Angiotensin-Converting Enzyme Inhibitors: New Orally Active 1,4-Thiazepine-2,5-diones, 1,4-Thiazine-2,5-diones, and 1,4-Benzothiazepine-2,5-diones Possessing Antihypertensive Activity

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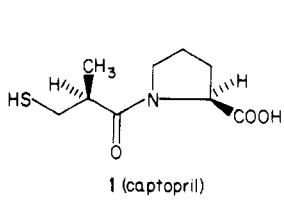
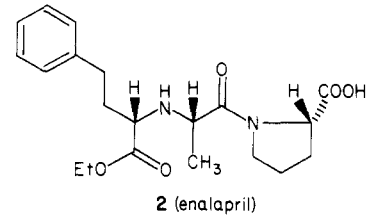
The preparation of a series of 1,4-thiazepine-2,5-diones, 1,4-thiazine-2,5-diones, and 1,4-benzothiazepine-2,5-diones and their ability in inhibiting the activity of angiotensin-converting enzyme (ACE) in vitro and in vivo were examined. These compounds are assumed to act as prodrugs since they undergo rapid ring-opening reactions to give the corresponding biologically active free SH compounds when incubated with rat plasma or when treated with aqueous 0.1 N HCl or phosphate buffer (pH 7.4). The thiazepines **23–25** and **30** are potent inhibitors of ACE when administered po to rats and are comparable in potency to captopril (**1**). The most active thiazines in rats, po, were **42** and **45**. Of the benzothiazepines studied, **22a** was the most active in inhibiting ACE in the conscious normotensive rat, ID_{50} = 0.15 mg/kg, po. The acute antihypertensive effects of oral administration of a number of these compounds on mean arterial pressure and heart rate were studied in spontaneously hypertensive rats (SHR) maintained on a sodium-deficient diet.

Inhibitors of the renin–angiotensin system have proven to be important agents for the control of high blood pressure in hypertensive diseases.[2-8] Angiotensin-converting enzyme (ACE), peptidyldipeptide hydrolase (EC 3.4.15.1), is a dipeptide-liberating exopeptide that plays an important physiological role in the regulation of blood pressure by virtue of two different reactions that it catalyzes. It catalyzes the cleavage of His-Leu from the COOH terminus of the inactive decapeptide angiotensin I to generate angiotensin II.[9,10] This octapeptide is a potent vasoconstrictor and salt-retaining agent that is the biologically active component of the renin–angiotensin system. ACE also catalyzes the release of Phe-Arg and Ser-Pro from the COOH terminus of bradykinin and thus inactivates the vasodilator and natriuretic activity of this nonapeptide.[11]

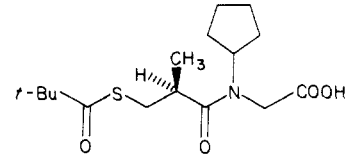
In recent years much attention has focused on the renin–angiotensin system as a means of controlling blood pressure and renal function. In particular it has been demonstrated that ACE inhibitors[12,13] such as captopril (**1**),[12a,12b] enalapril (**2**),[13e] and pivopril (**3**)[12n,12t] are orally effective antihypertensives when tested in the clinic.[2-8]



1 (captopril)



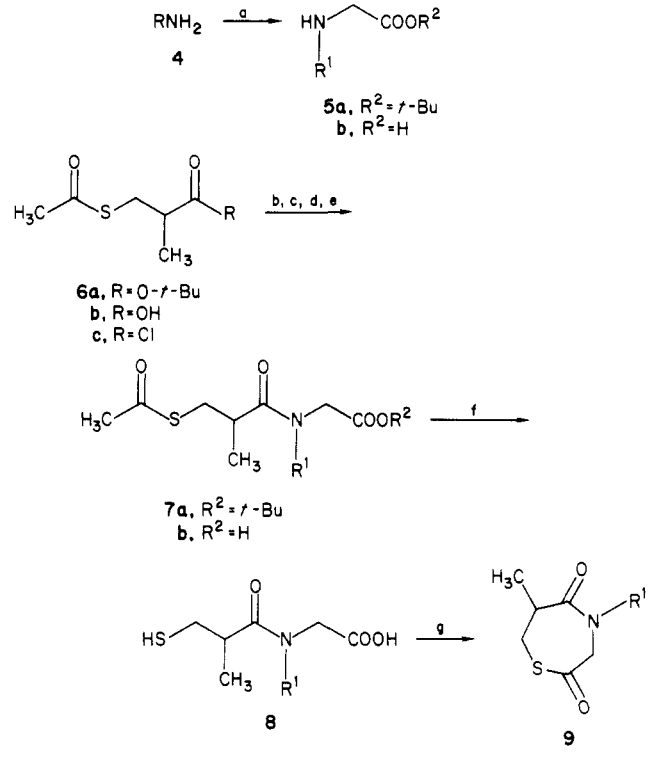
2 (enalapril)



3 (pivopril; REV 3659-(S))

Several undesirable clinical side effects of captopril administration have been reported.[14] These side effects, such as rashes and loss of taste, are diminished upon reduction or withdrawal of therapy. The toxicity associated with captopril (**1**) is generally agreed to be associated with

Scheme I. Synthesis of 1,4-Thiazepine-2,5-diones[a]



[a] Reagents: a, $BrCH_2CO_2R^2$; b, **6a**/anisole/TFA to give **6b** or $(CH_3)_3SiI/CH_2Cl_2$ to give **6b**; c, **6b**/$SOCl_2/CH_2Cl_2$/DMF to give **6c**; d, **6b**/**5a**/CH_2Cl_2/DCC to give **7a** or **6c**/**5a**/CH_2Cl_2/Et_3N to give **7a**; e, **7a**/$(CH_3)_3SiI/CH_2Cl_2$ to give **7b** or **7a**/anisole/TFA to give **7b**; f, **7b**/NH_3/CH_3OH; g, 8/PPA or 8/2,2′-dipyridyl disulfide/Ph_3P/toluene or 8/$ClCO_2Et/CH_2Cl_2/Et_3N$.

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(2) Fergusson, R. K.; Turini, G. A.; Brunner, H. R.; et al. Lancet *I* **1977**, 775.
(3) Gavras, H.; Brunner, H. R.; et al. *N. Engl. J. Med.* **1984**, *291*, 817.

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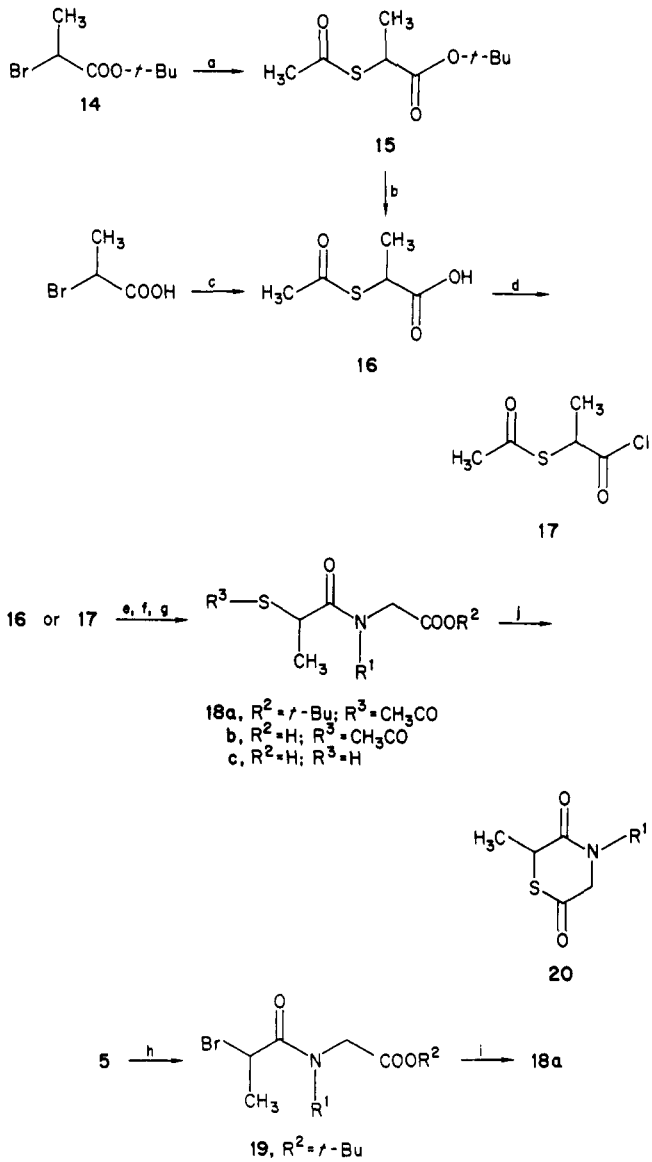
Scheme II. Synthesis of 1,4-Thiazine-2,5-diones[a]



[a] Reagents: a, CH_3COSH/p-dioxane/Et_3N; b, anisole/TFA or $(CH_3)_3SiI/CH_2Cl_2$; c, CH_3COSH/p-dioxane/Et_3N; d, $SOCl_2$/toluene/DMF; e, **16**/**5a**/DCC/CH_2Cl_2 or **17**/**5a**/Et_3N/CH_2Cl_2 to give **18a**; f, **18a**/anisole/TFA or **18a**/$(CH_3)_3SiI/CH_2Cl_2$ to give **18b**; g, **18b**/anhydrous NH_3/CH_3OH to give **18c**; h, **5a**/$BrCH(CH_3)$-$COCl/CH_2Cl_2/Et_3N$; i, CH_3COSH/p-dioxane/Et_3N; j, **18c**/$ClCO_2Et/CH_2Cl_2/Et_3N$.

the free sulfhydryl moiety since similar side effects are seen with penicillamine (12).

As an ongoing search for new and therapeutically useful antihypertensive agents we now report on the synthesis[15]

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(5) Gavras, H.; et al. *Lancet* **1981**, 543.

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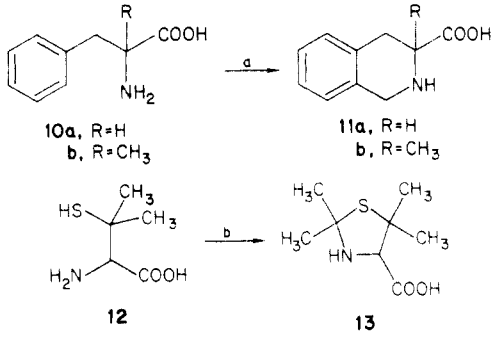
and biological activities of a series of novel 1,4-thiazepine-2,5-diones (**9**), 1,4-thiazine-2,5-diones (**20**), and 1,4-benzothiazepine-2,5-diones (**22**) that possess potent ACE inhibitory activity when tested in vitro and in vivo. Unlike captopril (**1**) this series of compounds lacks a free sulfhydryl group, and it was therefore our hope that potent ACE inhibition would be obtained while maintaining relatively low plasma levels of the biologically active corresponding free mercaptans.

