Angiotensin-Converting Enzyme Inhibitors. 9.[1] Novel [[N-(1-Carboxy-3-phenylpropyl)amino]acyl]glycine Derivatives with Diuretic Activity

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A series of molecules 1 having sulfonamide diuretic moieties covalently linked to non-sulfhydryl angiotensin-converting enzyme inhibitors (ACEI) were prepared and tested for both activities. IC$_{50}$ values for ACEI as low as 7 nM were observed. Discernable diuretic activity was seen for several hydrochlorothiazide-based molecules. Effects of the ACEI and diuretic structures on the respective potencies are discussed.

The creation of novel antihypertensive agents remains a principal focus of medical research. In the last decade a significant advance has been made with the introduction of angiotensin-converting enzyme (ACE) inhibitors. Compounds such as captopril[2] and enalapril[3] have captured a significant share of the market, and many similar compounds are currently under clinical investigation.[4]

It has been found that hypertensive patients do not always respond adequately[5] to simple treatment with an ACE inhibitor. In these cases therapeutic success often depends upon adding a diuretic[6] to the treatment regimen. Physiologically this has been explained as an elevation of circulating renin levels due to diuretic-induced sodium depletion.[7] The resulting angiotensin II (AII) production becomes a major contributor to blood pressure elevation. ACE inhibition under these conditions now removes this contribution, leading to a much higher treatment success rate. In general the necessary diuretic dosage has been found to be less than that required for an equivalent response to diuretics alone.

We considered it worthwhile to introduce diuretic activity into known ACE inhibitors from our own[8] and other[3] laboratories. Our goal was an agent with potent ACE activity and only mild diuretic activity. In this way we hoped to retain the beneficial effects of diuretic coadministration while minimizing the hypokalemic[9] and uricosuric[10] side effects frequently associated with diuretic therapy. Such an approach[11] would allow for improvement in initial treatment success rate without the need to titrate two dosage regimens. In addition the simpler dosage would favor patient compliance.

Consideration of the known structure–activity relationships of existing diuretics[12] led us to conclude that the

Scheme I
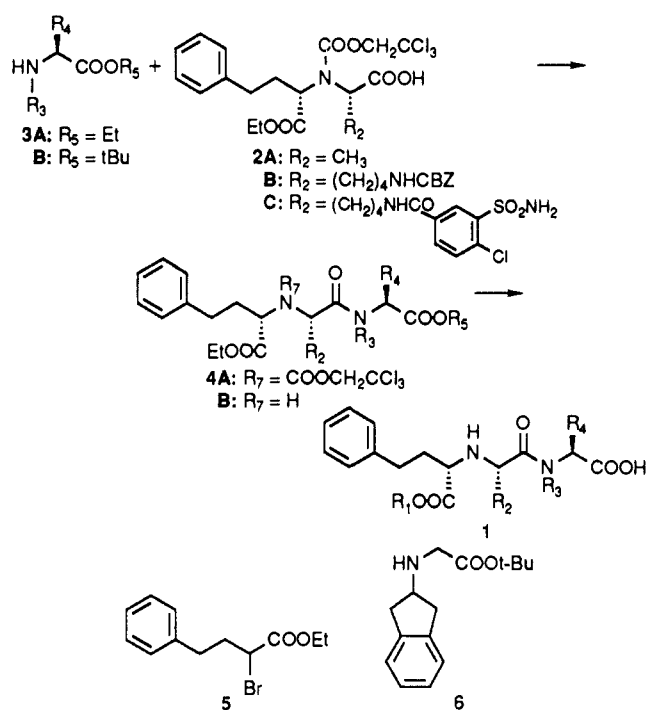
substitution pattern of indapamide,[13] hydrochlorothiazide,[14] furosemide,[15] and bumetanide[16] might readily

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(2) (a) Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science* **1977**, *196*, 441. (b) Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484. (c) Andren, L.; Karlberg, B. E.; Svensson, A.; Ohman, P.; Nilsson, O. R.; Hansson, L. *Acta Med. Scand.* **1985**, *217*, 155.

Table I

		activity								
		ip[a]			po[a]				diuretic[c]	
cmpd	ACEI in vitro: IC$_{50}$, µM	10	30	100	10	30	100	dur[b]	ip	po
1a	40%[d]					+		+		0
1b	0.68		0			+		+		0
1c	2.4				++			0		+
1d	NT[e]		+				+	++	++	
1e	2.2		++		0	0		++		0
1f	0.38			+++	+	+	++	+++		++
1g	28			+++	+	++	++	+	0	
1h	80			++		0			0	
1i	22			++		0		++	0	
1j	0.16			+++				++	0	
1k	0.23	+++		+++	+	+	+++	+++		++
1l	2.7				0		++	++		NT[e]
1m	3.5						++	++		NT[e]
1n	95						+	+	++	
1o	0.22	+++			++	++	+++	++		++
1p	15	++			++		++	++	++	
1q	38		NT[e]			NT[e]			0	
1r	0.0065	+++			++		+++	+++	0	0
1s	74%[d]	++					+	+		++
1t	0.40	+++			++		+++	+++		0
1u	0.007						++	++		0
1v	<20%[d]	+				0		+		0
1w	0.24			+++				+		+

[a] Inhibition of AI challenge at indicated mg/kg: 0, <30%; +, 30–50%; ++, 50–80%; +++, 80–100%. [b] Duration: +, <1 h; ++, 1–3 h; +++, >3 h. [c] Diuretic activity: 0, no increase; +, natriuresis; ++, natriuresis and diuresis (N = 8 or 16). [d] Percent inhibition at 100 µM. [e] Not tested.

be incorporated into our desired target structures with retained activity. In addition the simplicity and novelty

Scheme II

inherent in the structure of MK 447[17] recommended its inclusion. The current paper describes our results with glycine derivatives;[8] a companion article[18] addresses the

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(4) For a recent review, see: Kostis, J. B., DeFelke, E. A., Eds. Angiotensin Converting Enzyme Inhibitors; Alan R. Liss, New York, 1987; pp 213–262.

(5) This fraction has been reported as 75% for captopril[2c] and 35–45% for enalapril[3b] in long-term studies.

(6) For a recent review of the application of diuretics in antihypertensive therapy, see: Moser, M. Med. Clin. North Am. 1987, 71, 935.

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(10) Weinberger, M. H. J. Cardiovasc. Pharmacol. 1985, 7 (Suppl), S52. Weinberger, M. H. Hypertension 1983, 5 (Suppl. 3), 111.

