and the resulting mixture was stirred at room temperature for $1^1/_2$ h. The toluene was evaporated on a rotary evaporator and H_2O was added to the residue. The product was extracted three times with $CHCl_3$. The combined $CHCl_3$ extract was washed twice with H_2O. The $CHCl_3$ was dried over $MgSO_4$, filtered, and evaporated to afford (6.9 g, 98.3%) of **7b** as a pale yellow oil which was used directly without further purification.

Method A. *N*-Cyclopropylglycine *tert*-Butyl Ester (6, R^1 = c-C_3H_5; R^2 = *t*-Bu). Cyclopropylamine (19.5 g, 0.342 mol) was placed in a pressure bottle and EtOH (100 mL) was added. To the resulting solution was added *tert*-butyl bromoacetate (15.5 g, 0.0795 mol). The flask was stoppered, and the contents of the flask were stirred overnight at room temperature. Most of the solvent was removed on a rotary evaporator and H_2O was added

to the residue. The product was extracted several times with $CHCl_3$. The combined $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4), filtered, and evaporated to yield **6** as a pale yellow oil (12.5 g, 92%) which was used directly without further purification.

Method B. *N*-Cyclobutylglycine *tert*-Butyl Ester (6, R^1 = c-C_4H_7; R^2 = *t*-Bu). An ethanolic solution of *tert*-butyl bromoacetate (39 g, 0.2 mol) was added dropwise to a chilled ethanolic solution containing cyclobutylamine (28.4 g, 0.4 mol) and triethylamine (25.3 g, 0.25 mol). After stirring overnight at room temperature, the reaction mixture was concentrated and the residue redissolved in CH_2Cl_2 (300 mL). The solution was washed with H_2O (3 × 200 mL), dried ($MgSO_4$), filtered, and evaporated to dryness, yielding the titled compound as a light tan oil (24.9 g, 67%) which was used directly without further purification.

Method C. *N*-Nopinylglycine *tert*-Butyl Ester (6, R^1 = nopinyl; R^2 = *t*-Bu). Nopinylamine (64.2 g, 0.461 mol) was dissolved in a mixture of CH_3CN (250 mL), H_2O (110 mL), and concentrated NH_4OH (110 mL). *tert*-Butyl bromoacetate (90.4 g, 0.464 mol) in EtOH (200 mL) was added dropwise. The resulting mixture was stirred at room temperature overnight. Most of the solvent was evaporated and H_2O was added to the residue. The product was extracted several times with $CHCl_3$. The combined $CHCl_3$ extract was washed twice with H_2O, dried over $MgSO_4$, filtered, and evaporated to yield the titled product as a yellow oil (93.2 g). The product was purified by high-performance LC (AcOEt/*n*-C_6H_{14}/AcOH, 30:60:1) to give the pure product as a colorless oil (72 g, 62%). Anal. ($C_{15}H_{27}NO_2$) C, H, N.

Method D. *N*-[3-(Acetylthio)-2-methylpropanoyl]-*N*-cyclopropylglycine *tert*-Butyl Ester (8a, R^1 = c-C_3H_5; R^2 = *t*-Bu). To a solution of *N*-cyclopropylglycine *tert*-butyl ester (12 g, 0.072 mol) and 3-(acetylthio)-2-methylpropionic acid (**7a**; 8.1 g, 0.050 mol) in CH_2Cl_2 (200 mL) chilled in an ice–water bath was added DCC (14.4 g, 0.070 mol). The resulting mixture was stirred for 16 h at room temperature. The formed dicyclohexylurea was removed by filtration and washed with Et_2O. Evaporation of the filtrate yielded the titled compound as a pale oil which was used directly without further purification.

Method E. *N*-[3-(Acetylthio)-2-methylpropanoyl]-*N*-cyclohexylglycine *tert*-Butyl Ester (8a, R^1 = c-C_6H_{11}). To a solution of *N*-cyclohexylglycine *tert*-butyl ester (32.0 g, 0.15 mol), prepared by method B above, and triethylamine (18.2 g, 0.18 mol) in *p*-dioxane (500 mL) was added dropwise 3-(acetylthio)-2-methylpropanoyl chloride (**7b**; 27.1 g, 0.15 mol). After stirring at room temperature for 16 h, the mixture was filtered and concentrated to yield the crude titled product (47.6 g). This material was purified by high-performance LC, eluting with 14% AcOEt in *n*-C_6H_{14}, to yield pure product (36.4 g, 67.9%) as a colorless oil, R_f 0.21 (14% AcOEt in *n*-C_6H_{14}). Anal. ($C_{18}H_{31}NO_4S$) C, H, N.