Chemistry. The syntheses of the 1,4-thiazepine-2,5-diones (**9**)[15a] and 1,4-thiazine-2,5-diones (**20**)[15a] listed in Tables I and III were envisioned via cyclization of the corresponding β-mercaptoalkanoyl[16] and α-mercaptoalkanoyl amino acids exemplified by **8** and **18c**. Cushman et al. have previously described the syntheses[12b] of related β-mercaptoalkanoyl amino acids (**8**) by a means similar to that given in Scheme I. In our study for the most part nonnaturally occurring N-substituted glycines **5** were utilized. The general synthesis of the required N-substituted glycines **5** as well as the β-mercaptoalkanoyl amino acids **8** have previously been described by us.[12t,16] The required α-amino acids **5** were synthesized in a straightforward manner by reacting known primary amines with *tert*-butyl bromoacetate in a polar solvent such as EtOH or CH_3CN in the presence of a base such as Et_3N or $NaHCO_3$. A series of imino α-amino acids, **11a**, **11b**, and **13**, was also prepared by known methods. The 1,2,3,4-tetrahydroisoquinolines **11a**[17a-d] and **11b**[17e] were prepared by treatment of phenylalanine (**10a**) and α-methylphenylalanine (**10b**), respectively, with formaldehyde in the classical Pictet–Spengler fashion. The known thiazolidine **13**[18] was obtained by refluxing DL-penicillamine (**12**) in acetone with a catalytic amount of concentrated hydrochloric acid. In a manner similar to that previously described,[12t,16,19,20] 3-(acetylthio)-2-methylpropionic acid (**6b**) was prepared by the addition of thiolacetic acid to methacrylic acid in a Michael fashion. The corresponding acid chloride **6c** was prepared conveniently in toluene in the presence of $SOCl_2$ with a few added drops of pyridine or DMF as initiator. The appropriately substituted α-amino acids **5** were condensed with 3-(acetylthio)-2-methylpropionic acid (**6b**) in CH_2Cl_2 with use of dicyclohexylcarbodiimide (DCC) as the amide-generating reagent. The amides **7a** were also prepared by employing the acid chloride **6c** under standard Schotten–Baumann acylating conditions. In general the crude *tert*-butyl esters **7a** were directly converted to the free carboxylic acids **7b** by means of trifluoroacetic acid (TFA) in anisole or with trimethylsilyl iodide ($(CH_3)_3SiI$) in CH_2Cl_2 at room temperature. After the usual workup of the acidic products, the pure acids **7b** were obtained by high-performance LC over silica gel with use of the solvent system of *n*-C_6H_{14}/AcOEt/AcOH (60:40:1) and were fully characterized by standard methods: NMR, MS, and elemental analyses. In cases where the acids **7b** are liquids or low melting, the elemental analyses were generally performed on the corresponding dicyclohexylamine (DCHA) salts. In the case of the imino α-amino acids **11a**, **11b**, **13**, L-proline, and L-thioproline, the free carboxylic amides **7b** were obtained directly by direct acylation of the unprotected amino acids with the acid chloride **6c**. The free mercaptans **8** were generated from the thio esters **7b** by treatment with anhydrous NH_3 in CH_3OH followed by ion-exchange chromatography (AG-50W-X2, Bio-Rad Laboratories) with CH_3OH as the eluting solvent.



Reagents: a, **10a**/12 N HCl/37% formalin to give **11a** or **10b**/12 N HCl/paraformaldehyde to give **11b**; b, **12**/12 N HCl/acetone.

The first method employed by us for effecting the ring closure of **8** to **9** utilized 2,2′-dipyridyl disulfide[21] (Aldrithiol-2) in the presence of triphenylphosphine in refluxing toluene under high-dilution conditions. Although this method provided the desired cyclized product in 30–40% yield, the disadvantages of high-dilution conditions and the necessity of purifying the product from triphenylphosphine oxide warranted the further exploration of other ring-closure reactions. While several other syntheses were developed for effecting the ring closure of **8** to **9**, such as DCC in CH_2Cl_2 or neat PPA at 50 °C, the most efficient means utilized ethyl chloroformate in CH_2Cl_2 in the presence of 1 equiv of Et_3N at room temperature. By this latter method the ring-closure reaction gave typical yields of 30–50% after crystallization. The employment of ethyl chloroformate as the ring-closure reagent has the advantage over DCC in that a tedious separation of the product from dicyclohexylurea is not required.

The 1,4-thiazine-2,5-diones listed in Table III were prepared[15a] in an analogous manner to that described above for **9**. In this case the required starting materials for the cyclization reaction were the appropriately substituted 2-mercaptopropanoyl amino acids **18c**[16] listed in Table II. The synthesis of **18c** is outlined in Scheme II. *tert*-Butyl 2-bromopropionate (**14**) was treated with thiolacetic acid in *p*-dioxane in the presence of Et_3N to give

(14) (a) Atkinson, A. B.; Robertson, J. I. S. *Lancet* **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.

(15) (a) Suh, J. T.; Williams, B. E.; Skiles, J. W.; Loev, B. U.S. Patent 4 287 203, 1981. (b) Suh, J. T.; Williams, B. E.; Skiles, J. W.; Loev, B. U.S. Patent 4 347 246, 1982.

(16) (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.

(17) (a) Julian, P. L.; Karpel, W. J.; Magnani, A.; Mayer, E. W. *J. Am. Chem. Soc.* **1948**, *70*, 180. (b) Clemo, G. R.; Swan, G. A. *J. Chem. Soc.* **1946**, 617. (c) Percy, L. J.; Williams, J. K.; Arthur, M.; Edwin, W. M. *J. Am. Chem. Soc.* **1948**, *70*, 182. (d) Hein, G. E.; Niemann, C. *Ibid.* **1962**, *48*, 4487. (e) Ghosh, T. N.; Ghosh, B. K. *J. Sci. Ind. Res.* **1961**, *20B*, 400; *Chem. Abstr.* **1962**, *65*, 12430b.

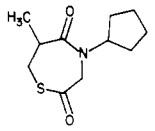
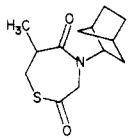
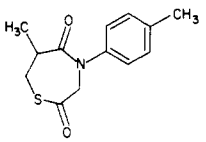
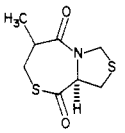
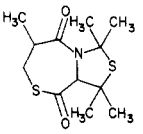
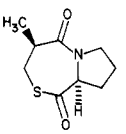
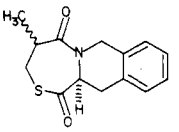
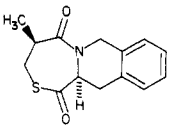
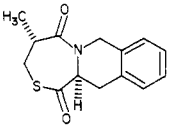
(18) King, F. E.; Clark-Lewis, J. W.; Smith, G. R.; Wade, R. *J. Chem. Soc.* **1959**, 2264.

(19) Optically pure forms of D-(−)- and D-(+)-β-(acetylthio)isobutyric acid (**6b**) can now be obtained commercially from Chemical Dynamics Corp., South Plainfield, NJ 07080.

(20) Fredga, A.; Martensson, O. *Ark. Kemi, Mineral. Geol.* **1942**, *16B*, 1.

(21) This reagent, $Ph_3P(PyS)_2$, commonly referred to as Mukaiyama's reagent, is well known as being useful for peptide synthesis through the oxidation–reduction condensation introduced in 1970. (a) Mukaiyama, T.; Matsueda, R.; Suzuki, M. *Tetrahedron Lett.* **1970**, 1801. (b) Mukaiyama, T. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 94.

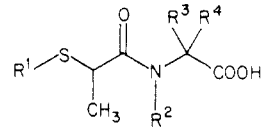
Table I. In Vitro ACE Inhibitory Activities of 1,4-Thiazepine-2,5-diones

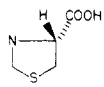
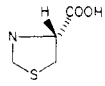
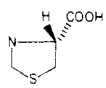
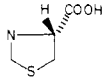
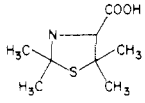
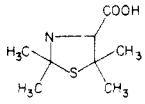
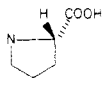
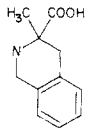
compd[a]	structure	mp,[b] °C	yield,[c] %	procedure[d]	formula[e]	I_{50},[f] μM	I_{50}(SH),[f,g] μM
23		77–79	27	A, G, H, L	$C_{11}H_{17}NO_2S$	0.013	0.018
24		134–136	36	C, H, L	$C_{13}H_{19}NO_2S$	0.012	0.025
25		80	42	B, G, H, K	$C_{13}H_{15}NO_2S$	0.021	0.005
26[h]		76–78	30	I, H, K	$C_8H_{11}NO_2S$	0.047	0.058
27		137	43	I, H, K	$C_{12}H_{19}NO_2S_2$	50	15
28		98–101[i]	60	I, H, K	$C_9H_{13}NO_2S$	0.026	0.017
29[j]		142	43	I, H, K	$C_{14}H_{15}NO_2S$	0.018	0.045
(4*S*,12a*S*)-**30**[k]		148–153[l]	42	I, H, K	$C_{14}H_{15}NO_2S$	0.012	
(4*R*,12a*S*)-**31**[m]		160–163	32	I, H, K	$C_{14}H_{15}NO_2S$	0.80	
1 (captopril)							0.017
2 (enalapril)[n]							
3 (pivopril)						3.60	0.017

[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, ^{1}H NMR, and MS spectra consistant with the assigned structures. [f] Concentration inhibiting 50% of the activity of rabbit lung ACE at pH 8.5 in 0.10 M KH_2PO_4 buffer containing 0.30 M NaCl with the substrate Hip-His-Leu at a concentration of 2 mM. [g] IC_{50} values of the corresponding ring-open free-SH forms **8**. [h] Corresponds to a mixture of 6*S*,9*R* and 6*R*,9*R* diastereomers (9:1) on the basis of TLC and NMR. [i] Corresponds to the cyclized analogue of captopril (**1**), $[\alpha]_D^{22}$ 119.0° (*c* 1.0, EtOH). The optical rotation of this compound prepared by an alternate route[12f] was reported as $[\alpha]^{24}_D$ −36.02° (*c* 0.66, EtOH) and having a melting point of 103–104 °C. Captopril (**1**)[12b] was prepared by us by means similar to methods[d] I and H. In Method I, H_2O was used as the reaction medium and $NaHCO_3$ (3 equiv) as the base. The *S*,*S* and *R*,*S* diastereomers of *N*-[3-(acetylthio)-2-methylpropanoyl]-L-proline were separated according to the literature procedure.[12b] The *S*,*S* diastereomer gave the following physical data: mp 81–83 °C; $[\alpha]^{22}_D$ −162° (*c* 1.0, EtOH) (lit.[12b] mp 83–85 °C; $[\alpha]^{22}_D$ −164.4° (*c* 2.4; EtOH)). Captopril (**1**) prepared by us and used to prepare **28** had the following physical data: mp 85–88 °C; $[\alpha]^{22}_D$ −127.1° (*c* 1.0, EtOH) (lit.[12b] mp 87–88 °C; $[\alpha]^{22}_D$ −131.0° (*c* 1.7, EtOH)). [j] On the basis of NMR spectra and TLC, this compound corresponds to a 4:1 racemic mixture of the respective optically pure diastereomers **30** and **31**. [k] Corresponds to the 4*S*,12a*S* diastereomer of **29**, $[\alpha]^{20}_D$ +34.6° (*c* 1.0, $CHCl_3$). This diastereomer was prepared by utilizing optically pure D-(−)-β-(acetylthio)isobutyric acid (**6b**)[19] and D-3-carboxy-1,2,3,4-tetrahydroisoquinoline, $[\alpha]^{20}_D$ −164.1° (*c* 1.0, H_2O) (lit.[17d] mp 327.5 °C dec; $[\alpha]_D$ −176.1° (*c* 1.8%, 1.4 N NaOH)). [l] Literature[12q] mp 163–165 °C. [m] Corresponds to 4*R*,12a*S* diastereomer of **29**, $[\alpha]^{20}_D$ +0.6° (*c* 1.0, $CHCl_3$). [n] I_{50} = 8.0 μM, I_{50}(diacid) = 0.0058 μM.