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(12) For a recent summary, see: Cragoe, E. J., Jr., Ed. Diuretics; Wiley: New York, 1983.

(13) Beregi, L. G. Curr. Med. Res. Opin. 1977, 5 (Suppl 1), 3.

(14) deStevens, G.; Werner, L. H.; Halamandaris, A.; Ricca, S. Experientia (Basel) 1958, 14, 463.
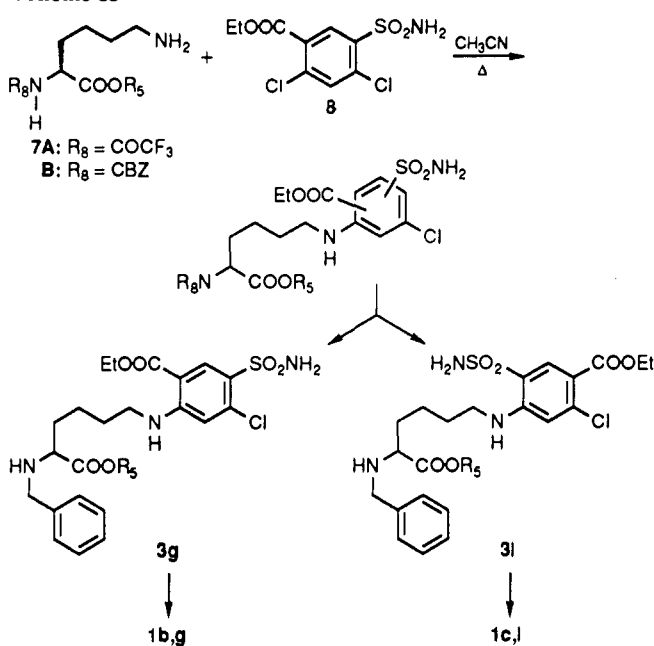
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(16) Nielsen, O. B. T.; Nielsen, C. K.; Feit, P. W. J. Med. Chem. 1973, 16, 1170.

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extension of our results to substituted proline derivatives.

Chemistry

The target compounds 1 (Table III) were prepared (Scheme I) by reaction of an appropriate glycine derivative 3^8 with the acid chloride of 2 to give 4A. Cleavage of the secondary urethane[19] and modification of R_2–R_4 as necessary (see the supplementary material for details) gave 4B. Certain reactive glycine derivatives could be reacted with the acylimidazole derived from unacylated 2A to give 4B directly. Acid cleavage of 4 (R_5 = tBu) gave monoesters 1a,d,g–i,p,s,t,v. Exhaustive saponification of 4 (R_5 = Et) gave diacids 1e,f,k–n,q and triacids 1b,c. Diacids 1j,u were prepared by saponification of 1h,t respectively. Diacid 1o was prepared by CBZ cleavage[20] of 1w. Diacid 1r was prepared by sequential saponification and acid cleavage of 4p (R_5 = tBu).

The preparation and use of 2A have been described elsewhere.[8] An analogous route was used to prepare 2B from N^6-CBZ-L-Lys-OtBu (see the Experimental Section). Reaction with 5, diastereomer separation, and urethane formation gave 2B-OtBu. This was deprotected to 2B and coupled with Pro-OtBu or 6 to give the precursors to 1p,r and 1t,u, respectively.

Regioisomers 1b,c and 1g,i were prepared (Scheme II) by reaction of α-protected lysines 7 with 8.[21] Chromatographic separation was achieved after replacement of R_8 with an N-benzyl substituent. The two regioisomers showed virtually identical spectral properties. The anthranilic acid regiochemistry (cf. 4b,g) was assigned to that isomer which showed a typical blue fluorescence upon UV illumination.

The diuretic moieties of 1a,e,h,j were introduced at the beginning of the synthetic sequence. Amides 1p,q,t were prepared from amines carried to the last stages as CBZ derivatives. Hydrochlorothiazide moieties were introduced into 3 by reaction of an appropriate acetal or ketal with 9 (eq 1).[22]
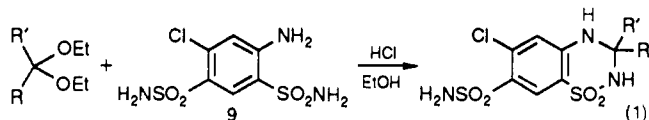
Results and Discussion

Many of the diacids tested (Table I) for ACE inhibition in vitro showed submillimolar activity, with the best (1r,u) approaching enalaprilat (MK 422) in potency. Not surprisingly none of the monoesters tested showed comparable activity, although some (1i,p,t) were modestly active.[23] Similar to our previous results[8,24] with ACE inhibitors there

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(20) Ben-Ishai, D.; Berger, A. *J. Org. Chem.* **1952**, *17*, 1564.
(21) In contrast to ref 15, we have found roughly equimolar ratios of displacement at each chlorine atom for a variety of primary amines using the published conditions.
(22) Topliss, J. G.; Sherlock, M. H.; Clarke, F. H.; Daly, M. C.; Pettersen, B. W.; Lipski, J.; Sperber, N. *J. Org. Chem.* **1961**, *26*, 3842. We have not attempted to separate the diastereomeric mixtures generated at this center.
(23) We have not determined whether the observed activity is due to traces of diacid in the product, esterase in the enzyme preparation, or intrinsic activity of the monoester.
(24) Stanton, J. L.; Gruenfeld, N.; Babiarz, J. E.; Ackerman, M. H.; Friedmann, R. C.; Yuan, A. M.; Macchia, W. *J. Med. Chem.* **1983**, *26*, 1267.

Table II

	dog		
compd	ACE inhibition[a]	diuretic activity[b]	SHR[c]
1f	~100	+	12–21
1k	>100	++	11–14
1o	~100	++	0
captopril	0.1	0	
enalapril	0.1	0	33–38[d]
hydrochlorothiazide	>100	++	0

[a] ED_{50}, mg/kg po. [b] See note c Table I, for activity ratings. [c] Maximum percent decrease in blood pressure, 100 mg/kg ip. [d] 1.0 mg/kg ip.

appears to be a significant tolerance for large substituents at R_3 and R_4. The limit appears to be exceeded with 1n, where a combination of otherwise allowable substituents (cf. 1m with 1b or 1c) shows significantly decreased activity. We believe that this effect is probably steric, rather than electrostatic, since we have shown[8,18] that charged groups can be accommodated in R_3/R_4. Within a series (1f,o,w) we see little effect in vitro with changes in R_2.