Method F. *N*-[3-(Acetylthio)-2-methylpropanoyl]-*N*-cyclopropylglycine (18). Crude **8a** (R^1 = c-C_3H_5; R^2 = *t*-Bu) (19.5 g, 61.9 mmol) was dissolved in a mixture of anisole (50 mL) and TFA (250 mL). The resulting red solution was stirred for $1^1/_2$ h at room temperature. The solvent was evaporated and the residue was distributed between AcOEt and saturated $NaHCO_3$. The aqueous $NaHCO_3$ layer was acidified cautiously with concentrated HCl to pH 4–5. The precipitated product was extracted into $CHCl_3$ and washed twice with H_2O. The organic phase was dried ($MgSO_4$), filtered, and evaporated to give initially a colorless oil which was crystallized from Et_2O to afford **18** as colorless crystals (9.7 g, 61%), mp 86–88 °C. The dicyclohexylamine (DCHA) salt was prepared by dissolving **18** in Et_2O and adding DCHA dropwise with stirring until pH 7–9. The precipitated salt was filtered and washed with Et_2O to afford colorless crystals, mp 68–70 °C. Anal. ($C_{11}H_{17}NO_4S \cdot C_{12}H_{23}N$) C, H, N.

Method G. *N*-[3-(Acetylthio)-2-methylpropanoyl]-*N*-cyclohexylglycine (26). To a solution of **8a** (R^1 = c-C_6H_{11}; R^2 = *t*-Bu) (36.4 g, 0.101 mol) in CH_2Cl_2 (300 mL) was added trimethylsilyl iodide ($(CH_3)_3SiI$; 20.4 g, 0. 102 mol). After the mixture was stirred at room temperature for 1.75 h, H_2O (50 mL) was added, followed after 10 min by saturated aqueous $NaHCO_3$ (500 mL). An emulsion formed, which was separated by centrifuging. The aqueous solution was separated, acidified with concentrated HCl to pH 3, and extracted several times with AcOEt. The combined organic extract was dried ($MgSO_4$), filtered, and concentrated to yield **26** (25.6 g, 83%) as a pale yellow oil. The compound was characterized as its DCHA salt, prepared by dissolving the acid in Et_2O and adding DCHA to pH 8–9. The salt was isolated as a white crystalline solid, mp 142–144 °C. Anal. ($C_{14}H_{23}NO_4S \cdot C_{12}H_{23}N$) C, H, N.

Method H. *N*-(3-Mercapto-2-methylpropanoyl)-*N*-(1-ethyl-2-pyrrolidinylmethyl)glycine (50). Crude **8a** (R^1 = 2-methylene-1-ethylpyrrolidine; R^2 = *t*-Bu) was dissolved in a mixture of anisole (20 mL) and TFA (60 mL). The resulting solution was stirred at room temperature for 2 h. The TFA was evaporated in vacuo and the residue was distributed between AcOEt and saturated aqueous $NaHCO_3$. The aqueous phase was separated and washed twice with AcOEt. The aqueous layer was saturated with NH_4Cl and placed in a heavier than water continuous liquid extractor. The product was continuously extracted over $CHCl_3$ over 16 h. The $CHCl_3$ was dried ($MgSO_4$), filtered, and evaporated to give the mercaptan **50** as a colorless oil (4.3 g, 57%). The product was characterized as its DCHA salt, which was prepared in Et_2O to give colorless crystals, mp 120–122 °C. Anal. ($C_{13}H_{24}N_2O_3S \cdot C_{12}H_{23}N$) C, H, N.

Method I. *N*-(3-Mercapto-2-methylpropanoyl)-*N*-cyclopropylglycine (17). Anhydrous NH_3 was bubbled for 15 min through CH_3OH (350 mL) and the resulting saturated solution was added to **18** (20 g, 77.2 mmol) and the system was placed under nitrogen. The reaction was stirred at room temperature for $1^1/_2$ h. The solvent was removed in vacuo and the residue was applied to a column of AG-50W-X2 (Bio-Rad Laboratories) cation-exchange resin eluting with CH_3OH. The CH_3OH was evaporated and the residue dissolved in $CHCl_3$. The $CHCl_3$ was washed once with a small amount of H_2O and dried ($MgSO_4$). Filtration and evaporation of the solvent afforded **17** as a colorless oil (15 g) which was crystallized from AcOEt/*n*-C_6H_{14} to give colorless crystals (14 g, 84%), mp 89–91 °C. The DCHA salt was prepared in ether as in method F to give colorless crystals, mp 123–125 °C. Anal. ($C_9H_{15}NO_3S \cdot C_{12}H_{23}N$) C, H, N.