tert-butyl 2-(acetylthio)propionate (**15**) as a pale yellow oil in 96% yield. The ester **15** was deesterified with either TFA in anisole or with $(CH_3)_3SiI$ in CH_2Cl_2 to give the carboxylic acid **16** in 50% and 82% yields, respectively. Alternatively the acid **16** could be prepared directly in 80% yield by treatment of 2-bromopropionic acid in *p*-dioxane with thiolacetic acid. The acid **16** was easily converted into the corresponding acid chloride **17** with $SOCl_2$ in refluxing

Table II. N-Substituted α-Mercaptopropanoyl and α-(Acylthio)propanoyl Acids and Inhibition of ACE in Vitro



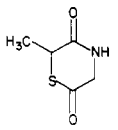
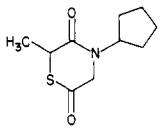
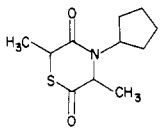
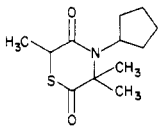
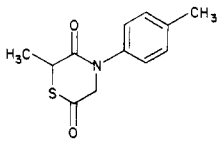
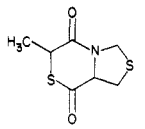
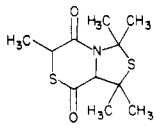
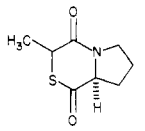
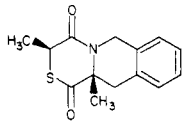
compd[a]	$R^2NCR^3R^4COOH$	R^1	mp,[b] °C	yield,[c] %	procedure[d]	formula[e]	IC_{50},[f] μM
32	$HNCH_2COOH$ (tiopronin)	H	91–96			$C_5H_9NO_3S$	1.9[g]
33a	c-$C_5H_9NCH_2COOH$	CH_3CO	157–158[h]	41, 84	A, F; D, E, G	$C_{12}H_{19}NO_4S$	0.51
33b	c-$C_5H_9NCH_2COOH$	H	163–164[h]	85	H	$C_{10}H_{17}NO_3S$	0.057
34a	c-$C_5H_9NCH(CH_3)COOH$	CH_3CO	118–120	75	B, G	$C_{13}H_{21}NO_4S$	6.8
34b	c-$C_5H_9NCH(CH_3)COOH$	H	118–120	86	H	$C_{11}H_{19}NO_3S$	6.8
35	c-$C_5H_9NC(CH_3)_2COOH$	CH_3CO	156–158	61	B, G	$C_{14}N_{23}NO_4S$	95
36a	4-$(CH_3)C_6H_4NCH_2COOH$	CH_3CO	105–107	86	B, G	$C_{14}H_{15}NO_4S$	0.18
36b	4-$(CH_3)C_6H_4NCH_2COOH$	H	101–104	90	H	$C_{12}H_{15}NO_3S$	0.026
37a(*R,R*)[i]		CH_3CO	129–131	39	I	$C_9H_{13}NO_4S_2$	37
37a(*S,R*)[j]		CH_3CO	103–105	45	I	$C_9H_{13}NO_4S_2$	1.7
37b(*R,R*)[k]		H	153–155	95	H	$C_7H_{11}NO_3S_2$	10[l]
37b(*S,R*)[m]		H	117–118	92	H	$C_7H_{11}NO_3S_2$	0.14[n]
38a		CH_3CO	150–153	65	I	$C_{13}H_{21}NO_4S_2$	>100
38b		H	165–168	92	H	$C_{11}H_{19}NO_3S_2$	21
39		CH_3CO	oil	74	I	$C_{10}H_{15}NO_4S$	
40		CH_3CO	171–174	29	J	$C_{16}H_{19}NO_4S$	>100
1 (captopril)							0.017
2 (enalapril)[o]							8.0
3 (pivopril)[p]							3.60

[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 exhibited satisfactory C, H, and N microanalyses and were within 0.4% of theoretical values. All compounds exhibited IR, ^1H NMR, and MS spectra consistent with the assigned structures. [f] Concentration inhibiting 50% of the activity of rabbit lung ACE at pH 8.3 in 0.10 M KH_2PO_4 buffer containing 0.30 M NaCl with the substrate Hip-His-Leu at a concentration of 2 mM. [g] Literature[12b] IC_{50} = 1.7 μM. [h] Dicyclohexylamine (DCHA) salt. [i] Corresponds to *R,R* diastereomer, $[\alpha]^{22}_D$ +22.25 (*c* 1.0, $CHCl_3$). [j] Corresponds to the *S,R* diastereomer, $[\alpha]^{22}_D$ −232.48° (*c* 1.0, $CHCl_3$). [k] Corresponds to the *R,R* diastereomer, $[\alpha]^{25}_D$ −162.18° (*c* 1.0, $CHCl_3$) (lit.[12d] mp 161–163 °C; $[\alpha]^{25}_D$ −166.2° (*c* 1.0, CH_3OH)). [l] Literature[12d] IC_{50} = 7.0 μM. [m] Corresponds to the *S,R* diastereomer, $[\alpha]^{25}_D$ −97.01° (*c* 1.0, $CHCl_3$) (lit.[12d] mp 122–123 °C; $[\alpha]^{25}_D$ −110.4° (*c* 1.0, CH_3OH)). [n] Literature[12d] IC_{50} = 0.37 μM. [o] IC_{50}(corresponding diacid) = 0.0058 μM. [p] IC_{50}(corresponding mercaptan) = 0.017 μM.

toluene with a catalytic amount of DMF. The acid chloride **17** was obtained as a pale yellow oil after vacuum distillation (56–67 °C, 0.1–0.2 mmHg). The appropriately substituted amino acid esters **5** were condensed with **16** in CH_2Cl_2 with use of DCC as the amide-generating reagent to give the amides **18a**. Alternatively the amides **18a** were also prepared with use of the acid chloride **17** and the appropriately substituted amino acids **5**. The *tert*-butyl amides **18a** could also be obtained by reaction of α-bromo amides **19** with thiolacetic acid. The α-bromo amides **19** were easily obtained by treatment of the corresponding α-amino acid esters **5** with 2-bromopropanoyl chloride. The *tert*-butyl esters **18a** were deprotected with $(CH_3)_3SiI$ in CH_2Cl_2 to give the acids **18b**. As analogy to the β-acylthioamide acids **7b**, the corresponding α-acylthioamide acids **18b** were obtained directly when the imino α-amino acids **11a**, **11b**, **13**, L-proline, and L-thioproline were employed. For example, treatment of L-proline with the acid chloride **17** gave **39** directly. The acids **18b** were fully characterized by NMR, MS, TLC, and elemental analyses. The free mercapto acids **18c** were generated from the thio esters **18b** by treatment with anhydrous NH_3 in CH_3OH followed by ion-exchange chromatography (AG-50W-X2) using CH_3OH as the eluting solvent. The α-mercaptoalkanoyl acids **18c** listed in Table II were fully characterized by means of NMR, MS, TLC, and microanalyses. Cyclization of the mercapto acids **18c** was accomplished with ethyl chloroformate to give the desired 1,4-thiazine-2,5-diones (**20**) in 30–45% yield after recrystallization.

We have recently reported on the syntheses[22] of a series

Table III. In Vitro ACE Inhibitory Activities of 1,4-Thiazine-2,5-diones

compd[a]	structure	mp,[b] °C	yield,[c] %	procedure[d]	formula[e]	I_{50},[f] μM	I_{50}(SH),[f,g] μM
41[h]		81–83	30	K	$C_5H_7NO_2S$	1.9	1.9
42		73–74	65.6	K	$C_{10}H_{15}NO_2S$	0.15	0.057
43		79.5–81	31	K	$C_{11}H_{17}NO_2S$	27	6.8
44		58.5–60.0	34	K	$C_{12}H_{19}NO_2S$	90	
45		93–94	41	K	$C_{12}H_{13}NO_2S$	0.085	0.026
46[i]		oil	41.9	M	$C_7H_9NO_2S_2$	0.38	0.14–10
47		93–95	38	K	$C_{11}H_{17}NO_2S_2$	>100	21
48[j]		90.5–92	32	K	$C_8H_{11}NO_2S$	33	
49[k]		170–172	41.1	K	$C_{14}H_{15}NO_2S$	>100	>100
1 (captopril)							0.017
2 (enalapril)[l]							
3 (pivopril)						3.60	0.017

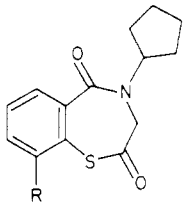
[a] Except where indicated all compounds are racemic. [b] Uncorrected. [c] Yield refers to the last step in each synthetic sequence. [d] See Experimental Section. [e] All compounds exhibited satisfactory C, H, and N microanalyses and were within 0.4% of theoretical values. All compounds exhibited IR, ^{1}H NMR, and MS spectra consistent with the assigned structures. [f] Concentration inhibiting 50% of the activity of rabbit lung ACE at pH 8.3 in 0.10 M KH_2PO_4 buffer containing 0.30 M NaCl with the substrate Hip-His-Leu at a concentration of 2 mM. [g] IC_{50} values of the corresponding ring-open free-SH forms; see Table II. [h] Corresponds to cyclized tiopronin (**32**). [i] Corresponds to a mixture of *R,R* and *S,R* diastereomers in the ratio of 1:1. [j] Corresponds to one diastereomer as judged by NMR and TLC analyses, the absolute configuration about C-6 is unknown, $[\alpha]^{22}_D$ −210.38° (*c* 1.445, $CHCl_3$). [k] Corresponds to 3*S*,11a*R* diastereomer. [l] IC_{50} = 8.0 μM, IC_{50}(diacid) = 0.0058 μM.

of novel aroylamino acids **21** possessing in vitro and in vivo ACE inhibitory activities. The aroylamino acid **21** class of ACE inhibitors is of interest because these compounds contain a mercaptan functionality directly bonded to an aromatic ring, in contrast to the aliphatic thiol moiety contained in captopril (**1**) and the vinylogous mercaptan of pivopril (**3**). Aromatic and alkyl thiols differ chemically in many respects, such as acidity and reactivity. It was our hope that these differences would result in a more advantageous pharmacological profile for these aromatic thiols. In particular, the clinical side effects thought to be due to the free aliphatic sulfhydryl group in captopril (**1**) may be reduced or eliminated in compounds such as **21**. Furthermore it was also our hope that by cyclization of **21** to give a series of 1,4-benzothiazepine-2,5-diones (**22**) we would maintain potent ACE inhibitory activity while reaching relatively low plasma levels of the biologically active mercaptans **21**.

The compounds **22** were easily prepared by treatment

(22) (a) Menard, P. R.; Suh, J. T.; Jones, H.; Loev, B.; Neiss, E. S.; Wilde, J.; Schwab, A.; Mann, W. S. *J. Med. Chem.* **1985**, *28*, 328. (b) Suh, J. T.; Menard, P. R.; Jones, H. U.S. Patent 4 440 941, 1984.

