temperature for 20 min and then poured into H_2O (300 mL). The mixture was extracted with ether (3 × 100 mL), and the combined ethereal extracts were washed with H_2O and dried over $MgSO_4$. Filtration and evaporation of the solvent gave an oil. On trituration with petroleum ether (40–60 °C) the oil solidified, and crystallization from $CHCl_3$ gave the product **37**: yield 1.0 g (62%); mp 140–141 °C. Anal. ($C_{10}H_8ClNO_3S_3$) C, H, N.

Compounds **33–36** and **38–42** (Table I) were all prepared by either method A or B.

5-(Phenylsulfonyl)thiophene-2-sulfonamide (43). A solution of 5-(phenylthio)thiophene-2-sulfonamide (**8**; 5.0 g, 0.018 mol) and 30% H_2O_2 (5.0 mL) in AcOH (50 mL) was heated on a steam bath for 1 h. H_2O was added to the hot solution until crystallization commenced and the mixture was then allowed to cool. The product was filtered off, washed with H_2O, dried, and crystallized from EtOAc/petroleum ether (60–80 °C) to give **43**: yield 4.2 g (75%); mp 137.5–139 °C. Anal. ($C_{10}H_9NO_4S_3$) C, H, N. A polymorph of mp 170–172 °C was also obtained.

Compounds **44–60** and **62–67** (Table I) were all prepared by this method.

5-[(4-Aminophenyl)sulfonyl]thiophene-2-sulfonamide (61). A mixture of 5-[[4-(isobutyrylamino)phenyl]sulfonyl]thiophene-2-sulfonamide (**65**; 1.8 g, 0.005 mol) and 15% aqueous HCl (20 mL) was heated under reflux until solution was complete (2 h). After cooling, the solution was neutralized by the addition of solid $NaHCO_3$, and the resultant precipitate was filtered off and crystallized from MeOH/H_2O to give **61**: yield 0.9 g (61%); mp 195–196 °C. Anal. ($C_{10}H_{10}N_2O_4S_3$) C, H, N.

4-(Phenylsulfonyl)benzenesulfonamide (68). Diphenyl sulfide (18.6 g, 0.1 mol) was dissolved in $CHCl_3$ (50 mL) and chlorosulfonic acid (11.7 g) was added dropwise with cooling to the solution over a period of 15 min. The solution turned deep violet and a vigorous evolution of HCl occurred. After the solution was stirred at room temperature for 30 min, the $CHCl_3$ was evaporated to give an oil. PCl_5 (21 g) was added in portions to this oil, and the mixture was warmed on the steam bath for 10 min. The resultant clear solution was then evaporated, the residual oil was dissolved in $CHCl_3$, and the solution was washed with H_2O and dried ($MgSO_4$). The residue obtained by filtration and evaporation of the filtrate was added in portions to a stirred solution of concentrated ammonia (150 mL), and the resultant solid was filtered off and dissolved in ether. The ether solution was dried ($MgSO_4$) and filtered, and the filtrate was evaporated. The residue was crystallized from EtOH/H_2O to give 4-(phenylthio)benzenesulfonamide: yield 12.0 g (45%); mp 138–140 °C (lit.[18] mp 129–130 °C). 4-(Phenylthio)benzenesulfonamide (5.0 g, 0.02 mol) was added to 30% H_2O_2 (10 mL) in AcOH (50 mL), and the solution was heated on a steam bath for 1 h. The solution was then cooled in ice, and the resultant solid was filtered off and crystallized from EtOH/H_2O to give **68**: yield 2.2 g (40%); mp 190–192 °C (lit.[11] mp 182 °C).