Results in the rat showed a wider range of activity. Early compounds were tested ip for ACE inhibition, showing good activity in some cases (1b,f,g,b). After further work revealed oral activity for 1b and 1d, all subsequent testing was done orally. This led to the identification of lysyl glycine derivatives (1o,p,r,t,u) as uniformly long-acting, potent ACE inhibitors po. There appeared to be little or no difference between monoester and diacid (1p vs 1r, 1t vs 1u) within this series. For a homologous series within R_3 the in vivo ACE inhibition does not appear to depend on the length (1f,k,l), flexibility (1k vs 1m), or charge (1s) of the intervening chain. Isobutyl derivative 1v may represent a steric problem from excessive branching.

Diuretic screening (Table I) was carried out by the route proven effective for in vivo ACE inhibition. Of 12 non-thiazide compounds tested only 1d showed evidence of diuresis. This was accompanied by only weak ACE inhibition. Those compounds containing thiazide moieties showed consistent diuretic activity, although in no case was it comparable to that of the parent hydrochlorothiazide. When the effect of an interviewing chain is examined (1f,k,m,s,v) a similar pattern to the ACE inhibition results above is noted.

Three compounds (1f,k,o) were considered sufficiently interesting to undergo further testing (Table II). In the low-sodium SHR both 1f and 1k showed a slight lowering of blood pressure at 100 mg/kg ip. At the same doses po these compounds were inactive. Compound 1o was tested po in the same assay and found to be inactive. In a separate experiment these compounds were tested for ACE inhibition orally in the dog; significantly less activity was found than in the rat.

Experimental Section

General conditions and instrumentation may be found in our previous paper.[8]

A. Preparation of Monoacids: Ethyl α-[[2-[2-[3-(Aminosulfonyl)-4-chlorobenzoyl]-1-(carboxymethyl)hydrazino]-1-methyl-2-oxoethyl]amino]benzenebutanoate (1a). A mixture containing 420 mg (0.672 mmol) of 4Ba-OtBu in 15 mL of 4 N HCl/dioxane was stirred for 15.5 h at room temperature. The mixture was concentrated in vacuo and the residue was triturated with ether and filtered to give 408 mg (100%) of 1a as a white solid. Mp: 144 °C (softens). NMR (DMSO-d_6): 8.45 (d, J = 2 Hz, 1 H), 8.0 (dd, J = 8 Hz, 2 Hz, 1 H), 7.5 (d, J = 8 Hz, 1 H) 7.3 (b s, 5 H), 4.1–4.7 (m, 6 H), 2.8 (m, 2 H), 2.0 (m, 2 H), 1.1–1.4 (m, 6 H). Anal. ($C_{24}H_{29}ClN_4O_8S$·HCl): C, H, N.

N^6-[3-(Aminosulfonyl)-4-chlorobenzoyl]-N^2-(2,3-dihydro-1H-inden-2-yl)-N^2-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-lysine monohydrochloride (1d) was synthesized from 4Bd-OtBu (1.14 g, 1.36 mmol) to yield 844 mg (80%) [mp: 158–162 °C (softens)] after trituration with hexane. NMR (DMSO-d_6): 8.4 (d, J = 2 Hz, 1 H), 7.95 (dd, J = 8 Hz, 2 Hz, 1 H), 7.5 (d, J = 8 Hz, 1 H), 7.1–7.4 (m, 9 H), 3.9–4.5 (m, 6 H), 2.8–3.6 (m, 8 H), 1.5–2.3 (m, 8 H), 1.0–1.5 (m, 6 H). Anal. ($C_{37}H_{45}ClN_4O_8S\cdot HCl\cdot 3.5H_2O$): C, H, N.

N-[[5-(1,1-Dimethylethyl)-2-hydroxy-3-iodophenyl]methyl]-N-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]glycine hydrochloride (1h) was synthesized from 4Bh-OtBu (1.82 g, 2.67 mmol) to yield 1.24 g (70%) (mp: 165–168 °C) after trituration with ether. NMR (DMSO-d_6): 7.7 (b s, 1 H), 7.3 (b s, 5 H), 6.9 (b s, 1 H), 4.0–4.9 (m, 8 H), 2.8 (m, 2 H), 2.0 (m, 2 H), 1.1–1.4 (m, 15 H). Anal. ($C_{28}H_{37}IN_2O_6\cdot HCl$): C, H, N.

N^6-[4-(Aminosulfonyl)-5-chloro-2-(ethoxycarbonyl)-phenyl]-N^2-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-N^2-(phenylmethyl)-L-lysine hydrochloride (1g) was synthesized from 4Bg-OtBu (4.0 g, 4.91 mmol) at 0 °C to give 3.5 g (87.6%) (mp: 115–120 °C) after trituration with Et$_2$O/petroleum ether. NMR (DMSO-d_6): 8.4 (s, 1 H), 7.1–7.5 (m, 6 H), 4.7–5.1 (m, 3 H), 4.0–4.5 (m, 8 H), 3.1 (m, 2 H), 2.8 (m, 2 H), 1.4–2.1 (m, 8 H), 1.1–1.4 (m, 12 H). Anal. ($C_{37}H_{47}ClN_4O_9S\cdot HCl\cdot H_2O$): H; C: calcd, 54.61; found, 54.05; N: calcd, 6.88; found, 6.47.

N^6-[2-(Aminosulfonyl)-5-chloro-4-(ethoxycarbonyl)-phenyl]-N^2-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-N^2-(phenylmethyl)-L-lysine hydrochloride (1i) was synthesized from 4Bi-OtBu (1.5 g, 1.84 mmol) to yield 1.3 g (88.7%) (mp: 106–110 °C) after trituration with Et$_2$O. NMR (DMSO-d_6): 8.3 (s, 1 H), 7.1–7.5 (m, 6 H), 4.7–5.1 (m, 3 H), 4.0–4.5 (m, 8 H), 3.1 (m, 2 H), 2.8 (m, 2 H), 1.4–2.1 (m, 8 H), 1.1–1.4 (m, 12 H). Anal. ($C_{37}H_{47}ClN_4O_9S\cdot HCl$): H, N; C: calcd, 55.85; found, 55.40.