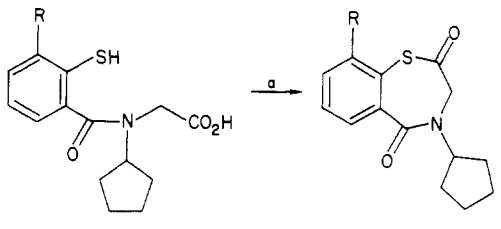
Table IV. In Vitro ACE Inhibitory Activities of 1,4-Benzothiazepine-2,5-diones



compd	R	mp,[a] °C	yield,[b] %	procedure[c]	formula[d]	I_{50},[e] μM	I_{50}(SH),[e,f] μM
22a	H	109–110	29	N	$C_{14}H_{15}NO_2S$	4.5	4.8
22b	Cl	116–117	31.9	N	$C_{14}H_{14}ClNO_2S$	0.21	0.28
22c	OCH_3	156–158	35	N	$C_{15}H_{17}NO_3S$	0.40	0.38
1 (captopril)							0.017
2 (enalapril)[g]							
3 (pivopril)						3.60	0.017

[a] Uncorrected. [b] Yield refers to the last step in each synthetic sequence. [c] See Experimental Section. [d] All compounds exhibited satisfactory C, H, and N microanalyses and were within 0.4% of theoretical values. All compounds exhibited IR, ^1H NMR, and MS spectra consistent with the assigned structures. [e] Concentration inhibiting 50% of the activity of rabbit lung ACE at pH 8.3 in 0.10 M KH_2PO_4 buffer containing 0.30 M NaCl with the substrate Hip-His-Leu at a concentration of 2 mM. [f] Corresponds to the IC_{50} values of the corresponding ring-open SH compounds **21**; see ref **22**. [g] I_{50} = 8.0 μM, IC_{50} = 0.058 μM.

Scheme III. Synthesis of 4-Cyclopentyl-1,4-benzothiazepine-2,5-diones[a]



21a, R=H
b, R=Cl
c, R=OCH_3

22a, R=H
b, R=Cl
c, R=OCH_3

[a] Reagents: **21**/$ClCO_2Et/CH_2Cl_2/Et_3N$.

of the corresponding aromatic thiols **21**[22] with ethyl chloroformate in CH_2Cl_2. In this fashion the desired 1,4-benzothiazepine-2,5-diones were obtained in 30–40% crystalline yields. The physical characteristics and the in vitro ACE inhibitory activities of **22** are listed in Table IV.

Biological Results and Discussion

The compounds of Tables I, III, and IV represent a novel class of structures that exhibit potent in vitro and in vivo ACE inhibitory activities comparable to that of captopril (**1**). The most active in vitro ACE inhibitors in the 1,4-thiazepine-2,5-dione series (Table I) were found to be **23**, **24**, **25**, **28**, and **30**. The IC_{50} values of these compounds were in the range of 0.012–0.026 μM. In the case of the 1,4-thiazine-2,5-diones (Table III), the most active compounds of the series were **42** and **45**, which possessed IC_{50} values of 0.15 and 0.085 μM, respectively. The most active member of the 1,4-benzothiazepine-2,5-dione series was **22b** having an IC_{50} of 0.21 μM. For comparison captopril (**1**), when tested in a likewise manner by us, gave an IC_{50} value of 0.017 μM.

Unfortunately, many compounds of the discussed three series possess a high degree of instability in rat plasma[23] and also in acidic and basic media. For example, incubation of **26** with rat plasma at 37 °C leads to conversion of 42% of the compound to the corresponding free thiol after 5 min. Incubation of **25**, **30**, and **43** with rat plasma at 37 °C leads to rapid disappearance of the thiolactone moiety of each compound. The time required for 50% loss of the original thiolactone concentration was 5 min, <5 min, and 8 min, respectively, for these compounds. Similar results were obtained upon dissolving the compounds in either aqueous 0.1 N HCl or phosphate buffer (pH 7.4). The drugs were found to be unstable in these aqueous media at 37 °C and exhibited half-lives ($t_{1/2}$) of 0.5–2.0 h. It is presumed that the drugs undergo rapid hydrolysis of the thiolactone to give the corresponding free SH compounds.

In terms of stability in plasma and in aqueous acid and base media, the 1,4-benzothiazepine-2,5-diones listed in Table IV were no better than the alicyclic thiazepines and thiazines in Tables I and III. For example, compounds **22b** and **22c** are prone to rather rapid hydrolytic ring opening under various conditions. It is assumed that the activity of the cyclic compounds **22b** and **22c** is due to rather facile ring opening to give the active aromatic thiols **21b** and **21c**. From Table IV it is seen that the in vitro ACE inhibitory activities of **22** correlate well with their corresponding ring-open forms.

The stability of 1,4-benzothiazepine-2,5-dione (**22c**) solutions at various pH values and in CH_3OH was studied by a UV spectral method. At room temperature, the half-lives between pH 1 and 4 ranged from 50 to 95 min, and those between pH 6 and 7.8 were less than 18 min. The half-lives at pH 2 and 7.3 at 37 °C were 60 and 5 min, respectively. The half-life of **22c** in distilled H_2O at room temperature was 45 min. The half-life of **22c** in human serum in vitro was less than 15 min. The half-life of **22b** in human serum in vitro was less than 5 min.

Steric compression at the carbon α to the thiolactone carbonyl group was introduced in compounds **27**, **43**, **44**, **47**, and **49** in an attempt to make the carbonyl group less susceptible to nucleophilic attack and therefore to decrease the rate of hydrolysis. However, these synthetic efforts did not increase the stability toward hydrolysis to any appreciable degree. For example, the $t_{1/2}$ of **42** in rat plasma was ca. 20 min whereas, for **44**, in which geminal dimethyls were placed adjacent to the thiolactone carbonyl, the $t_{1/2}$ was only 1.3 h. Unfortunately all of these compounds containing steric compression at the moiety designed to react with the S_2' subsite[24] of ACE were also

(23) See the Experimental Section for a method of measuring the thiol concentration of rat plasma solutions of thiols, thio esters, thiazepines **9**, thiazines **20**, and benzothiazepines **22**. This procedure has been adapted from a described method: Ellman, G.; Lysko, H. *Anal. Biochem.* **1979**, *93*, 98.

(24) This nomenclature is derived from the following ACE inhibitors review: Petrillo, E.; Ondetti, M. *Med. Res. Rev.* **1982**, *2*, 1.

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

compd	dose,[b] mg/kg, po	N[c]	% inhibn[d] (range)	duration,[e] min (range)
1 (captopril)	0.15[f] (0.30)	5	50 (65–68)[g]	(100)[g]
2 (enalapril)	0.08[h] (0.30)	5	50 (80–85)[g]	(210)[g]
3 (pivopril)	0.058[i] (0.30)	5	50 (65–70)[g]	(60)[g]
22a	0.15	3	(5)	(10–20)
22b	0.60	3	(50–55)	(40–50)
22c	0.80	3	(50–55)	(35–50)
23	0.15	3	(65–69)	(10–50)
24	0.15	4	(85–96)	(60–140)
25	0.15	2	(55–76)	(10–40)
26	1.5	2	(43–64)	(20–80)
27	1.5	4	(0–29)	(0–30)
28	0.15	4	(64–100)	(30–100)
30(*S,S*)	0.30	2	(45–50)	(10–30)
30(*R,S*)	0.30	2	(15–17)	(10)
42	0.15	4	(24–49)	(10–30)
	1.50	3	(73–79)	(30–50)
43	1.5	2	(3–5)	
44	1.5	2	(20–34)	(20)
45	1.5	4	(71–81)	(70–90)
47	1.5	3	(14–21)	(20–50)
48	1.5	2	(17–24)	(10)
49	1.5	2	(14–16)	(10–60)

[a] See Experimental Section. [b] Doses equal to or greater than the ED_{50} (0.15 mg/kg, po) of captopril (1) were selected. [c] N = number of animals. [d] Corresponds to the percent inactivation of the angiotensin I induced vasopressor response in normotensive conscious rats at a specified dose. [e] The time to 50% recovery of the angiotensin I response. [f] Literature[31] ID_{50} = 0.015 mg/kg, po. [g] Corresponds to a dose of 0.30 mg/kg, po. [h] Literature[13e] ID_{50} = 0.014 mg/kg, po. [i] Corresponds to REV 3659-(S), previously referred to as pivalopril. The approved USAN name for 3 is pivopril.

relatively inactive in inhibiting this enzyme in vitro, IC_{50} values of 15–100 μM being obtained. It should be pointed out that the corresponding ring-open forms were also all relatively inactive in inhibiting ACE in vitro. This seems to imply that the addition of an α-methyl or an α,α-dimethyl substituent at the S_2' receptor binding site of a substrate of ACE leads to sterical constraints that are not easily accommodated by the S_2' receptor cavity of ACE. This results in higher binding constraints and therefore higher IC_{50} values.

Because of the lack of stability of the thiazepines **9**, thiazines **20**, and benzothiazepines **22** and since acyclic thiols with structures similar to **8**, **18c**, and **22** are known to be potent ACE inhibitors[12t,12a,22a] in vitro, it is likely that the activity exhibited by the cyclic species is due to their corresponding ring-open SH forms. As seen in Table I the IC_{50} values of the thiazepines **9** are close in value and direction to their corresponding ring-open β-mercaptoalkanoyl amino acids **8**. As seen in Table IV, the benzothiazepines **22** gave IC_{50} values that were virtually identical with their corresponding ring-opened aromatic thiols **21**. When the IC_{50} values for the α-mercaptopropanoyl amino acids **18c** of Table II are compared to those for the corresponding thiazines **20** of Table III, similar observations are obtained. It is seen that the most potent α-mercaptopropanoyl amino acids **18c** of Table II gave rise to the most potent thiazines **20** of Table III. Because of the low stability in rat and human plasma and because of the instability in both basic and acidic media, it is assumed that all biological activities observed for the prodrug ring-closed materials **9**, **20**, and **22** are in all likelihood due to their corresponding ring-open counterparts **8**, **18c**, and **21**, respectively.

The compounds of Table I, III, and IV were also evaluated for their ability to inhibit the pressor response to angiotensin I when administered orally to unanesthetized normotensive male rats. 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.

The oral inhibition of ACE in conscious normotensive rats for selected representative agents is given in Table V. Those agents that were the most active in vitro were also the most active in vivo. From Table V it is seen that the 1,4-thiazepine-2,5-diones **23–25** and **30** are potent inhibitors of ACE when administered po to rats and are comparable in potency to captopril (**1**). The most active 1,4-thiazine-2,5-diones were **42** and **45**. In general the thiazines **20** were much less potent inhibitors of ACE than their corresponding seven-membered ring analogues as seen when **42** and **45** are compared to **23** and **25**, respectively. However, as mentioned above, it is assumed that the biologically active components of the thiazepine and thiazine series are their corresponding β-mercapto and α-mercapto ring-open forms. Cushman et al. have previously shown that β-mercapto acids **8** are much more potent inhibitors of ACE than their corresponding α-series **18c**.[12b] Therefore it is not surprising that the thiazepine prodrugs are much more potent inhibitors of ACE than their related thiazine prodrug analogues.

The benzothiazepines **22a–c** were also studied for their ability in inhibiting ACE in the conscious normotensive rat. As seen in Table V, ID_{50} values of 0.6 and 0.8 mg/kg, po, were obtained for **22b** and **22c**, respectively. When the cyclized compound **22c** was compared in a dose–effect study with its ring-open form **21c**, the ID_{50} obtained for both compounds was 0.8 mg/kg, po, and at 3 mg/kg, po, the duration of time in which inhibition was greater than or equal to 50% was 1.3 h for **22c** and 1.6 h for **21c**. At their ID_{50} doses **22c** and **21c** exhibited durations of action of 50 and 60 min, respectively. At 30 mg/kg, po, the durations of action for **22c** and **21c** were greater than 3 h. While only one-fifth as potent as captopril (**1**), the durations of action for **22c** and **21c** were comparable to that found with captopril at equiefficacous doses.