(*S*)-3-(Acetylthio)-2-methylpropanoyl Chloride (7b). This material was prepared in 91.5% yield as a pale yellow oil in a manner similar to the corresponding *dl* compound **7b** with use of optically pure (*S*)-acetyl-β-mercaptoisobutyric acid.[20a] Crude **7b** was used directly without further purification.

(*S*)-*N*-[3-(Acetylthio)-2-methylpropanoyl]-*N*-cyclopentylglycine *tert*-Butyl Ester (8a). This material was prepared in a manner similar to method E. To a solution of **6** (R^1 = cyclopentyl; R^2 = *t*-Bu) (8.37 g, 0.042 mol) and triethylamine (4.64 g, 0.046 mol) dissolved in CH_2Cl_2 and chilled to 0–10 °C was added dropwise **7b**-(*S*) (7.6 g, 0.042 mol) in a small amount of CH_2Cl_2 (15 mL). The resulting mixture was stirred for $2^1/_2$ h at room temperature. The CH_2Cl_2 was evaporated and the residue was dissolved in AcOEt. The AcOEt was washed consecutively with saturated aqueous $NaHCO_3$, brine, 10% aqueous HCl, and H_2O. The AcOEt was dried ($MgSO_4$), filtered, and evaporated to afford **8a** as a pale yellow oil which was used directly without further purification.

Method J. (*S*)-*N*-(3-Mercapto-2-methylpropanoyl)-*N*-cyclopentylglycine *tert*-Butyl Ester (10). Nitrogen gas was bubbled through a methanolic solution of corresponding **8a** (14.8 g, 43.1 mmol) in 100 mL of CH_3OH for 10 min followed by anhydrous NH_3 for 30 min. The reaction mixture was stirred under nitrogen for 30 min. The solvent was evaporated and the residue was dissolved in AcOEt. The AcOEt was washed twice with 1 N HCl and twice with H_2O. The AcOEt was dried ($MgSO_4$), filtered, and evaporated to give crude **10**. The crude product was purified by HPLC using the solvent system of AcOEt/*n*-C_6H_{14} (5:95) as eluent. The pure product **10** (R^1 = c-C_5H_9) was obtained as a colorless oil: $[\alpha]^{CHCl_3}_D$ −27.01° (*c* 1.0). Anal. ($C_{15}H_{27}NO_3S$) C, H, N.

Method K. (*S*)-*N*-Cyclopentyl-*N*-[3-[(2,2-dimethyl-1-oxopropyl)thio]-2-methyl-1-oxopropyl]glycine *tert*-Butyl Ester (11a). To a mixture of corresponding **10** (4.5 g, 15 mmol) and triethylamine (2 g, 20 mmol) in CH_2Cl_2 (150 mL) was added slowly dropwise under nitrogen a solution of pivaloyl chloride (2 g, 16 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred under nitrogen at room temperature for 16 h. The reaction mixture was washed consecutively with H_2O, 1 N HCl, and H_2O. The organic phase was dried ($MgSO_4$), filtered, and evaporated in vacuo to afford crude **11a** as a pale yellow oil. The crude

product was purified by chromatography over silica gel with AcOEt/n-C_6H_{14} (5:95) to give **11a** as colorless crystals (5.5 g, 95%) after crystallization from AcOEt/n-C_6H_{14}: mp 58 °C; $[\alpha]^{CHCl_3}_D$ −83.62° (c 1.0). Anal. ($C_{20}H_{35}NO_4S$) C, H, N.