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Novel Synthesis of (*S*)-1-[5-(Benzoylamino)-1,4-dioxo-6-phenylhexyl]-L-proline and Analogues: Potent Angiotensin Converting Enzyme Inhibitors

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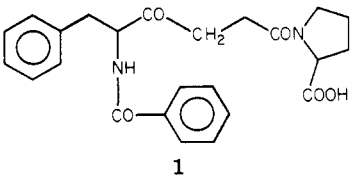
A new approach was developed for the synthesis of (*S*)-1-[5-(benzoylamino)-1,4-dioxo-6-phenylhexyl]-L-proline (**1**) and 23 analogues. The δ-(acylamino)-γ-keto acid intermediates were obtained by a modified Dakin–West reaction using 3-carbomethoxypropionyl chloride. Acylation of L-proline and recrystallization of the mixture of diastereomers gave the optically pure title compound in three reaction steps. The in vitro angiotensin converting enzyme (ACE) inhibitory activity of **1** was confirmed. Some of the novel analogues (**6**, **11**, **13**, and **17**) were also found to be potent inhibitors of ACE in vitro with an IC_{50} of 1.4–8.8 × 10^{-9} M (IC_{50} for captopril = 0.9 × 10^{-8} M). In vivo these compounds (**6**, **11**, **17**, and **18**) were much less active than captopril, especially by the oral route. Against angiotensin I (AI) challenge in normotensive conscious rats, **1** and **6** produced <50% inhibition at 30 mg/kg po but 57 to 82% inhibition at 3 mg/kg iv. Inhibition by both routes lasted <1 h. In renal hypertensive rats, **1** and 15 of its analogues failed to produce significant blood pressure lowering effects, in contrast to the marked effects of captopril. Near maximum inhibition of AI was achieved by continuous intravenous infusions of **1** and **20**, suggesting that limited oral activity may be due to degradation and/or clearance.

Angiotensin converting enzyme (ACE) inhibitors hold great promise in the treatment of hypertension.[1] Recently a very potent ACE inhibitor (**1**), an analogue of the tripeptide Bz-Phe-Gly-Pro, was disclosed[2] in which the NH of the amide portion of Phe-Gly was replaced by a methylene group.



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In the original synthesis[2] the key intermediate, (*S*)-δ-(benzoylamino)-γ-oxobenzenehexanoic acid, was obtained via a lengthy procedure in 11% yield. In order to explore the structure–activity relationsip of this series, a more

(1) (a) D. W. Cushman, H. S. Cheung, E. F. Sabo, and M. A. Ondetti, *Biochemistry*, **16**, 5484 (1977); (b) D. Gross, A. A. Patchett, and C. S. Sweet, National Medicinal Chemistry Symposium, of the American Chemical Society, 17th, Troy, NY, June 15–19, 1980, American Chemical Society, Washington, DC; (c) A. A. Patchett et al., *Nature (London)*, **288**, 280 (1980).

(2) R. G. Almquist, Wan-Ru Chao, M. E. Ellis, and H. L. Johnson, *J. Med. Chem.*, **23**, 1392 (1980).

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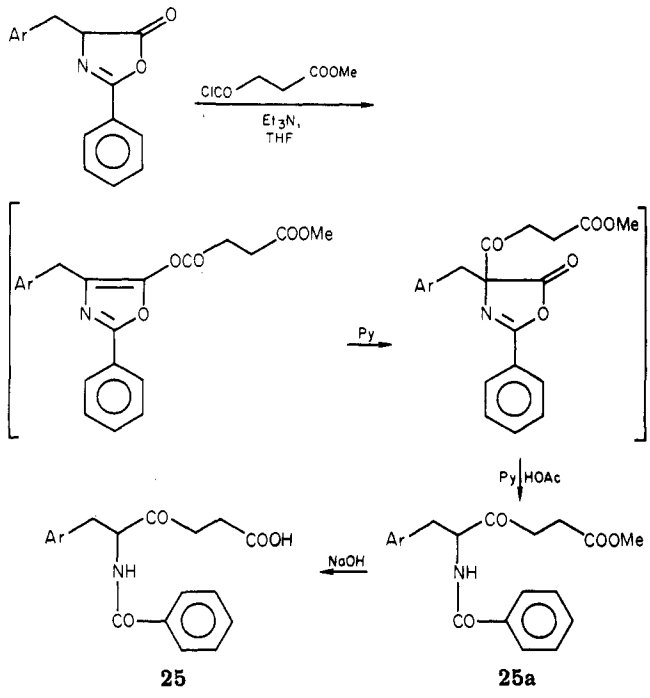
Scheme I



practical synthesis was required. Thus, a modification of the Dakin–West reaction[3] was utilized as shown in Scheme I.