The acute antihypertensive effects of oral administration of the compounds in Tables I, III, and IV on mean arterial pressure (MAP) and heart rate were studied in spontaneously hypertensive rats (SHR) maintained on a sodium-deficient diet. From Table VI it is seen that the thiazepines **23**, **25**, and **30** (SR) caused decreases in MAP from 27% to 45% with durations of 12.5 h to greater than 24 h at doses ranging from 50 to 100 mg/kg, po. The

Table VI. Antihypertensive Effects of Selected Agents in Low-Sodium SHR[a]

compd	dose, mg/kg, po	N[b]	max[c] % ΔMAP (range)	duration,[d] h (range)
22a	100 (ip)	2	+ (9–14)	(2.5)
22b	100	3	− (17–29)	(5.5–15)
22c	100	2	− (32–40)	(>24)
23	100	2	− (15–27)	(2–12.5)
25	100	2	− (37)	(>24)
30(*S,S*)	100	2	− (27–45)	(15–>24)
45	50	3	− (31–34)	(3–19.5)
1 (captopril)	30	5	− (30, ±5), SD[e]	(>22)
2 (enalapril)	30	7	− (38, ±6), SD[e]	(>24)
3 (pivopril)	30	6	− (30, ±3), SD[e]	(>24)

[a] See the Experimental Section. [b] *N* = number of animals. [c] Maximum percent change in mean arterial pressure (MAP) over control at a specified dose. [d] Time that an agent changes arterial pressure outside the "control area". The control area is defined as the area between the means (±2 standard deviations) of arterial pressure and heart rate readings taken from 45 control rats. [e] SD = 1 standard deviation.

thiazine **45** at 50 mg/kg, po, caused a decrease of 34% in MAP with a duration of 19.5 h. The most active compound in the sodium-deficient SHR in the benzothiazepine series is **22c**. This compound at 100 mg/kg, po, resulted in a 40% decrease in MAP with a duration of greater than 24 h.

One of the most active compounds in inhibiting ACE in vitro is **30**. The antihypertensive effects of this thiazepine were compared to those of captopril (**1**) in the sodium-deficient SHR. Groups of seven animals were orally administered **30** in doses of 2–60 mg/kg or captopril (**1**) in doses of 0.6–20 mg/kg or methocel suspension (vehicle control). Two and 6 mg/kg of **30** had no significant effect compared to the methocel treatment. Twenty milligrams/kilogram decreased MAP a maximum of 11% and had a duration of 3 h. The 60 mg/kg dose was effective for 24 h, causing a maximum decrease in MAP of 17%. No dose significantly altered heart rate. In contrast, captopril (**1**) was effective for 24 h at 20 mg/kg but not at 6 mg/kg. The 20 mg/kg dose of captopril caused a maximum decrease in MAP of 21%. On the basis of these results, **30** appears to be approximately one-third as potent as captopril (**1**) on a weight basis.

For the benzothiazepine **22c**, 10 mg/kg, po, was the minimum dose, decreasing MAP by 16% and having a duration of less than 2 h in the sodium-deficient SHR. Thirty, 100, and 300 mg/kg, po, caused progressively larger decreases in MAP. The 30 mg/kg dose had an antihypertensive duration of 22 h, while the 100 and 300 mg/kg doses had durations of more than 24 h. For comparison 10 mg/kg, po, of captopril (**1**) decreased mean arterial pressure in the sodium-deficient SHR by 25% with a duration of over 24 h.

In conclusion, the thiazepines **9**, thiazines **20**, and benzothiazepines **22** in Tables I, III, and IV have resulted in a series of compounds that are potent inhibitors of ACE in vitro and in vivo. These compounds are assumed to act as prodrugs since they undergo rapid ring-opening reactions to give the corresponding biologically active free thiol compounds when incubated with rat plasma or exposed to aqueous 0.1 N HCl or phosphate buffer (pH 7.4). A number of these compounds also proved to be antihypertensive agents when tested in the sodium-deficient SHR model.

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 a 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 on a Perkin-Elmer 589 or 298 spectrophotometer. Solid samples were taken in KBr pellets. Liquid samples were taken neat on NaCl salt plates. ^{1}H 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 GS–MS equipped with an SS 100 data system at an 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 60F–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.

***tert*-Butyl 2-Bromopropionate (14).** α-Bromopropionic acid (500 g, 3.26 mol) was dissolved in CH_2Cl_2 (1400 mL) and concentrated H_2SO_4 (3–5 mL) was added. The resulting solution was cooled to ca. −5 to −10 °C by means of a dry ice/acetone bath. Isobutylene was bubbled into the solution with stirring until ca. 400 mL of isobutylene liquified. The resulting mixture was stirred for 18 h at room temperature. The CH_2Cl_2 was washed twice with 10% aqueous K_2CO_3 and twice with H_2O, dried over $MgSO_4$, filtered, and concentrated in vacuo to give pure **14** (561 g, 82%) as a colorless liquid (lit.[25] bp 80.3–83 °C (30 mm)), which was used directly.

***tert*-Butyl 2-(Acetylthio)propionate (15).** *tert*-Butyl 2-bromopropionate (**14**; 202 g, 1.967 mol) was dissolved in *p*-dioxane (1 L) and Et_3N (109 mL) was added. The reaction was placed under nitrogen and then thiolacetic acid (77.8 g, 1.024 mol) in *p*-dioxane (150 mL) was added dropwise with vigorous stirring. After all the thiolacetic acid was added (1 h), stirring was continued for 16 h at room temperature. Precipitated triethylamine hydrobromide was filtered and washed with a small amount of *p*-dioxane. The filtrate was concentrated in vacuo to give **15** as a reddish oil (189.5 g, 96%). The crude product **15** was used directly without further purification.

2-(Acetylthio)propionic Acid (16). A solution of **15** (354 g, 1.74 mol) in anisole (100 mL) was chilled in an ice-water bath to ca. 10 °C. To this solution was added TFA (370 mL) dropwise over $1^1/_2$ h. After all the TFA was added, the reaction mixture was stirred for 30 min with external cooling and then for 16 h at room temperature. Most of the TFA and anisole were evaporated in vacuo while the temperature was maintained below 60 °C. The residue was dissolved in AcOEt (500 mL) and the product was extracted several times into saturated aqueous $NaHCO_3$. The combined aqueous $NaHCO_3$ extract was washed twice with AcOEt and then acidified cautiously to pH 2 by the dropwise addition of concentrated HCl. The product was extracted several times into $CHCl_3$. The combined $CHCl_3$ extract was washed twice with H_2O and dried over $MgSO_4$. Filtration and evaporation of the solvent afforded the desired product (126 g, 49%) as a pale yellow oil. Anal. ($C_5H_8O_3S$) C, H.

2-(Acetylthio)propionic Acid (16). To **15** (86.3 g, 0.423 mol) in CH_2Cl_2 (500 mL) under nitrogen was added $(CH_3)_3SiI$ (84 g, 0.420 mol) dropwise. The reaction was stirred at room temperature for $1^1/_2$ h and then another portion of $(CH_3)_3SiI$ (60 g, 0.300 mol) was added. The reaction was stirred for an additional hour and then H_2O (ca. 100 mL) was added and stirring was continued for 5 min. The product was extracted several times into saturated aqueous $NaHCO_3$. The combined $NaHCO_3$ layer was washed twice with AcOEt and then acidified to pH 2–3 by the dropwise addition of concentrated hydrochloric acid. The precipitated product was extracted several times into AcOEt, washed with H_2O, dried over $MgSO_4$, filtered, and concentrated to afford **16** (49.8 g, 79.6%) as an orange oil, which was used directly without further purification and was identical in all respects to that described above.

(25) Carson, J.; Rinehart, K. L.; Thornton, S. D. *J. Org. Chem.* **1953**, *18*, 1594.

2-(Acetylthio)propionic Acid (16). A mixture of *p*-dioxane (200 mL) and Et_3N (161 g, 1.58 mol) was chilled in an ice–water bath to ca. 10 °C. To this solution thiolacetic acid (120 g, 1.58 mol) was added dropwise while the temperature was maintained between 10 and 15 °C. The resulting brick red solution of thiolacetic acid and Et_3N was added dropwise to a solution of 2-bromopropionic acid (242 g, 1.58 mol) in *p*-dioxane (500 mL) with vigorous stirring (mechanical stirrer). A resulting precipitate of triethylamine hydrobromide slowly formed. During the course of addition, the reaction became warm and was cooled intermittently with an ice–water bath. The reaction was stirred at room temperature for 16 h. The reaction was filtered to remove triethylamine hydrobromide. The filtrate was evaporated and the residue was dissolved in saturated aqueous K_2CO_3. The aqueous K_2CO_3 was washed twice with Et_2O. The aqueous phase was separated and acidified cautiously to pH 3 by the dropwise addition of concentrated HCl. The product was extracted several times into Et_2O. The combined Et_2O extract was washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to give **16** as an orange liquid (184.8 g, 78.9%), which was identical in all respects to that described in the example above.

2-(Acetylthio)propionyl Chloride (17). To a solution of **16** (125.5 g, 0.848 mol) in toluene (1400 mL) and DMF (3–5 mL) was added $SOCl_2$ (47 mL) dropwise at room temperature. After all the $SOCl_2$ was added (15–20 min), the reaction mixture was heated to a gentle reflux for 3 h and then stirred for 16 h at room temperature. Toluene was evaporated in vacuo and the product was vacuum distilled (56–67 °C (0.1–0.2 mmHg)) to give **17** as a pale yellow oil (57.6 g, 41%). The product was stored in a freezer until needed and used without further purification.

Method A. *tert*-Butyl *N*-[2-(Acetylthio)propanoyl]-*N*-cyclopentylglycinate (18a, R^1 = c-C_5H_9). A mixture of **16** (22.2 g, 0.150 mol) and *tert*-butyl *N*-(cyclopentyl)glycinate[26] (29.6 g, 0.149 mol) in CH_2Cl_2 (200 mL) was cooled in an ice–water bath to ca. 10 °C. To this solution was added DCC (31 g, 0.150 mol) portionwise over 10–15 min. After all the DCC was added, stirring was continued for 15 min with external cooling and then for 2 h at room temperature. Precipitated dicyclohexylurea was filtered and washed with a small amount of cold CH_2Cl_2. Evaporation of the filtrate yielded crude **18a** as a pale yellow oil (47 g), which was used directly without further purification.

Method B. *tert*-Butyl *N*-[2-(Acetylthio)propanoyl]-*N*-*p*-tolylglycinate (18a, R^1 = *p*-$CH_3C_6H_4$). *tert*-Butyl *N*-*p*-tolylglycinate[26] (25 g, 0.113 mol) was dissolved in CH_2Cl_2 (500 mL) and Et_3N (12 g, 0.119 mol) was added. The resulting solution was chilled in an ice–H_2O bath to 5–10 °C and then **17** (19 g, 0.114 mol) in CH_2Cl_2 (50 mL) was added dropwise over 15 min. After all of **17** was added, the reaction mixture was stirred for 1 h with external cooling followed by 2 h at room temperature. The reaction was concentrated in vacuo on a rotary evaporator. To the residue was added AcOEt. The AcOEt was washed consecutively twice with 10% aqueous $NaHCO_3$, twice with 10% aqueous HCl, and twice with H_2O. The organic extract was dried over $MgSO_4$, filtered, and evaporated to give crude **18a** as an orange oil. The crude product **18a** was further purified by high-performance LC employing the solvent system of $CHCl_3/CH_3OH$ (99:1) and used directly.