1-[N^6-[3-(Aminosulfonyl)-4-chlorobenzoyl]-N^2-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-lysyl]-L-proline hydrochloride (1p) was synthesized from 4Bp-OtBu at 0 °C (1.50 g, 2.12 mmol) to yield 0.95 g (63%) (mp: 165–170 °C) after trituration with EtOAc. NMR (DMSO-d_6) 8.9 (b s, 1 H), 8.55 (s, 1 H), 8.1 (d, 1 H), 7.65 (s, 2 H), 7.4–7.1 (m, 5 H), 4.4 (m, 1 H), 4.2–4.1 (m, 3 H), 3.8–3.35 (m, 4 H), 3.3 (m, 2 H), 2.9–2.5 (m, 2 H), 2.2 (m, 3 H), 1.9 (m, 5 H), 1.6 (m, 6 H), 1.20 (t, 3 H). Anal. ($C_{30}H_{39}ClN_4O_8S\cdot HCl\cdot H_2O$): C, H; N: calcd, 7.94; found, 7.46.

1-[N^6-[3-(Aminosulfonyl)-4-chlorobenzoyl]-N^2-[1-carboxy-3-phenylpropyl]-L-lysyl]-L-proline hydrochloride (1r) was synthesized from 4Bp-OtBu (1.15 g, 1.69 mmol) after sequential saponification (vide infra) and reaction at 0 °C to yield 0.96 g (86%) (mp: 177–185 °C) after trituration with EtOAc. NMR (DMSO-d_6): 7.6 (b s, 1 H), 7.3 (b s, 5 H), 6.8 (b s, 1 H), 4.5–4.9 (m, 2 H), 4.0–4.3 (m, 4 H), 2.8 (m, 2 H), 1.9 (m, 2 H), 1.1–1.3 (m, 12 H). Anal. ($C_{28}H_{35}ClN_4O_8S\cdot HCl\cdot H_2O\cdot 0.5EtOAc$): C, H, N.

Ethyl α-[[2-[2-[[7-(aminosulfonyl)-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazin-3-yl]methyl]-2-methyl-1-(carboxymethyl)hydrazino]-1-methyl-2-oxoethyl]amino]benzenebutanoate S,S-dioxide hydrochloride (1s) was synthesized from 4Bs-OtBu (2.0 g, 2.73 mmol) at 0 °C to yield 1.2 g (62%) (mp: 170–172 °C dec) from 10:1 hexane/EtOH. NMR (DMSO-d_6): 8.4 (s, 1 H), 7.3 (b s, 5 H), 7.0 (s, 1 H), 3.9–4.5 (m, 5 H), 2.3–3.4 (m, 11 H), 1.2–1.8 (m, 6 H). Anal. ($C_{26}H_{35}ClN_6H_9S_2\cdot 1.5HCl$): C, H, N.

N-[N^6-[3-(Aminosulfonyl)-4-chlorobenzoyl]-N^2-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-lysyl]-N-(2,3-dihydro-1H-inden-2-yl)glycine hydrochloride (1t) was synthesized from 4Bt-OtBu (1.49 g, 1.82 mmol) at 0 °C to yield 0.90 g (60%) (mp: 148–155 °C) after trituration with EtOAc. NMR (DMSO-d_6): 8.88 (m, 2 H), 8.25 (m, 1 H), 7.6 (d, 1 H), 7.5–7.0 (m, 9 H), 5.1 (m, 2 H), 4.6–3.9 (m, 6 H), 3.6–2.70 (m, 9 H), 2.6–2.0 (m, 4 H), 1.75 (m, 4 H), 1.25 (t, 3 H). Anal. ($C_{36}H_{43}ClN_4O_8S\cdot HCl\cdot H_2O$): C, H, N.

N-[1-[7-(Aminosulfonyl)-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazin-3-yl]-3-methylbutyl]-N-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]glycine S,S-dioxide hydrochloride (1v) was synthesized from 4Bv-OtBu (1.3 g, 1.71 mmol) at 0 °C to yield 0.94 g (68%) (mp: 168–170 °C) after trituration with Et$_2$O. NMR (acetone-d_6): 8.05 (s, 1 H), 7.1–7.5 (m, 6 H), 4.0–4.6 (m, 7 H), 3.5 (m, 1 H), 2.8 (m, 2 H), 0.9–2.1 (m,

12 H). Anal. ($C_{29}H_{40}ClN_5O_9S_2\cdot HCl\cdot H_2O\cdot Et_2O$): C, H, N.

B. Preparation of Diacids. N-[[7-(Aminosulfonyl)-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazin-3-yl]methyl]-N-[N-(1-carboxy-3-phenylpropyl)-L-alanyl]glycine S,S-dioxide (1f). A solution of 2.63 g (3.91 mmol) 4Bf-OEt in 35 mL (35 mmol) of 1 N NaOH and 50 mL of EtOH was stirred at room temperature for 22 h. Acidification with 1 N HCl was followed by extraction into EtOAc. The extracts were washed with brine, dried (Na$_2$SO$_4$), and concentrated. The residue was triturated with Et$_2$O to give 2.44 g (98%) of 1f. Mp: 163 °C. NMR (CD$_3$OD): 8.2 (s, 1 H), 7.2 (s, 5 H), 7.0 (s, 1 H), 4.8–5.2 (m, 3 H), 4.2 (b s, 2 H), 3.6–3.9 (m, 2 H), 2.6–2.9 (m, 2 H), 2.0–2.41 (m, 2 H), 1.6 (b d, 3 H). A sample was recrystallized from H$_2$O for analysis. Anal. ($C_{23}H_{28}ClN_5O_9S_2\cdot H_2O$): C, H, N.

N^6-[4-(Aminosulfonyl)-2-carboxy-5-chlorophenyl]-N^2-[N-(1-carboxy-3-phenylpropyl)-L-alanyl]-N^2-(phenylmethyl)-L-lysine hydrochloride (1b) was synthesized from 4Bd-OEt (0.326 g, 0.396 mmol) to yield 0.291 g (99%) (mp: 172–176 °C) after trituration with Et$_2$O. NMR (DMSO-d_6): 8.2 (s, 1 H), 7.1–7.5 (m, 1 H), 4.7–5.0 (m, 3 H), 4.2 (m, 2 H), 3.2 (m, 2 H), 2.7 (m, 2 H), 1.5–2.1 (m, 8 H), 1.3 (d, 3 H). Anal. ($C_{23}H_{39}ClN_4O_9S\cdot HCl\cdot 0.5Et_2O$): C, H, N.

N^6-[2-(Aminosulfonyl)-4-carboxy-5-chlorophenyl]-N^2-[N-(1-carboxy-3-phenylpropyl)-L-alanyl]-N^2-(phenylmethyl)-L-lysine hydrochloride (1c) was synthesized from 4Bc-OEt (0.434 g, 0.527 mmol) to yield 0.24 g (59%) (mp: 163–165 °C) after trituration with Et$_2$O. NMR (DMSO-d_6): 8.2 (s, 1 H), 7.1–7.5 (m, 11 H), 4.7–5.0 (m, 3 H), 4.2 (m, 2 H), 3.2 (m, 2 H), 2.7 (m, 2 H), 1.5–2.1 (m, 8 H), 1.3 (d, 3 H). Anal. $C_{33}H_{39}ClN_4O_9S\cdot HCl\cdot 0.5$ Et$_2$O): C, H, N.