Chemistry. The required oxazolones are readily obtained from the acylalanines by treatment with Ac_2O[4] or with dicyclohexylcarbodiimide (DCC)[5] or by catalytic hydrogenation of the corresponding unsaturated oxazolones[6] obtained from the reaction of aldehydes and hippuric acid. The Dakin–West reaction and the base hydrolysis to the racemic acylamino keto acids of type **25** usually proceeded in excellent yields. Acylation of L-proline with **25** gave a mixture of the *R*, L and *S*, L diastereomers of **1**. Repeated recrystallization from EtOAc gave pure *S*, L diastereomer, identical in every respect with an authentic sample.[2] For the final acylation procedure, two methods were used. The acylation of L-proline benzyl ester in the presence of hydroxybenzotriazol (HBT) and DCC, followed by catalytic debenzylation, as described,[2] gave after repeated recrystallization from EtOAc a 24.6% yield of optically pure **1**. Alternatively, the imidazolide method was somewhat more convenient[7,8] but gave a somewhat lower yield (20%) of optically pure **1**.

It was assumed that crystallization of the mixture of diastereomers of the analogues gave the desired *S*, L diastereomer as in the case of **1**. When the final product was amorphous or an oil, the diastereomeric mixture was tested.

The methyl ester **2** and amide **3** of **1** were prepared from optically pure **1**. The oxime **4** was obtained from **1** and hydroxylamine. Compound **6** was obtained from **9** by catalytic debenzylation in the presence of Pd/C in MeOH. The 3-pyridinyl compound **13**, in contrast to all the others, was quite water soluble. The key intermediate **28** was obtained from crude 2-phenyl-4-(3-pyridinylmethyl)-5-(4*H*)-oxazolone, which was prepared from 2-phenyl-4-(3-pyridinylmethylene)-5(4*H*)-oxazolone[9] by hydrogenation in THF with 20% Pd/C as catalyst.

Acid hydrolysis of **25** gave the α-amino ketone hydrochloride **34**. N-Acetylation was accomplished readily to give **30**, but acylation with benzyl chloroformate apparently gave a stable mixed anhydride. This acylation worked well, however, when the methyl ester of **34** was used. Mild hydrolysis of **29a** gave **29**, which was converted to **15** using DCC and HBT as described for the preparation of **1**.[2] Catalytic debenzylation of **15** gave **14**.

The tetrahydro-2-furanylcarbonyl compound **18** was obtained from the 2-furanylcarbonyl compound **17** by hydrogenation in MeOH with Pd/C as catalyst.

The final products are tabulated in Table I and the intermediates are shown in Table II.

Biological Results and Discussion

In Vitro Results. It has been previously shown[2] that **1** fits the hypothetical active site of angiotensin converting enzyme proposed by Ondetti et al.,[1] and it is not surprising that the structural requirements for inhibitory activity in this portion of the molecule agree with previous findings. Thus, a free carboxyl group on the terminal amino acid is required for maximum activity (**1**, **7**, **13**, **20**, and **23**). Since an unpurified enzyme preparation was utilized in this assay, it appears likely that the activity shown by the corresponding esters (**2**, **8**, **9**, **12**, and **19**) and amide **3** can be attributed to partial hydrolysis. The pyrrolidino compound **24** was void of activity.

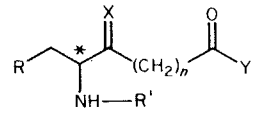
Conversion of the ketone (**1**) to an oxime (**4**) greatly lowers the activity.

The importance of additional binding sites on the converting enzyme located at some distance beyond the projected site of the zinc ion is demonstrated by this series of compounds. Essential requirements for optimal activity is this series appear to be an *aromatic* 5-(acylamino) moiety (**1** vs. **14** or **16**) and an aromatic group in the 6 position, as shown by the lack of activity of **10**. Substitution on this aryl group seems to have little effect on activity (**1** vs. **6**, **11**), and even the pyridyl compound **13** shows comparable activity.