***exo*-*N*-Bicyclo[2.2.1]hept-2-ylglycine Ethyl Ester (5a).**[26] *exo*-2-Aminonorbornane (100 g, 0.901 mol) was dissolved in EtOH (1500 mL) and then Et_3N (100 g, 1.465 mol) was added. The resulting solution was chilled in an ice–water bath to 5–10 °C. Ethyl bromoacetate (150.4 g, 0.901 mol) was added dropwise over 30 min while the temperature was maintained between 5 and 10 °C. After the addition was complete, the reaction was stirred for 16 h at room temperature. The reaction was concentrated in vacuo, and then $CHCl_3$ (1 L) and H_2O (250 mL) were added to the residue. The $CHCl_3$ was separated and the aqueous layer was extracted once more with $CHCl_3$. The combined $CHCl_3$ extract was washed twice with H_2O, dried over $MgSO_4$, filtered, and evaporated to give a colorless oil. The crude product was vacuum distilled at 75–80 °C (0.1 torr) to give the titled compound **5a** as a colorless oil (108.2 g, 61%). Anal. ($C_{11}H_{19}NO_2$) C, H, N.

***exo*-*N*-Bicyclo[2.2.1]hept-2-ylglycine Sodium Salt (5b).** Sodium hydroxide (19.8 g, 0.495 mol) was added to absolute EtOH (1.5 L) and the resulting mixture was stirred at room temperature for 1 h and then chilled to 10 °C by means of an ice–water bath. To this mixture *exo*-*N*-bicyclo[2.2.1]hept-2-ylglycine ethyl ester (**5a**; 97.5 g, 0.495 mol) was added dropwise over 30 min while the temperature was maintained at 10 °C. The resulting mixture was vigorously stirred for 16 h at room temperature. The precipitated sodium salt was filtered and washed with a small amount of cold EtOH to give the titled compound **5b** as a colorless solid (56.5 g). The filtrate was chilled in an ice–water bath and the solid that formed was filtered and then washed with cold EtOH to give the titled compound **5b** (33.5 g). A combined yield of 90 g (95%) of the titled sodium salt **5b** was obtained, mp 255–258 °C. Anal. ($C_9H_{15}NO_2Na$) C, H, N.

Method C. *exo*-*N*-[3-(Acetylthio)-2-methyl-1-oxopropyl]-*N*-bicyclo[2.2.1]hept-2-ylglycine (7b, R^1 = *exo*-Norbornyl).[12t] The sodium salt of *N*-*exo*-norbornylglycine (**5b**, R^1 = *exo*-norbornyl; 29 g, 0.140 mol) was dissolved in CH_3OH (300 mL) and then triethylamine (33.5 g, 0.332 mol) was added. The resulting solution was cooled in a dry ice/acetone bath to −5 to 0 °C and then **6c**[12t] (30.3 g, 0.168 mol) was added dropwise over 10 min. After all **6c** was added, stirring was continued at −5 to 0 °C for 30 min and then at room temperature for $2^1/_2$ h. Methanol was evaporated and the product was extracted several times into saturated aqueous $NaHCO_3$. The combined aqueous $NaHCO_3$ extract was washed twice with AcOEt and then acidified to pH 3–4 by the dropwise addition of concentrated hydrochloric acid. The precipitated product was extracted into CH_2Cl_2, washed with H_2O, dried over $MgSO_4$, filtered, and concentrated to afford pure **7b**, R^1 = *exo*-norbornyl, as a viscous colorless oil (38.6 g, 88%). The DCHA salt was prepared by dissolving **7b** in Et_2O and then adding DCHA dropwise with stirring until pH 7–9. The precipitated salt was filtered and washed with a small amount of Et_2O to give colorless crystals, mp 123–125 °C (lit.[12t] mp 125–126 °C). Anal. ($C_{15}H_{23}NO_4S \cdot C_{12}H_{23}N$) C, H, N.

Method D. *tert*-Butyl *N*-(2-Bromopropanoyl)-*N*-cyclopentylglycinate (19, R^1 = c-C_5H_9). *tert*-Butyl *N*-cyclopentylglycinate[26] (56.9 g, 0.286 mol) was dissolved in *p*-dioxane (500 mL) and Et_3N (26.3 g, 0.260 mol) was added. 2-Bromopropionyl chloride (44.5 g, 0.260 mol) was added dropwise. After all the acid chloride was added, stirring was continued for 16 h. Precipitated triethylamine hydrochloride was filtered and washed with *p*-dioxane. The filtrate was evaporated in vacuo to give a thick pale orange oil. This material was dissolved in CH_2Cl_2. The CH_2Cl_2 was washed consecutively with H_2O, twice with 10% aqueous $NaHCO_3$, twice with 10% aqueous HCl, and twice with H_2O. The CH_2Cl_2 was dried over $MgSO_4$, treated with decolorizing charcoal, filtered, and evaporated to give **19** (80 g, 92%) as off white crystals. The analytical sample was prepared by recrystallization from isooctane, mp 48–49 °C. Anal. ($C_{14}H_{24}BrNO_3$) C, H, N.

Method E. *tert*-Butyl *N*-[2-(Acetylthio)propanoyl]-*N*-cyclopentylglycinate (18a, R^1 = c-C_5H_9). To **19** (R^1 = c-C_5H_9; 38.9 g, 0.116 mol) in *p*-dioxane (500 mL) was added dropwise at room temperature a mixture of thiolacetic acid (8.9 g, 0.116 mol) and Et_3N (11.8 g, 0.116 mol) in *p*-dioxane (100 mL). Several minutes after the addition a white precipitate of triethylamine hydrobromide formed. The reaction mixture was stirred at room temperature for 16 h. Precipitated triethylamine hydrobromide was filtered and washed with a small amount of cold *p*-dioxane. The filtrate was evaporated in vacuo to yield the crude product as an orange oil. The crude product was further purified by high-performance LC utilizing the solvent system of 15% AcOEt in *n*-C_6H_{14} to give **18a**, R^1 = c-C_5H_9, as a pale yellow oil (25.5 g, 66.6%), which was identical in all respects to that described above. Anal. ($C_{16}H_{27}NO_4S$) C, H, N.

Method F. *N*-[2-(Acetylthio)propanoyl]-*N*-cyclopentylglycine (33a). A solution of **18a** (R^1 = c-C_5H_9; 52 g, 0.158 mol) in anisole (30–40 mL) was chilled in an ice–water bath to 5–10 °C. TFA (100 mL) was added in one portion and the resulting mixture was stirred for 15 min with external cooling and then for 2 h at room temperature. Most of the TFA and anisole were evaporated in vacuo (T < 60 °C), and the residue was dissolved in AcOEt. The product was extracted several times into saturated

(26) See ref 12t for a general method of preparing N-substituted glycine esters from *tert*-butyl or ethyl bromoacetates and primary amines.

aqueous $NaHCO_3$. The combined aqueous extract was washed twice with AcOEt. The aqueous $NaHCO_3$ extract was acidified cautiously to pH 3 by the dropwise addition of concentrated HCl. The product was extracted several times into $CHCl_3$, washed with H_2O, dried over $MgSO_4$, filtered, and evaporated to give the crude product. The crude product was further purified by high-performance LC eluting with AcOEt/n-C_6H_{14}/AcOH (10:20:1) to give pure **33a** as a pale yellow oil (16.5 g, 41%). The DCHA salt was prepared by dissolving **33a** in Et_2O/n-C_6H_{14} and adding DCHA dropwise with stirring until pH 7–9. The precipitated salt was filtered and washed with a small amount of cold Et_2O/n-C_6H_{14} to give colorless crystals, mp 157–158 °C. Anal. ($C_{12}H_{19}NO_4S \cdot C_{12}H_{23}N$) C, H, N.

Method G. *N*-[2-(Acetylthio)propanoyl]-*N*-cyclopentylglycine (33a). To **18a** (R^1 = c-C_5H_9; 25.5 g, 0.0774 mol) in CH_2Cl_2 (400 mL) and under N_2 was added $(CH_3)_3SiI$ (15.5 g, 0.0774 mol). The reaction was stirred at room temperature for $1^1/_2$ h. A small amount of H_2O (50 mL) was added. The layers were separated, and the organic phase was washed twice with 10% aqueous $NaHSO_3$, twice with 10% aqueous HCl, and twice with H_2O. The CH_2Cl_2 was dried over $MgSO_4$, filtered, and evaporated to give pure **33a** (17.8 g, 84%) as a pale yellow oil, which was identical in all respects to that described above in method F.

Method H. *N*-(2-Mercaptopropanoyl)-*N*-cyclopentylglycine (33b). Anhydrous NH_3 was bubbled through CH_3OH (200 mL) for 15 min and then the saturated NH_3/CH_3OH solution was added to **18b** (R^1 = c-C_5H_9; 16.5 g, 0.060 mol) and placed under N_2. The resulting solution was stirred at room temperature for $1^1/_2$ h. The CH_3OH was evaporated and the residue was applied to a column of 130 g of AG-50W-X2 (Bio Rad Laboratories) cation-exchange resin and eluted with CH_3OH. The CH_3OH was evaporated in vacuo and the residue was dissolved in $CHCl_3$. The $CHCl_3$ was washed with H_2O, dried over $MgSO_4$, filtered, and evaporated to give the pure product as a pale yellow oil (11.8 g, 85%). The DCHA salt was prepared in Et_2O to give colorless crystals, which were filtered and washed with cold Et_2O, mp 163–164 °C. Anal. ($C_{10}H_{17}NO_3S \cdot C_{12}H_{23}N$) C, H, N.

3-Carboxy-1,2,3,4-tetrahydroisoquinoline (11a).[17a-d] A suspension of L-phenylalanine (75 g, 0.455 mol) in concentrated HCl (488 mL) and 37% formalin (165 mL) was heated to a gentle reflux with vigorous stirring for 30 min. After this time another portion of formalin (75 mL) and concentrated HCl (165 mL) were added. Stirring and heating were continued for 4 h. The reaction mixture was cooled to room temperature and the solid that formed was filtered and washed with a small amount of CH_3OH to afford the hydrochloride of **11a** as a colorless solid (68.9 g, 71%): mp 309–310 °C dec (lit.[17d] mp 327.5 °C dec); $[\alpha]^{22}_D$ −78.24° (*c* 1.01, CH_3OH) (lit.[17d] $[\alpha]_D$ −176.1° (*c* 1.8%, 1.4 N NaOH)). This partially racemized product was used directly without further purification in order to synthesize **29**. Anal. ($C_{10}H_{11}NO_2 \cdot HCl$) C, H, N.