N-[2-[[3-(Aminosulfonyl)-5-carboxy-2-chlorophenyl]amino]-2-oxoethyl]-N-[N-(1-carboxy-3-phenylpropyl)-L-alanyl]glycine (1e) was synthesized from 4Be-OEt (0.486 g, 0.71 mmol) in 90% aqueous MeOH to yield 0.24 g (56%) (mp: 174 °C) by direct filtration. NMR (DMSO-d_6): 8.2 (b s, 1 H), 7.6 (b s, 1 H), 7.2 (b s, 5 H), 4.3–4.9 (m, 6 H), 2.8 (m, 2 H), 1.9 (m, 2 H), 1.2 (b d, 3 H). Anal. ($C_{24}H_{27}ClN_4O_{10}S\cdot 2H_2O$): C, H, N.

N-[N-(1-Carboxy-3-phenylpropyl)-L-alanyl]-N-[[5-(1,1-dimethylethyl)-2-hydroxy-3-iodophenyl]methyl]glycine (1j) was synthesized from 1h (0.82 g, 1.24 mmol) in 60% aqueous EtOH to yield 0.664 g (85%) (mp: 138–141 °C) after trituration with Et$_2$O. NMR (DMSO-d_6): 7.6 (b s, 1 H), 7.3 (b s, 5 H), 6.8 (b s, 1 H), 4.5–4.9 (m, 2 H), 4.0–4.3 (m, 4 H), 2.8 (m, 2 H), 1.9 (m, 2 H), 1.1–1.3 (m, 12 H). Anal. ($C_{26}H_{33}IN_2O_6\cdot HCl\cdot 0.5H_2O$): C, H; N: calcd, 4.36; found, 3.94.

N-[3-[7-(Aminosulfonyl)-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazin-3-yl]propyl]-N-[N-(1-carboxy-3-phenylpropyl)-L-alanyl]glycine S,S-dioxide (1k) was synthesized from 4Bk-OEt (6.7 g, 9.54 mmol) to yield 5.76 g (86%) (mp: 187–189 °C) after sequential trituration with Et$_2$O and EtOAc. NMR (DMSO-d_6): 8.30 (s, 1 H), 7.2–7.5 (m, 5 H), 7.0 (s, 1 H), 4.9 (m, 1 H), 4.1–4.4 (m, 4 H), 3.6 (m, 2 H), 2.7 (m, 2 H), 1.9 (m, 6 H), 1.2 (m, 3 H). Anal. ($C_{25}H_{32}ClN_5O_9S_2\cdot 0.6HCl\cdot 0.4EtOAc$): C, H, N.

N-[1-[7-(Aminosulfonyl)-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazin-3-yl]ethyl]-N-[N-(1-carboxy-3-phenylpropyl)-L-alanyl]glycine S,S-dioxide hydrochloride (1l) was synthesized from 4Bl-OEt (1.0 g, 1.45 mmol) to yield 0.72 g (73%) (mp: 178–180 °C) after trituration with 1:1 Et$_2$O/EtOAc. NMR (DMSO-d_6): 7.98 (s, 1 H), 7.1–7.7 (m, 6 H), 4.96 (m, 5 H), 2.85 (m, 1 H), 2.60 (m, 1 H), 2.15 (m, 2 H), 1.2–1.5 (m, 6 H). Anal. ($C_{24}H_{30}ClN_5O_9S_2\cdot HCl\cdot 0.5EtOAc$): H; C: calcd, 43.82; found, 43.33; N: calcd, 9.83; found, 9.13.

N-[7-(Aminosulfonyl)-6-chlorospiro[2H-1,2,4-benzothiadiazine-3(4H),1'-cyclohexan-4'-yl]-N-[N-(1-carboxy-3-phenylpropyl)-L-alanyl]glycine S,S-dioxide hydrochloride (1m) was synthesized from 4Bm-OEt (0.50 g, 0.688 mmol) to yield 0.41 g (84%) (mp: 160 °C) after trituration with EtOH. NMR (acetone-d_6): 8.30 (s, 1 H), 7.2 (s, 5 H), 6.95 (s, 1 H), 3.9–4.5 (m, 4 H), 2.8–3.1 (7, 3 H), 1.5–2.1 (m, 10 H), 1.3 (m, 3 H). Anal. ($C_{27}H_{34}ClN_5O_9S_2\cdot 2HCl\cdot C_2H_5OH$): C, N; H: Calcd, 5.12; found, 4.66.

N-[7-(Aminosulfonyl)-6-chlorospiro[2H-1,2,4-benzothiadiazine-3(4H),1'-cyclohexan-4'-yl]-N-[N-(1-carboxy-3-phenylpropyl)-L-alanyl]lysine S,S-dioxide (1n) was synthesized from 4Bn-OEt (0.60 g, 0.644 mmol) in 65% aqueous EtOH to give 0.36 g (64%) (mp: 130–132 °C) upon direct acid-
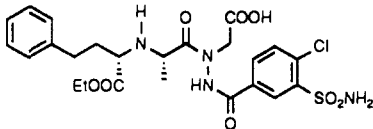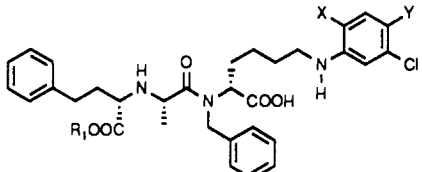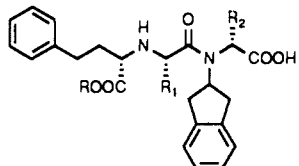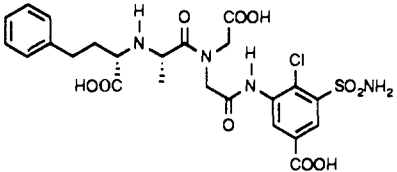
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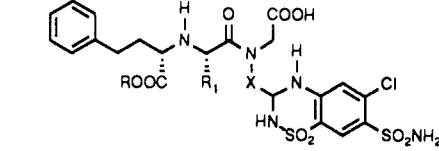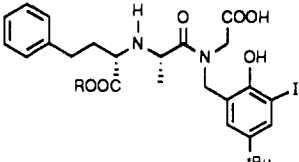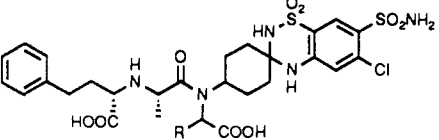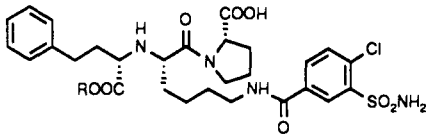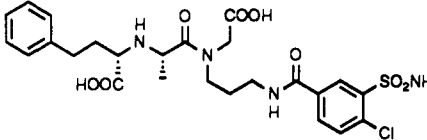
compound				formula	mp, °C	solvent[a]