Method L. (*S*)-*N*-Cyclopentyl-*N*-[3-[(2,2-dimethyl-1-oxopropyl)thio]-2-methyl-1-oxopropyl]glycine (74c). To an ice-cold solution of corresponding **11a** (2.6 g, 6 mmol) in CH_2Cl_2 (30 mL) was slowly added a solution of $(CH_3)_3SiI$ (1.24 g, 6 mmol) in CH_2Cl_2 (10 mL) under nitrogen. The reaction mixture was stirred 1 h at room temperature. Ice–water was added and the product was extracted several times into 5% aqueous $NaHCO_3$. The aqueous extract was acidified to pH 4 with concentrated HCl and the precipitated product was extracted several times into AcOEt. The AcOEt was washed with H_2O, dried ($MgSO_4$), filtered, and evaporated to afford pure **74c** as a colorless crystalline solid (1.5 g, 75.1%): mp 155–156 °C; $[\alpha]^{CHCl_3}_D$ −104.64° (c 1.0). Anal. ($C_{16}H_{27}NO_4S$) C, H, N.

(*R*)-3-(Acetylthio)-2-methylpropanoyl Chloride (7b). This material was prepared in 94% yield as a pale yellow oil in a manner similar to the corresponding dl compound **7b** with use of optically pure (*R*)-acetyl-β-mercaptoisobutyric acid (**7a**).[20a] Crude **7b**-(*R*) was used directly without further purification.

(*R*)-*N*-[3-(Acetylthio)-2-methylpropanoyl]-*N*-cyclopentylglycine *tert*-Butyl Ester (8a). This material was obtained in 84% yield as a pale yellow oil in a manner similar to method E and was used directly without further purification.

(*R*)-*N*-(3-Mercapto-2-methylpropanoyl)-*N*-cyclopentylglycine *tert*-Butyl Ester (10). This material was obtained in 87% yield as a colorless oil by means analogous to method J: $[\alpha]^{CHCl_3}_D$ +27° (c 1.0). Anal. ($C_{12}H_{27}NO_3S$) C, H, N.

(*R*)-*N*-Cyclopentyl-*N*-[3-[(2,2-dimethyl-1-oxopropyl)thio]-2-methyl-1-oxopropyl]glycine *tert*-Butyl Ester (11a). This material was obtained in 94.4% yield as a colorless crystalline solid in a manner analogous to method K: mp 50 °C; $[\alpha]^{CHCl_3}_D$ +84. 21° (c 1.0). Anal. ($C_{20}H_{35}NO_4S$) C, H, N.

(*R*)-*N*-Cyclopentyl-*N*-[3-[(2,2-dimethyl-1-oxopropyl)thio]-2-methyl-1-oxopropyl]glycine (74b). This material was obtained in 62% yield in a manner analogous to method L as a color less crystalline solid after crystallization from Et_2O/n-C_6H_{14}: mp 156 °C; $[\alpha]^{CHCl_3}_D$ +111.05° (c 1.0). Anal. ($C_{16}H_{27}NO_4S$) C, H, N.

(*S*)-3-(Benzoylthio)-2-methylpropanoyl Chloride (7d). D-(−)-3-(Benzoylthio)isobutyric acid (**7c**; 27 g, 0.121 mol; $[\alpha]^{CHCl_3}_D$ −61.90°, obtained from Chemical Dynamics Corp.[20b]) was dissolved in a mixture of CH_2Cl_2 (75 mL) and DMF (2.5 mL). To this solution was added dropwise $SOCl_2$ (12.6 mL) under nitrogen at room temperature. After all the $SOCl_2$ was added, the reaction mixture was stirred for 4 h at room temperature. The CH_2Cl_2 and excess $SOCl_2$ were evaporated in vacuo, and the residue was dissolved in Et_2O. The Et_2O was washed twice with H_2O, dried ($MgSO_4$), filtered, and evaporated to afford **7d** as a pale yellow oil (16.0 g, 55%) which was used directly in the subsequent reaction without further purification.

Method M. (*S*)-*N*-Cyclopentyl-*N*-[3-(benzoylthio)-2-methyl-1-oxopropyl]glycine *tert*-Butyl Ester (8c). To CH_2Cl_2 (259 mL) were added **6** (12.9 g, 65 mmol) and triethylamine (6.5 g, 65 mmol). The resulting solution was chilled in an ice–water bath and then **7d** (16.0 g, 65 mmol) in CH_2Cl_2 (25 mL) was added dropwise. The reaction was stirred for 30 min with external cooling and then for $2^1/_2$ h at room temperature. The CH_2Cl_2 was washed twice with H_2O, dried ($MgSO_4$), filtered, and evaporated to give **8c** as initially a pale yellow oil (22.2 g, 84.4%). An analytical sample was prepared by chromatography over silica gel ($CHCl_3$) to give pure **8c** as a colorless solid after crystallization from Et_2O/n-C_6H_{14}: mp 54–56 °C; $[\alpha]^{CHCl_3}_D$ −77.27° (c 1.0). Anal. ($C_{22}H_{31}NO_4S$) C, H, N.