In Vivo Results. The in vivo ACE inhibitory activity of **1** and its analogues was evaluated in conscious normotensive rats ($N = 2$ for each compound). In this test, the compunds were given orally and then challenged wtih angiotensin I (AI) until the responses to AI returned to the predose values. The test compounds were then administered intravenously in the same rats and the AI challenges were repeated. Compound **1** at 30 mg/kg po produced only a 48% inhibition of AI, and the inhibition lasted only 49 min (time required for the AI response to return to 70% of the pretreatment response). Compound **1** at 3 mg/kg administered intravenously as a single bolus injection produced only a 57% inhibition of AI, whereas captopril at 0.3 mg/kg produced 83% inhibition. Compounds **6**, **11**, **13**, **17**, and **18** were all less effective orally than the parent compound, but when given intravenously, they produced significant (64–84%) inhibition of AI (Table III).

(3) W. Steglich and G. Hoefle, *Chem. Ber.*, **102**, 883 (1969).
(4) H. E. Carter, *Org. React. (NY)*, **3**, 205 (1946).
(5) R. S. Lott, E. G. Breitholle, and C. H. Stammer, *J. Org. Chem.*, **45**, 1151 (1980).
(6) M. Ali, N. H. Khan, and A. A. Siddiqui, *Synth. Commun.*, **6**(3) 227 (1976).
(7) L. Birkhofer, W. Konkel, and A. Ritter, *Chem. Ber.*, **94**, 1263 (1961).
(8) K. Ruehlman, *Chem. Ber.*, **94**, 1263 (1961), prepared *N,O*-bis(trimethylsilyl)-L-proline using the expensive (trimethylsilyl)diethylamine. Jack Hinkley of our laboratory worked out a less expensive procedure using a slight excess of hexamethyldisilazane in MeCN solution to give a 80+% yield of distilled bissilylated proline.
(9) R. K. Griffith and H. J. Harwood, *J. Org. Chem.*, **29**, 2659 (1964).

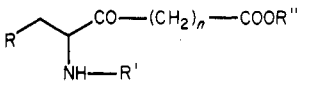
Table I. Chemical and Pharmacological Data of Title Compound and Analogues