| **1a** | | | | $C_{24}H_{29}ClN_4O_8S \cdot HCl$ | 144 | E |

	R_1	X	Y			
1b:	$R_1 = H$	$X = COOH$	$Y = SO_2NH_2$	$C_{33}H_{39}ClN_4O_9S \cdot HCl \cdot 0.5E$	172-176	E
1c:	$R_1 = H$	$X = SO_2NH_2$	$Y = COOH$	$C_{33}H_{39}ClN_4O_9S \cdot HCl \cdot 0.5E$	163-165	E
1g:	$R_1 = Et$	$X = COOEt$	$Y = SO_2NH_2$	$C_{37}H_{47}ClN_4O_9S \cdot HCl \cdot H_2O^b$	115-120	E/H
1i:	$R_1 = Et$	$X = SO_2NH_2$	$Y = COOEt$	$C_{37}H_{47}Cl_4N_4O_9S \cdot HCl^c$	106-110	E

	R	R_1	R_2			
1d:[j]	$R = Et$	$R_1 = Me$	$R_2 = A$	$C_{37}H_{45}ClN_4O_8S \cdot HCl \cdot 3.5H_2O^d$	158-162	H
1t:[j]	$R = Et$	$R_1 = A$	$R_2 = H$	$C_{36}H_{43}ClN_4O_8S \cdot HCl \cdot H_2O$	148-155	EA
1u:[j]	$R = H$	$R_1 = A$	$R_2 = H$	$C_{34}H_{39}ClN_4O_8S \cdot HCl \cdot 1.5H_2O$	178-184	EA

| **1e** | | | | $C_{24}H_{27}ClN_4O_{10}S \cdot 2H_2O$ | 174 | W/M |

	R	R_1	X			
1f:	$R = H$	$R_1 = Me$	$X = CH_2$	$C_{23}H_{28}ClN_5O_9S_2 \cdot H_2O$	163	E
1k:	$R = H$	$R_1 = Me$	$X = (CH_2)_3$	$C_{25}H_{32}ClN_5O_9S_2 \cdot 0.6HCl \cdot 0.4EA$	187-189	EA
1l:	$R = H$	$R_1 = Me$	$X = (S)\text{-}CH(CH_3)$	$C_{24}H_{30}ClN_5O_9S_2 \cdot HCl \cdot 0.5EA^e$	178-180	EA
1o:	$R = H$	$R_1 = (CH_2)_4NH_2$	$X = CH_2$	$C_{26}H_{35}ClN_6O_9S_2 \cdot 2HBr \cdot 1.5H_2O^f$	165-167	C
1s:	$R = Et$	$R_1 = Me$	$X = N(CH_3)CH_2$	$C_{26}H_{35}ClN_6O_9S_2 \cdot 1.5HCl$	170-172	Et/H
1v:	$R = Et$	$R_1 = Me$	$X = (S)\text{-}CH(CH_2iPr)$	$C_{29}H_{40}ClN_5O_9S_2 \cdot HCl \cdot H_2O \cdot E$	168-170	E
1w:	$R = H$	$R_1 = (CH_2)_4NHCBZ$	$X = CH_2$	$C_{33}H_{41}ClN_6O_{11}S_2 \cdot H_2O$	149-152	E

	R					
1h:	$R = Et$			$C_{28}H_{37}IN_2O_6 \cdot HCl$	165-168	E
1j:	$R = H$			$C_{26}H_{33}IN_2O_6 \cdot HCl \cdot 0.5H_2O^g$	138-141	E

	R					
1m:	$R = H$			$C_{27}H_{34}ClN_5O_9S_2 \cdot HCl \cdot Et^h$	160	Et
1n:	$R = (CH_2)_4NH_2$			$C_{31}H_{43}ClN_6O_9S_2 \cdot 3HBr$	130-132	

Table III (Continued)

compound	formula	mp, °C	solvent[a]
1p: R = Et	$C_{30}H_{39}ClN_4O_8S \cdot HCl \cdot H_2O$[j]	165–170	EA
1r: R = H	$C_{28}H_{35}ClN_4O_8S \cdot HCl \cdot H_2O \cdot 0.5EA$	177–185	EA
1q	$C_{25}H_{31}ClN_4O_8S \cdot HCl \cdot 1.5Et$	120–126	Et

[a] For trituration: E = Et_2O, H = hexane, EA = EtOAc, W = H_2O, M = MeOH, C = $CHCl_3$, Et = EtOH. [b] C: calcd, 54.61; found, 54.05; N: calcd, 6.88; found, 6.47. [c] C: calcd, 55.85; found, 55.40. [d] N: calcd, 6.65; found, 5.99. [e] C: calcd, 43.82; found, 43.33; N: calcd, 9.83; found, 9.13. [f] N: calcd, 9.73; found, 9.18. [g] N: calcd, 4.36; found 3.94. [h] H: calcd, 5.12; found, 4.66. [i] N: calcd, 7.94; found, 7.46. [j] A = $(CH_2)_4$-NCO-3-SO_2NH_2-4-Cl-C_6H_3.

ification. NMR (acetone-d_6): 8.30 (s, 1 H), 7.25 (m, 10 H), 6.95 (s, 1 H), 5.05 (s, 2 H), 3.9–4.5 (m, 3 H), 3.15 (m, 2 H), 2.85 (m, 2 H), 1.9–2.7 (m, 7 H), 1.35–1.85 (m, 10 H), 1.3 (m, 3 H). Anal. ($C_{31}H_{43}ClN_6O_9S_2 \cdot 3HBr$): C, H, N.