Method N. (*S*)-*N*-(3-Mercapto-2-methylpropanoyl)-*N*-cyclopentylglycine *tert*-Butyl Ester (10). Nitrogen gas was bubbled through a methanolic solution (250 mL) of **8c** (21.0 g, 51.9 mmol) for 30 min followed by anhydrous NH_3 for 30 min. The reaction was stirred at room temperature under nitrogen for 16 h. The solvent was evaporated and the residue was dissolved in AcOEt. The AcOEt was washed consecutively with H_2O, 1 N HCl, and again with H_2O. The AcOEt was dried ($MgSO_4$), filtered, and evaporated to afford crude **10**. The crude product was purified by chromatography over silica gel with AcOEt/n-C_6H_{14} (5:95) as eluent to give pure **10** as a colorless oil (12.8 g, 82%) which was identical in all respects with that previously prepared by method J.

Preparation of Angiotensin-Converting Enzyme. A crude preparation of ACE was obtained by extracting rabbit lung acetone powder (Pel-Freez Biologicals, Inc., Rogers, AR) with cold 0.05 M potassium phosphate buffer at pH 8.3. The homogenate was centrifuged at 40000g and the clear supernatant, containing the ACE, was dialyzed against 0.05 M phosphate buffer to remove low molecular weight inhibitors. This preparation has been described in detail by Cushman and Cheung.[24] The activity of the crude ACE was determined in 0.1 M KH_2PO_4–0.3 M NaCl–2% Me_2SO at pH 8.3 and 37 °C with hippuryl-histidyl-leucine (HHL), 2 mM, as substrate by the method of Cushman and Cheung.[25] The quantity of enzyme used was sufficient to catalyze the hydrolysis of 10–15% of the substrate in 10 min. To determine IC_{50} values, assays were initiated by adding enzyme to a buffered solution of substrate ± inhibitor. After 10 min the reaction was terminated by addition of 0.25 mL of 1 M HCl and one of the reaction products, hippuric acid, was extracted with ethyl acetate. A 1.0-mL aliquot of the extract was evaporated to dryness and the residue was dissolved in 1.0 mL of H_2O. The hippuric acid concentration was determined from the absorbance at 228 nm. Enzyme activity is expressed as nanomoles of hippuric acid formed per minute per milligram of protein.

Inhibition of ACE in Normotensive Conscious Rats. 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 tranducers for continuous monitoring of pressure. Angiotensins I and 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 orally given one dose of inhibitor, suspended in an 0.5% gum tragacanth suspension. The angiotensin I injections were repeated every 10 min for at least 2 h except for occasional injections of angiotensin II. For each rat, the maximum inhibition of the angiotensin I pressor response following the test agent was determined as a percent of the initial response to angiotensin I. The time to 50% recovery of the angiotensin I response ($t_{1/2}$) was also determined. For a selected number of inhibitors, a dose–response plot was drawn and the ID_{50} values calculated.

Antihypertensive Effects in Sodium-Deficient SHR. Eleven-week-old, male, spontaneously hypertensive rats were maintained on a sodium-deficient diet (ICN Pharmaceutical, Inc.) and distilled water for 3 weeks to elevate their renin levels. Polyethylene catheters were then implanted in their abdominal aortae. Three to four days later, the rats were harnessed and their catheters attached to a recording system via swivels that allowed the animals to roam freely within individual cages while their arterial pressures were monitored. By means of an electronic switching system and computer, the arterial pressures from these rats were measured for 10 s, every 5 min for 24 h. The average values over half-hour periods were recorded. At least 1 h after recording began, the rats were dosed po or ip with inhibitor in a 0.5% gum tragacanth suspension or with the suspension alone. The doses of **74a** were 2, 6, 20, or 60 mg/kg. Captopril (**2**) was given at 0.6, 2, 6, or 20 mg/kg. Each dose was given to seven to nine rats. Eight rats received the gum tragacanth suspension as a control.

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pertensive effects in SHR and for ACE inhibition evaluations in conscious normotensive dogs.