Method I. *N*-[3-(Acetylthio)-2-methylpropanoyl]-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (7b). To a stirred suspension of partially resolved **11a** (40.0 g, 187.2 mmol; $[\alpha]^{22}_D$ −78.24° (*c* 1.01, CH_3OH)) in *p*-dioxane (1 L) and H_2O (200 mL) was added Et_3N (78 mL, 560.8 mmol) followed by the dropwise addition of 3-(acetylthio)-2-methylpropionyl chloride[12t] (**6c**, 33.8 g, 187.2 mmol). The mixture became mostly homogeneous as the reaction proceeded. After ca. 3 h a small amount of insoluble material was filtered off and the filtrate was concentrated in vacuo on a rotary evaporator. To the residue was added H_2O (500 mL) and then concentrated aqueous HCl was added until a pH of 2–3 was obtained. The product was extracted several times into AcOEt. The combined AcOEt extract was washed consecutively with H_2O, twice with 10% aqueous HCl, and once with brine. The AcOEt was dried over $MgSO_4$, filtered, and evaporated to give crude **7b** (55 g) as an orange oil. Crude **7b** was further purified by high-performance LC over silica gel, employing the solvent system of n-C_6H_{14}/AcOEt/AcOH (30:20:1) to give pure **7b**[27] (30.3 g, 50.2%) as a pale yellow oil. The DCHA salt of **7b** was prepared by dissolving **7b** in Et_2O and then adding DCHA dropwise with stirring until a pH of 7–9 was obtained. The precipitated salt was filtered and washed with Et_2O to afford colorless crystals, mp 161–163 °C. Anal. ($C_{16}H_{19}NO_4S \cdot C_{12}H_{23}N$) C, H, N.

***N*-(3-Mercapto-2-methylpropanoyl)-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (8).** This compound was obtained in 98% yield as a colorless oil by method H. The DCHA salt was prepared by dissolving **8** in Et_2O and then adding DCHA dropwise with stirring until a pH of 7–9 was achieved. The precipitated salt was filtered and washed with Et_2O to afford colorless prisms of **8**,[27] mp 145–146 °C (lit.[12q,28] mp 191–192 °C). Anal. ($C_{14}H_{17}NO_3S \cdot C_{12}H_{23}N$) C, H, N.

3-Carboxy-3-methyl-1,2,3,4-tetrahydroisoquinoline (11b).[17e] A suspension of α-methyl-DL-phenylalanine (25.0 g, 0.14 mol) and paraformaldehyde (3.0 g, 0.10 mol) in 12 N HCl (150 mL) was heated to a gentle reflux. The suspension was treated with further paraformaldehyde at 1-h intervals (2 g and then 1 g) and then refluxed for 1 h after the final addition. The reaction was then stirred for 16 h at room temperature. The precipitated solid was collected and dried in vacuo to give the hydrochloride of **11b** (20.1 g, 63.3%) as a colorless solid, mp >300 °C (lit.[17e] mp not given), which was used directly without further purification.

Method J. 2-[2-(Acetylthio)propanoyl]-3-methyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (40). A suspension of **11b**·HCl (1.7 g, 7.47 mmol) in pyridine (20 mL) was treated dropwise with **17** (1.7 g, 10.2 mmol) over 5 min. The mixture was stirred for 16 h at room temperature and then concentrated in vacuo. The residue was chromatographed over silica gel (100 g), utilizing the solvent system of 20% HOAc in n-C_6H_{14} to recover excess **17** followed by the elution of **40** with 30% HOAc in n-C_6H_{14}. In this manner **40** (0.7 g, 29%) was obtained as a colorless solid, mp 173–179 °C. Anal. ($C_{16}H_{19}NO_4S$) C, H, N.

***N*-[2-(Acetylthio)propanoyl]thiazolidine-4(*R*)-carboxylic Acid (37a).** This compound was prepared in 84% yield as a colorless oil by means similar to method I. In this case H_2O was used as the reaction solvent and $NaHCO_3$ (3 equiv) was used as the base instead of Et_3N. The product was isolated as a mixture of *S,R* and *R,R* diastereomers as a colorless solid. The mixture of diastereomers was fractionally recrystallized from Et_2O to give the respective pure, diastereomers on the basis of TLC analyses. The faster moving spot by TLC (AcOEt/n-C_6H_{14}/AcOH, 60:40:1) afforded the *S,R* diastereomer as a colorless crystalline solid: mp 103–105 °C; $[\alpha]^{25}_D$ −232.48° (*c* 1.0, $CHCl_3$). Anal. ($C_9H_{13}NO_4S_2$) C, H, N. The more soluble material and the lower spot on TLC afforded the pure *R,R* diastereomer as a colorless crystalline solid: mp 129–131 °C; $[\alpha]^{25}_D$ +22.25° (*c* 1.0, $CHCl_3$). Anal. ($C_9H_{13}NO_4S_2$) C, H, N.

(4*R*)-3-[(2*S*)-2-Mercaptopropanoyl]thiazolidine-4-carboxylic Acid (37b). Method H was employed to give the titled compound **37b** (*SR*) in 95% yield as colorless crystals: mp 117–118 °C; $[\alpha]^{25}_D$ −97.01° (*c* 1.0, $CHCl_3$) (lit.[12d] mp 122–123 °C; $[\alpha]^{25}_D$ −110.4° (*c* 1.0, CH_3OH)). Anal. ($C_7H_{11}NO_3S_2$) C, H, N.

(4*R*)-3-[(2*R*)-2-Mercaptopropanoyl]thiazolidine-4-carboxylic Acid (37b). Method H was employed to give the titled compound **37b**(*RR*) in 92% yield as colorless crystals: mp 153–155 °C; $[\alpha]^{25}_D$ −162.18° (*c* 1.0, $CHCl_3$) (lit.[12d] mp 161–163 °C; $[\alpha]^{25}_D$ −166.2° (*c* 1.0, CH_3OH)). Anal. ($C_7H_{11}NO_3S_2$) C, H, N.

3,3,5,5-Tetramethyl-*dl*-thiazolidine-4-carboxylic Acid (13).[18] DL-Penicillamine (50.0 g, 0.335 mol) was suspended in acetone (500 mL) and then concentrated HCl (15 mL) was added. The mixture was heated to reflux for $3^1/_2$ h and then allowed to cool slowly while standing overnight under nitrogen. The crystalline product was collected by filtration and was washed with a small amount of acetone. The product was dried several hours under vacuum at 65 °C to afford the hydrochloride of **13** as a colorless solid (60.0 g, 79.5%), mp 200–204 °C (lit.[18] mp 199 °C).

***N*-[3-(Acetylthio)-2-methylpropanoyl]-2,2,5,5-tetramethylthiazolidine-4-carboxylic Acid (7b).** The titled compound was prepared in 65% yield as colorless prisms by method I, mp 145–146 °C. Anal. ($C_{14}H_{23}NO_4S_2$) C, H, N.

***N*-(3-Mercapto-2-methylpropanoyl)-2,2,5,5-tetramethylthiazolidine-4-carboxylic Acid (8).** This compound was obtained in 92% yield as a colorless crystalline material by method

(27) On the basis of TLC, this compound consists of a mixture of 2′*S*,3*S* and 2′*R*,3*S* diastereomers in which the former is highly predominant (9:1).

(28) Refers[12q] to fully resolved material: 2′*S*,3*S* diastereomer; $[\alpha]_D$ −21.0° (*c* 1.0, CH_3OH).

H, mp 163–164 °C. Anal. ($C_{12}H_{21}NO_3S_2$) C, H, N.

N-[3-(Acetylthio)-2-methylpropanoyl]thiazolidine-4-(R)-carboxylic Acid (7b). This compound was prepared in 85% yield as a colorless oil by means similar to method I. The reaction was done in H_2O and employed $NaHCO_3$ (3 equiv) instead of Et_3N as the base. The product was isolated as a mixture of *S,R* and *R,R* diastereomers (60:40). The DCHA salt was prepared as described in method C to give colorless crystals, mp 178–181 °C. Anal. ($C_{10}H_{15}NO_4S_2 \cdot C_{12}H_{23}N$) C, H, N.

N-(3-Mercapto-2-methylpropanoyl)thiazolidine-4(R)-carboxylic Acid (8). This compound was obtained from the corresponding acetylthio compound, mixture of *S,R* and *R,R* diastereomers, in 92% yield by method H. The product was isolated as a colorless oil, mixture of diastereomers. The DCHA salt was prepared as described in method C, mp 190–191 °C (lit.[12d] mp 190–191 °C). Anal. ($C_8H_{13}NO_3S_2 \cdot C_{12}H_{23}N$) C, H, N.

Method K. N-Cyclopentyl-6-methyl-1,4-thiazine-2,5-dione (42). A solution of **33b** (11.6 g, 0.050 mol) in CH_2Cl_2 (300 mL) was cooled to 0 °C by means of a dry ice/acetone bath and then Et_3N (5 g, 0.050 mol) was added. Ethyl chloroformate (5.4 g, 0.050 mol) in CH_2Cl_2 (20 mL) was added dropwise. The reaction was allowed to warm slowly to room temperature and then stirred for 16 h. The CH_2Cl_2 was evaporated and the residue was partitioned between H_2O and Et_2O. The layers were separated, and the Et_2O layer was washed once each with saturated aqueous $NaHCO_3$, H_2O, and brine. The Et_2O was dried over $MgSO_4$, filtered, and evaporated to afford the crude product as an orange oil. The product was further purified by high-performance LC using the solvent system of 25% AcOEt in *n*-C_6H_{14} to yield **42** (7.0 g, 65.6%) as pale yellow crystals. The analytical sample was prepared by recrystallization from 30% isooctane in *n*-C_6H_{14}, mp 73–74 °C. Anal. ($C_{10}H_{15}NO_2S$) C, H, N.

Method L. 4-exo-Bicyclo[2.2.1]hept-2-yl-6,7-dihydro-6-methyl-1,4-thiazepine-2,5(3H,4H)-dione (24). A solution of *N-exo*-2-bicyclo[2.2.1]heptyl-*N*-(3-mercapto-2-methylpropanoyl)glycine (**8**;[12t] 3.5 g, 13 mmol) in toluene (150 mL) was deoxygenated by bubbling nitrogen through it for 10 min. To this solution was then added 2,2′-dipyridyl disulfide[21] (4.28 g, 19.5 mmol) and triphenylphosphine (5.1 g, 19.5 mmol) and the resulting solution was stirred under N_2 for 5 h. This solution was then diluted to 225 mL with toluene and added at a rate of 15 mL/h to 2.5 L of refluxing toluene. The resulting mixture was stirred at reflux for 48 h and was then cooled to room temperature. The solution was consecutively washed with 0.1 N HCl, saturated aqueous $NaHCO_3$, H_2O, and brine. The toluene was dried over $MgSO_4$, filtered, and evaporated in vacuo. The resulting solid was purified by high-performance LC employing the solvent system of 35% AcOEt in *n*-C_6H_{14} to afford pure **24** (1.2 g, 36%) as colorless crystals, mp 134–136 °C. Anal. ($C_{13}H_{19}NO_2S$) C, H, N.

Method M. 6-Methyl-1H,3H-thiazolo[4,3-c][1,4]thiazine-5,8(6H,8aH)-dione (46). Polyphosphoric acid (36 g) was added to a 1:1 mixture of **37b**(*RR*) and **37b**(*SR*) (1.5 g, 6.78 mmol) and the mixture was stirred at 50 °C for a period of 4 h. The reaction mixture was then dissolved in H_2O and the product was extracted several times into AcOEt. The combined organic extract was washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to give **46** (0.7 g, 41.9%) as a pale yellow oil and as a mixture of diastereomers. Anal. ($C_7H_9NO_4S_2$) C, H, N.