N-[3-[[3-(Aminosulfonyl)-4-chlorobenzoyl]amino]-propyl]-*N*-[*N*-(1-carboxy-3-phenylpropyl)-L-alanyl]glycine hydrochloride (1q) was synthesized from 4Bq-OEt (1.5 g, 2.28 mmol) to yield 0.30 g (21.8%) (mp: 120–126 °C) after chromatography (0–20% MeOH/$CHCl_3$) and acidification (HCl/EtOH). NMR (CD$_3$OD): 8.7 (s, 1 H), 8.0 (d, J = 8 Hz, 1 H), 7.4 (b d, J = 8 Hz, 1 H), 7.2 (b s, 5 H), 4.4–4.8 (2 H), 3.9–4.3 (m, 2 H), 2.8–3.4 (m, 6 H), 1.2–1.6 (m, 7 H). Anal. ($C_{25}H_{31}ClN_4O_8S \cdot HCl \cdot 1.5EtOH$): C, H, N.

N-[[7-(Aminosulfonyl)-6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazin-3-yl]methyl]-*N*-[*N*^{2}-(1-carboxy-3-phenyl-propyl)-*N*^{6}-[(phenylmethoxy)carbonyl]-L-lysyl]glycine *S,S*-dioxide (1w) was synthesized from 4Bw-OEt (2.2 g, 2.54 mmol) to yield 1.26 g (42%) (mp: 149–152 °C dec) after trituration with EtOAc, then Et_2O. NMR (DMSO-d_6): 8.0 (s, 1 H), 7.3 (m, 11 H), 5.05 (s, 2 H), 4.45 (b, 1 H), 4.1–4.4 (m, 6 H), 3.3–3.9 (m, 4 H), 2.9–3.2 (m, 2 H), 2.3 (m, 2 H), 1.90 (m, 2 H), 1.55 (m, 4 H), 1.1–1.4 (m, 8 H). Anal. ($C_{33}H_{41}ClN_6O_{11}S_2 \cdot H_2O$): C, H, N.

N-[*N*^{6}-[3-(Aminosulfonyl)-4-chlorobenzoyl]-*N*^{2}-(1-carboxy-3-phenylpropyl)-L-lysyl]-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine hydrochloride (1u) was synthesized from 4Bt-OtBu (0.80 g, 1.02 mmol) by saponification as above, followed by HCl/dioxane as previously described, to yield 0.41 g (55%) (mp: 178–184 °C) after trituration with EtOAc. NMR (acetone-d_6): 8.75 (b s, 1 H), 8.19 (m, 1 H), 7.58 (m, 1 H), 7.4–6.95 (m, 11 H), 5.1 (m, 1 H), 4.1 (m, 1 H), 3.5–2.8 (m, 11 H), 2.6–2.0 (m, 6 H), 1.9–1.4 (m, 6 H). Anal. ($C_{34}H_{39}ClN_4O_8S \cdot HCl \cdot 1.5H_2O$): C, H, N.

N-[[7-(Aminosulfonyl)-6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazin-3-yl]methyl]-*N*-[*N*^{2}-(1-carboxy-3-phenyl-propyl)-L-lysyl]glycine *S,S*-Dioxide Dihydrobromide (1o). To a solution of 0.5 g (0.62 mmol) of 1w in 5 mL of CH_2Cl_2 was added 0.5 mL of 36% HBr/HOAc. The solution was stirred for 1 h at room temperature and then diluted with Et_2O and filtered. The solid was triturated with $CHCl_3$ to give 0.34 g (68%). Mp: 165–167 °C. NMR (DMSO-d_6): 8.05 (s, 1 H), 7.1–7.3 (m, 6 H), 4.45 (b s, 1 H), 4.20 (s, 2 H), 3.3–3.9 (m, 4 H), 2.28 (m, 2 H), 1.90 (m, 2 H), 1.55 (m, 4 H), 1.3 (m, 2 H). Anal. ($C_{26}H_{35}ClN_6O_9S_2 \cdot 2HBr \cdot 1.5H_2O$): C, H; N: calcd, 9.73; found, 9.18.

N^6-[(Phenylmethoxy)carbonyl]-L-lysine *tert*-Butyl Ester. A mixture of N^6-CBZ-L-Lys (30 gm), 30 mL of concentrated H_2SO_4, 300 mL of isobutylene, and 300 mL of dioxane was kept in a closed container for 18 h and then poured onto 500 mL of 2 N NaOH at 0–5 °C. The aqueous layer was extracted with ether. The combined organic extracts were washed with brine and dried (MgSO$_4$). Removal of the volatiles in vacuo provided 26.2 g (73%) of an oil which was taken forward without further purification. NMR (CDCl$_3$): 7.28 (s, 5 H), 5.06 (s, 2 H), 3.17 (m, 3 H), 1.45 (m, 15 H).

N^2-[1-(Ethoxycarbonyl)-3-phenylpropyl]-*N*^{6}-[(phenyl-methoxy)carbonyl]-L-lysine *tert*-Butyl Ester. A mixture of the above amine (26.2 g, 77.9 mmol), bromide 5 (25.4 g, 93.5 mmol), and anhydrous K_2CO_3 (12.9 g, 93.5 mmol) in 250 mL of anhydrous acetonitrile was heated at reflux for 18 h and the volatiles were then removed in vacuo. The residue was diluted with ethyl acetate and water. The organic layer was washed with brine and dried (MgSO$_4$). Removal of the volatiles in vacuo provided a residue which was purified by HPLC using 25% ethyl acetate in hexanes. The fractions containing the more polar diastereomer were collected and the volatiles were removed in vacuo to provide 14.4 g (28%) of the oily product. NMR (CDCl$_3$): 7.26 (s, 5 H), 7.13 (s, 5 H), 5.05 (s, 2 H), 4.12 (q, 3 H), 3.35–3.0 (m, 4 H), 2.65 (t, 2 H), 2.1–1.8 (m, 2 H), 1.27 (t, 3 H).

N^2-[1-(Ethoxycarbonyl)-3-phenylpropyl]-*N*^{6}-[(phenyl-methoxy)carbonyl]-*N*^{2}-[(2,2,2-trichloroethoxy)carbonyl]-L-lysine *tert*-Butyl Ester. To a solution of the above compound (28.4 g, 53.9 mmol) in 8 mL of pyridine and 80 mL of anhydrous THF was added (2,2,2-trichloroethyl)chloroformate (12.3 g, 58.2 mmol). After 2 h the mixture was cooled and filtered. The volatiles were removed in vacuo. The residue was diluted with ethyl acetate, washed with aqueous HCl and brine, and dried (MgSO$_4$). Removal of the volatiles in vacuo provided a residue, which was purified by HPLC using 20% ethyl acetate in hexanes. Concentration in vacuo of the product-rich fractions provided 34.4 g (91%) of the oily product. NMR (CDCl$_3$): 7.30 (s, 5 H), 7.20 (s, 5 H), 5.02 (s, 2 H), 4.9–4.2 (m, 4 H), 4.13 (q, 2 H), 3.15 (m, 2 H), 2.70 (m, 2 H), 2.15–1.8 (m, 2 H), 1.40 (m, 15 H), 1.23 (t, 3 H).