Registry No. **6** (R^1 = c-C_4H_7, R^2 = *t*-Bu), 78773-49-6; **6** (R^1 = c-C_3H_5, R^2 = *t*-Bu), 78773-38-3; **6** (R^1 = nopinyl, R^2 = *t*-Bu), 78773-60-1; **6** (R^1 = C_5H_9, R^2 = *t*-Bu), 78773-69-0; (±)-**7a**, 74407-69-5; (*R*)-**7a**, 74431-52-0; (*S*)-**7a**, 76497-39-7; (±)-**7b**, 70354-87-9; (*R*)-**7b**, 74345-73-6; (*S*)-**7b**, 69570-39-4; **7c**, 72679-02-8; **7d**, 74654-91-4; (*R*)-**8a** (R^1 = C_5H_9), 93040-19-8; **8a** (R^1 = c-C_3H_5), 93040-13-2; **8a** (R^1 = c-C_6H_{11}), 93040-14-3; **8a** (R^1 = 2-methylene-1-ethylpyrrolidine), 93040-15-4; (*S*)-**8a** (R^1 = C_5H_9), 93040-16-5; (*S*)-**8c** (R^1 = C_5H_9), 93040-22-3; (*S*)-**10**, 93040-17-6; (*R*)-**10**, 93040-20-1; (*R*)-**11a**, 93040-21-2; (*S*)-**11a**, 93040-18-7; **12**, 89021-98-7; **13**, 93039-48-6; **14**, 93039-49-7; **14**·DCHA, 93039-50-0; **15**, 93040-23-4; **15**·DCHA, 93061-40-6; **16**, 93039-51-1; **17**, 93039-52-2; **18**, 93039-53-3; **18**·DCHA, 93039-54-4; **19**, 93039-55-5; **19**·DCHA, 93039-56-6; **20**, 93039-57-7; **21**, 93039-58-8; **22**, 93039-59-9; **22**·DCHA, 93039-60-2; **23a**, 86324-07-4; **23a**·DCHA, 93061-31-5; **23b**, 93133-32-5; **23b**·DCHA, 93133-31-4; **24**, 93039-61-3; **24**·DCHA, 93039-62-4; **25**, 82017-44-5; **25**·DCHA, 86324-06-3; **26**, 82017-39-8; **26**·DCHA, 82017-40-1; **27**, 86324-15-4; **28**, 93039-63-5; **28**·DCHA, 93039-64-6; **29**, 78773-46-3; **29**·DCHA, 78773-47-4; **30**, 78773-45-2; **30**·DCHA, 93039-65-7; **32**, 78773-59-8; **33**, 78773-58-7; **36**, 78773-62-3; **36**·DCHA, 93039-66-8; **37**, 86324-23-4; **37**·DCHA, 86324-24-5; **38**, 93132-65-1; **38**·DCHA, 93218-53-2; **39**, 93039-67-9; **39**·DCHA, 93039-68-0; **40**, 93039-69-1; **40**·DCHA, 93039-70-4; **41**, 93039-71-5; **41**·DCHA, 93039-72-6; **42**, 78773-86-1; **42**·DCHA, 78773-87-2; **43**, 81379-77-3; **43**·DCHA, 93039-73-7; **44**, 93039-74-8; **44**·DCHA, 93039-75-9; **45**, 93039-76-0; **45**·DCHA, 93039-77-1; **46**, 93061-32-6; **46**·DCHA, 93061-33-7; **47**, 93039-78-2; **47**·DCHA, 93039-79-3; **48**, 93061-34-8; **49**, 93061-35-9; **49**·DCHA, 93061-36-0; **50**, 93039-80-6; **50**·DCHA, 93039-81-7; **51**, 93039-82-8; **51**·DCHA, 93039-83-9; **52**, 93039-84-0; **52**·DCHA, 93039-85-1; **53**, 86323-76-4; **54**, 93039-86-2; **55**, 93039-87-3; **55**·Benz, 93039-88-4; **56**, 93039-89-5; **56**·Benz, 93039-90-8; **57**, 93039-91-9; **57**·Benz, 93039-92-0; **58**, 93039-93-1; **58**·Benz, 93039-94-2; **59**, 93039-95-3; **59**·Benz, 93039-96-4; **60**, 93039-97-5; **60**·Benz, 93039-98-6; **61**, 93039-99-7; **62**, 93040-00-7; **63**, 86324-00-7; **63**·Benz, 93040-01-8; **64**, 93040-02-9; **64**·Benz, 93040-03-0; **65**, 93061-37-1; **65**·Benz, 93061-38-2; **66**, 93040-04-1; **66**·Benz, 93040-05-2; **67**, 93040-06-3; **68**, 86323-77-5; **69**, 93040-07-4; **70**, 86323-79-7; **70**·Benz, 93040-08-5; **71**, 93040-24-5; **71**·Benz, 93061-39-3; **72**, 93040-09-6; **72**·Benz, 93040-10-9; **73**, 93040-11-0; **74a**, 93132-64-0; **74b**, 93040-12-1; **74c**, 81045-50-3; thiolacetic acid, 507-09-5; methacrylic acid, 79-41-4; *tert*-butyl bromoacetate, 5292-43-3; cyclobutylamine, 2516-34-9; nopinylamine, 64284-82-8; *N*-cyclohexylglycine *tert*-butyl ester, 66937-55-1; pivaloyl chloride, 3282-30-2; cyclopropylamine, 765-30-0; angiotensin-converting enzyme, 9015-82-1.