(3S,11aR)-6,11-Dihydro-3,11a-dimethyl-1,4-thiazino[4,3-b]isoquinoline-1,4(3H,11aH)-dione (49). The titled compound was prepared in a manner analogous to methods H and K. Anhydrous NH_3 was bubbled through CH_3OH (125 mL) for 15 min and then a solution of **40** (2.3 g, 7.17 mmol) in CH_3OH (20 mL) was added and the resulting solution was placed under nitrogen. The reaction was stirred at room temperature for $1^1/_2$ h. The CH_3OH was evaporated and the residue was applied to a column of AG-50W-X2 (Bio-Rad Laboratories) cation-exchange resin and eluted with CH_3OH. The CH_3OH was evaporated to afford *N*-(2-mercaptopropanoyl)-3-methyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (1.8 g) as a colorless oil, which was used directly without further purification. The thiol acid (1.8 g, 6.45 mol) was dissolved in $CHCl_3$ (60 mL) and then Et_3N (0.88 mL) was added. The resulting solution was cooled to −5 °C by means of a dry ice/acetone bath. Ethyl chloroformate (0.61 mL, 6.38 mmol) in $CHCl_3$ (10 mL) was added dropwise over 20 min. At the end of the addition the reaction mixture was warmed slowly to room temperature over 3 h. The reaction was washed with H_2O and 2 N HCl, dried over $MgSO_4$, filtered, and concentrated to give crude **49** as a yellow oil. The product was chromatographed over silica gel, using a gradient system of 0–10% AcOEt in $CHCl_3$. In this manner pure **49** (0.69 g, 41.1%) was obtained as colorless crystals, mp 170–172 °C. Anal. ($C_{14}H_{15}NO_2S$) C, H, N.

Method N. 9-Chloro-4-cyclopentyl-1,4-benzothiazepine-2,5(3H)-dione (22b). A solution of **21b**[22] (5.5 g, 17.5 mmol) in CH_2Cl_2 (150 mL) was cooled to 0 °C by means of a dry ice/acetone bath and then Et_3N (1.8 g, 17.5 mmol) was added. Ethyl chloroformate (1.9 g, 17.5 mmol) in CH_2Cl_2 (15 mL) was added dropwise. After all the ethyl chloroformate was added, the reaction was stirred for 30 min at 0 °C and then for 2 h at room temperature. The CH_2Cl_2 was washed twice with 10% aqueous HCl and twice with brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified over silica gel by high-performance LC employing the solvent system of AcOEt/*n*-C_6H_{14} (40:60) and a small amount of HOAc (1%). In this manner pure **22b** (1.65 g, 31.9%) was obtained as colorless needles, mp 117–118 °C. Anal. ($C_{14}H_{14}ClNO_2S$) C, H, N.

Preparation of Angiotensin-Converting Enzyme. A crude preparation of ACE was obtained by extracting rabbit lung acetone powder (Pel-Freez Biologicals, Inc., Rogers, AZ) with cold 0.05 M KH_2PO_4 buffer at pH 8.3. The homogenate was centrifuged for 30 min at 37000*g* and the clear supernatant, containing the ACE, was dialyzed against 0.05 M KH_2PO_4 buffer to remove low-molecular-weight inhibitors. This preparation has been described by Cushman and Cheung.[29] 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, using hippuryl-histidyl-leucine (HHL), 2 mM, as substrate by the method of Cushman and Cheung.[30] 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 AcOEt. 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 was 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 were connected to transducers for the 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 a 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 average two initial responses 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 were calculated.

Antihypertensive Effects in Sodium-Deficient SHR. The method used in the antihypertensive evaluation of an agent in the sodium-deficient SHR has previously been described.[12t]

Assay for Thiol Concentrations. The assay for thiol concentrations of solutions of **9**, **20**, and **22** incubated in rat plasma has been adapted from a method described by Ellman and Lysko.[23] Rats were killed by decapitation and blood (7–9 mL per rat) was

(29) Cushman, D. W.; Cheung, H. S. "Hypertension"; Springer Verlag: Berlin, 1972; p 532.
(30) Cushman, D. W.; Cheung, H. S. *Biochem. Pharmacol.* **1971**, *20*, 637.
(31) Rubin, B.; Laffan, R. J.; Kotler, D. G.; O'Keefe, E. H.; DeMaio, D. A.; Goldberg, M. E. *J. Pharmacol. Exp. Ther.* **1978**, *204*, 271.

collected in 15-mL Corex centrifuge tubes that contained 0.1 mL of Na_2 EDTA, 100 mg/mL. Rat plasma was obtained by centrifuging the blood at 3000*g* for 5 min at 4 °C.

In order to determine the effect of rat plasma on the thiol concentration of a solution of test compounds, 0.03 mL of a solution of test compound in Me_2SO or an appropriate solvent was incubated with 0.6 mL of rat plasma at 37 °C. At appropriate time intervals, 0.05-mL aliquots were removed and diluted with 1.8 mL of 5 mM NaH_2PO_4/1 mM disodium EDTA, pH 7.4. Within 30 min, 0.2 mL of a solution of 5,5′-dithiobis(2-nitrobenzoic acid) sodium salt, 10 mg/mL in 5 mM NaH_2PO_4/1 mM disodium EDTA, pH 7.4, was added to the diluted plasma aliquot. After 10 min at room temperature, the absorbance of the sodium was determined at 410 nm against a water blank. A sample blank was obtained by adding 0.01 mL of a saturated solution of *N*-ethylmaleimide to the sample and determining the absorbance at 410 mM. For thiols, a parallel sample containing 0.6 mL of rat plasma and 0.03 mL of Me_2SO or appropriate solvent was carried through the above procedure to determine the thiol concentration of rat plasma. For non-thiols like **9**, **20**, and **22** that were unstable under assay conditions an additional parallel sample consisting of 0.6 mL of solvent plus 0.03 mL of dry solution was carried through the same procedure described for rat plasma. The solvent chosen for this sample must be one in which the compound is stable. The absorbance at 410 nm of a Me_2SO blank consisting of Me_2SO (0.05 mL), sodium phosphate (5 mM), EDTA (1 mM, pH 7.4), and 5,5′-dithiobis(2-nitrobenzoic acid) sodium salt solution was also determined.

Calculations. (a) For compounds that contain thiol groups, the concentration of thiol in the plasma–drug solution that is due to the presence of drug is obtained as follows:

$$\text{drug (SH) in plasma} = [\text{net } A_{410}(\text{plasma–drug})] \times \frac{2.05}{0.05} \times \frac{1000}{13600} - [\text{net } A_{410}(\text{plasma–solvent})]$$

(b) For non-thiol compounds that may generate thiols under conditions of the thiol assay, first the drug (SH) in plasma is determined as above. The drug (SH) in solvent is determined as follows:

$$\text{drug (SH) in solvent} = [\text{net } A_{410}(\text{solvent–drug})] \times \frac{2.05}{0.005} \times \frac{1000}{13600} - [\text{net } A_{410}(\text{solvent})]$$

(c) Determinations of the "corrected drug (SH) in plasma" were necessitated by the observation that some thiazepines appeared to generate thiol under the assay conditions. The corrected drug (SH) in plasma was determined as follows:

$$\text{corrected drug (SH) in plasma} = \text{drug (SH) in plasma} - \text{drug (SH) in solvent}$$

(d) The number 13 600 that appears in the calculations is the molar extinction coefficient of thionitrobenzoate, a product of the reaction of thiol with 5,5′-dithiobis(2-nitrobenzoic acid). The percent SH present is determined as follows:

$$\%\ \text{SH present} = (\text{corrected drug (SH) in plasma})/(\text{potential (SH) of the plasma–drug solution}) \times 100$$

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Registry No. **5a**, 84827-39-4; **5b**·Na, 100486-30-4; (±)-**6c**, 70354-87-9; **7b** (R^1 = *exo*-norbornyl), 78773-45-2; **7b** (R^1 = tetramethylthiazolidine), 100486-35-9; **7b** (R^1 = thiazolidine), 100486-63-3; **7b** (R^1 = isoquinoline; isomer 1), 100486-33-7; **7b** (R^1 = isoquinoline; isomer 2), 100486-65-5; **7b** (isomer 1)·DCHA, 100486-64-4; **7b** (isomer 2)·DCHA, 73642-36-1; **8** (R^1 = *exo*-norbornyl), 78773-46-3; **8** (R^1 = tetramethylthiazolidine), 100486-62-2; **8** (R^1 = thiazolidine; isomer 1), 67714-45-8; **8** (R^1 = thiazolidine; isomer 1)·DCHA, 67714-50-5; **8** (R^1 = thiazolidine; isomer 2), 67714-46-9; **8** (R^1 = thiazolidine; isomer 2)·DCHA, 67714-51-6; **8** (R^1 = isoquinoline; isomer 1), 77832-18-9; **8** (R^1 = isoquinoline; isomer 1)·DCHA, 77832-19-0; **8** (R^1 = isoquinoline; isomer 2), 77832-17-8; **8** (R^1 = isoquinoline; isomer 2)·DCHA, 100486-66-6; (±)-**11a**, 100486-32-6; (±)-**11b**, 100486-34-8; (±)-**13**, 33078-43-2; (±)-**14**, 32821-07-1; (±)-**15**, 100486-38-2; (±)-**16**, 97643-42-0; (±)-**17**, 80058-08-8; (±)-**18a** (R^1 = C-C_5H_9), 100486-36-0; (±)-**18a** (R^1 = *p*-MeC_6H_4), 100486-37-1; (±)-**19** (R^1 = C-C_5H_9), 100486-31-5; **21b**, 83596-90-1; **22a**, 100486-61-1; **22b**, 83751-35-3; **22c**, 83751-36-4; (±)-**23**, 100486-39-3; **24**, 80142-31-0; (±)-**25**, 100486-40-6; **26** (isomer 1), 75527-07-0; **26** (isomer 2), 100570-31-8; **27**, 100486-41-7; **28**, 74190-08-2; **29**, 77972-29-3; **30**, 78962-34-2; **31**, 100570-28-3; (±)-**32**, 33068-82-5; (±)-**33a**, 100486-42-8; (±)-**33b**, 100486-43-9; **34a**, 100486-44-0; **34b**, 100486-45-1; (±)-**35**, 100486-46-2; (±)-**36a**, 100486-47-3; (±)-**36b**, 100486-48-4; **37a** (isomer 1), 100486-49-5; **37a** (isomer 2), 100486-50-8; **37b** (isomer 1), 67714-43-6; **37b** (isomer 2), 67714-44-7; **38a**, 100486-51-9; **38b**, 100486-52-0; **39**, 100486-53-1; **40**, 81089-91-0; (±)-**41**, 100486-54-2; (±)-**42**, 100486-55-3; **43**, 100486-56-4; (±)-**44**, 100486-57-5; (±)-**45**, 100486-58-6; **46** (isomer 1), 100570-29-4; **46** (isomer 2), 100570-30-7; **47**, 100486-59-7; **48**, 77171-92-7; **49**, 100486-60-0; CH_3COSH, 507-09-5; (±)-2-bromopropionic acid, 10327-08-9; *tert*-butyl *N*-cyclopentylglycinate, 78773-69-0; *tert*-butyl *N*-*p*-tolylglycinate, 84827-47-4; *exo*-2-aminonorbornane, 7242-92-4; (±)-2-bromopropionyl chloride, 71425-59-7; thiazolidine-4-carboxylic acid, 34592-47-7; 2-(2-mercaptopropanoyl)-3-methyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 81089-89-6.