N^2-[1-(Ethoxycarbonyl)-3-phenylpropyl]-*N*^{6}-[(phenyl-methoxy)carbonyl]-*N*^{2}-[(2,2,2-trichloroethoxy)carbonyl]-L-lysine (2B). A solution of the above ester (34.4 g) in 200 mL of 4 M HCl dioxane was kept at –5 °C overnight. Removal of the volatiles in vacuo provided a quantitative yield of the product. NMR (CDCl$_3$): 10.5 (b s, 1 H) 7.28 (s, 5 H), 7.16 (m, 5 H), 5.05 (s, 2 H), 4.9–4.3 (m, 4 H), 4.15 (q, 2 H), 3.1 (m, 2 H), 2.7 (m, 2 H), 2.45–1.7 (m, 3 H), 1.45 (m, 6 H). Dicyclohexylamine salt mp: 42–45 °C. Anal. ($C_{29}H_{35}Cl_3N_2O_8 \cdot C_{12}H_{23}N$): C, H, N.

C. Antihypertensive Activity in Sodium-Depleted, Spontaneously Hypertensive Rats. Sixteen week old, male, spontaneously hypertensive rats were used. They were maintained on a sodium-deficient diet and distilled water for 4 weeks prior to experimentation.

One week prior to experimentation, polyethylene catheters were implanted in the rats' abdominal aortae, with the external ends emerging from between the scapulae. At the time of experimentation, the rats were harnessed and their catheters were attached to a recording system that allowed the animals to roam freely in individual cages while their pressures were monitored. Water and food were available throughout the experiment.

After a 24-h acclimation period, two rats were dosed with the test compound. Compounds were administered ip or po at 100

mg/kg, dissolved or suspended in 0.5% [(hydroxypropyl)-methyl]cellulose in distilled water, 10 mL/kg. If a compound was incompatible with this vehicle, an alternative vehicle was used.

Mean arterial pressures (MAP) and heart rates were recorded for 10 s, every 5 min, with an electronic switching computer system. Fifteen or 30 min averages were tabulated and reported as percent changes from the 0.5-h average values just prior to dosing. Compounds were evaluated at each time period by comparing the mean percent changes oberved for MAP with those of a cumulative control group composed of all rats which received the vehicle alone in previous runs. Values which fell outside the range of the mean ± 2SD were considered significantly different from controls. The duration of action was the continuous length of time during which test values were below or above those of the historical controls. If the results in the two test rats were inconsistent, one or two additional rats were used.

D. Diuretic Testing in the Rat. Male rats weighing 175–250 g were used. Water and food were withheld for 18 h prior to and during the experiment. The rats were housed in metabolism cages, two to a cage.

Eight rats each were dosed with the test compound or vehicle alone. Compounds were administered iv, ip, or po at the indicated dose in Tris buffer (pH 8.5, 3.5 mL/kg). Each animal was given a fluid load of 0.9% saline, 20 mL/kg, by gavage after the test drug.

Urine was collected from each cage for 0–5- and 5–24-h postdosing. Volume (mL), Na^+ (mequiv), and K^+ (mequiv) per kilogram of body weight were recorded and reported as the means ± SD.

Nonpaired "*t*" tests were used to determine a significant difference in the volume or Na^+ or K^+ excretions between the test and control groups.

E. Diuretic Activity in Dogs. The procedure is a modification of ref 25. Mongrel dogs of either sex were fasted overnight and then anesthetized with pentobarbital sodium (35 mg/kg, iv) and respired with 20 mg/kg of room air at 12 breaths/min. Arterial pressure (femoral artery; Statham Instruments Model P23ID pressure transducer) was recorded.

After a ventral midline incision of the lower abdomen was made, the left and right ureters were cannulated for urine collection. Mannitol (4% solution, 2.2 mL/min) was infused via the left external jugular vein to maintain urine flow. Urine samples were collected every 15 min and analyzed for electrolytes with NOVA 4+4 ion sensitive electrodes.

Tests compounds were infused in 3.5 mL of saline or pH 8.5 Tris buffer through a right femoral venous cannula at 1.5 mL/min. Drug effects were monitored for a minimum for 1 h. Mean values of $N \geq 3$ animals (volume, Na^+, K^+) were compared to mean ± 2SD of pooled historical controls. Values outside the historical range were deemed significant. Duration of action refers to the time of return of treated animals to control values.

Supplementary Material Available: Synthetic procedures for the intermediates 4 (41 pages). Ordering information is given on any current masthead page.

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Angiotensin Converting Enzyme Inhibitors. 10. Aryl Sulfonamide Substituted N-[1-Carboxy-3-phenylpropyl]-L-alanyl-L-proline Derivatives as Novel Antihypertensives[†]

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Compounds 1a–g consisting of enalaprilat covalently bonded to aryl sulfonamides, including several known thiazide diuretics, were synthesized and tested for ACE inhibitory and diuretic and overall antihypertensive effects. All compounds were potent ACE inhibitors in vitro, with IC_{50} = 6.5–85 nM. At 10 mg/kg iv or ip in the rat, 1a–g inhibited the AI pressor response by 76–100%; inhibition declined significantly upon oral dosing. Compounds 1a and 1f at 100 mg/kg ip in the sodium-depleted, spontaneously hypertensive rats reduced blood pressure 28–35% and 41–42%, respectively. Compounds 1a and 1f elicited natriuresis and kaliuresis without accompanying volume increases in the rat; 1c at 25 mg/kg iv induced delayed diuresis. Compound 1f has been chosen for further development.

Angiotensin converting enzyme (ACE) inhibitors have been safely and effectively applied in the treatment of nearly all forms of hypertension, regardless of severity or etiology, and have also become the major therapy in the treatment of refractory congestive heart failure.[1] Numerous reviews of the chemistry and pharmacology[2] and clinical applications[3] of these drugs have appeared.

Recently, synergism between ACE inhibitors and diuretics has been investigated. The effects of diuretics on plasma renin levels have long been of interest.[4] It has been shown that diuretics could act to potentiate an enhanced antihypertensive response to ACE inhibitors by stimulating the renin–angiotensin–aldosterone system.[5] Clinical in-

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