3-(1-Indolinyl)benzylamines: A New Class of Analgesic Agents

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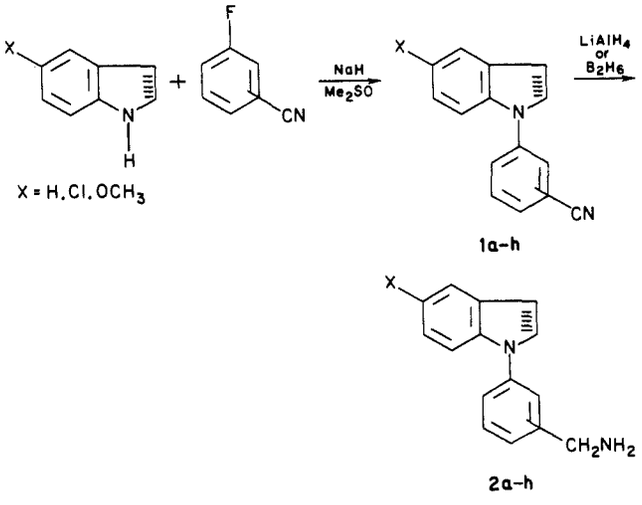
An extensive series of 3-(1-indolinyl)benzylamines and related compounds was synthesized and tested for analgesic activity. After a detailed study of structure–activity relationships, 3-(1-indolinyl)benzylamine (**2b**) was selected for further investigation as the most interesting member of this novel class of compounds. It was active in both the phenylquinone writhing and tail-flick assays for analgesic activity. No motor deficits were observed in the rotorod test, and **2b** was found to be free of any other effects on the central nervous system. The compound did not bind to opiate receptors, since it was inactive in inhibiting the stereospecific binding of [^{3}H]naloxone in rat brain homogenates. Thus, 3-(1-indolinyl)benzylamine represents a novel analgesic with an unusual chemical structure and biological profile.

Serendipity often plays a key role in the discovery of biological activity in a previously unexplored class of molecules.[1] During routine pharmacological screening of intermediates connected with another project,[2] a modest analgesic effect was observed for 1-(2-aminophenyl)-indoline (I). This compound produced a 43% inhibition of phenyl-*p*-quinone-induced writhing (PQW) in rats at the screening dose of 25 mg/kg, sc. We thought this result was quite interesting, since there was no literature precedent for this structural type of molecule within the numerous classes of compounds reported to possess analgesic properties.[3-5] In addition, the simple synthesis of I in two steps[2] from relatively cheap and readily available starting materials encouraged us to initiate an intensive synthetic study of structure–activity relationships within this novel structural type. By pursuing this lead, our objective was to find a chemically simple and unique pain-relieving agent with nonnarcotic properties.

The first structural variation of I to be investigated was a molecule (IIa) in which the *o*-amino group was separated from the phenyl ring by one carbon atom. This minor change produced a major enhancement of anti-PQW activity (ED_{50} = 14.1 mg/kg, sc). Next, the aminomethyl group was moved synthetically around the pendant phenyl ring to the meta (IIb) and para (IIc) positions. The para-substituted derivative was found to be much less active than IIa while the meta-substituted analogue IIb

Scheme I



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