compd	R	R'	X	n	Y[a]	mp, °C	recrystn solv	formula	anal.	optical confign at C*	$[\alpha]^{23}_D$ (c, solv),[b] deg	IC_{50}[c]
1	Ph	PhCO	O	2	A	155-157[d]	EtOAc	$C_{24}H_{26}N_2O_5$	C, H, N	S	−81.2[e]	3.2×10^{-9}
2	Ph	PhCO	O	2	B	~40		$C_{25}H_{28}N_2O_5$	C, H, N	S	−35.8	8.2×10^{-8}
3	Ph	PhCO	O	2	C	204-205	*i*-PrOH	$C_{24}H_{27}N_3O_4$	C, H, N	S	−123	1.5×10^{-7}
4	Ph	PhCO	NOH	2	A	~100	EtOAc/ *i*-Pr_2O	$C_{24}H_{27}N_3O_5$	C, H, N	S	−57.1	1.2×10^{-6}
5	Ph	PhCO	O	2	D	83-101		$C_{24}H_{26}N_2O_6$[f]	C, H, N	SR	−0.98 (1, MeOH)	5.4×10^{-7}
6	4-HO-Ph	PhCO	O	2	A	113-116		$C_{24}H_{26}N_2O_6$	C, H, N	SR	−131.0	4.7×10^{-9}
7	4-Bzl-O-Ph	PhCO	O	2	A	70-78		$C_{31}H_{32}N_2O_6$[h]	C, H, N	SR	−56.6	1×10^{-8}
8	4-Bzl-O-Ph	PhCO	O	2	E	47-50		$C_{35}H_{40}N_2O_6$	C, H, N	SR	−32.6	8.6×10^{-6}
9	4-Bzl-O-Ph	PhCO	O	2	F	52-55		$C_{38}H_{38}N_2O_6$	C, H, N	SR	−36.2 (0.45, $CHCl_3$)	8.6×10^{-8}
10	H	PhCO	O	2	A	60-63		$C_{18}H_{20}N_2O_5$[g]	C, H, N	SR	−38.6 (1, (MeOH)	3×10^{-7}
11	3,4-$(MeO)_2$-Ph	PhCO	O	2	A	152-153	EtOH	$C_{26}H_{30}N_2O_7$	C, H, N	S	−65.4	1.4×10^{-9}
12	3-pyridyl	PhCO	O	2	F	35-45		$C_{30}H_{31}N_3O_5$	C, H, N	SR	−37.4	1.5×10^{-8}
13	3-pyridyl	PhCO	O	2	A	80-100		$C_{23}H_{25}N_3O_5$[g]	C, H, N	SR	−58.4	5.7×10^{-9}
14	Ph	H, HCl	O	2	A	100-120		$C_{17}H_{23}N_2O_4Cl$[h]	C, H, N	SR	−52.0 (1.1, H_2O)	2.5×10^{-5}
15	Ph	BzlOCO	O	2	A	67-69		$C_{43}H_{63}N_{3.5}O_6$[i]	H, N; C[i]	SR	−4.08	2.4×10^{-7}
16	Ph	CH_3CO	O	2	A	156-157	EtOAc	$C_{19}H_{24}N_2O_5$	C, H, N	S	−85.6	3.3×10^{-7}
17	Ph		O	2	A	159-161	EtOAc	$C_{22}H_{24}N_2O_6$	C, H, N	S	−85.3	1.4×10^{-9}
18	Ph		O	2	A	~40		$C_{22}H_{28}N_2O_6$[g]	C, H, N	S	−77.5	2×10^{-8}
19	Ph	2-MePhCO	O	2	F	38-41		$C_{32}H_{34}N_2O_5$[k]	C, H, N	SR	−48.0	1.6×10^{-6}
20	Ph	2-MePhCO	O	2	A	150-158		$C_{25}H_{28}N_2O_5$	C, H, N	SR	−75.0	4.6×10^{-8}
21	Ph	PhCO	O	3	A	40-50		$C_{25}H_{28}N_2O_5$[k]	C, H, N	SR	−29.3	4.2×10^{-8}
22	Ph	PhCO	O	2	G	175-178	*i*-PrOH	$C_{35}H_{34}N_2O_5$	C, H, N	S	+21.1	1.8×10^{-7}
23	Ph	PhCO	O	2	H	65-70		$C_{28}H_{28}N_2O_5$[k]	C, H, N	S	+47.2	2.8×10^{-8}
24	Ph	PhCO	O	2	I	162-163	*i*-PrOH	$C_{23}H_{26}N_2O_3$	C, H, N	SR	0	inactive
captopril (Squibb)												0.9×10^{-8}

[a] A = L-proline; B = L-proline methyl ester; C = L-prolinamide; D = 4-hydroxy-L-proline; E = L-proline *tert*-butyl ester; F = L-proline benzyl ester; G = L-phenylalanine benzyl ester; H = L-phenylalanine; I = pyrrolidine. [b] c, 1, $CHCl_3$, unless stated otherwise. [c] Molar concentration required for 50% inhibition. [d] Literature[2] mp 156-160 °C. [e] Literature[2] rotation $[\alpha]^{21}_D$ −83.2° (c 0.98, $CHCl_3$). [f] 0.125 $CHCl_3$. [g] 0.5H_2O. [h] H_2O. [i] 1.5-dicyclohexylamine. C: calcd, 71.24; found, 71.73. [k] 0.25H_2O.

Table II. Acylamino Keto Acid and Ester Intermediates



no.	intermed for compd no.	R	R′	R″	*n*	mp or bp (mmHg), °C	recrystn solv	yield, %	formula	anal.
25	1-5	Ph	PhCO	H	2	182-184	EtOH	76	$C_{19}H_{19}NO_4$	C, H, N
25a		Ph	PhCO	Me	2	102-103	*i*-PrOH	78	$C_{20}H_{21}NO_4$	C, H, N
26	6-9	4-Bzl-O-Ph	PhCO	H	2	146-149	EtOH	98	$C_{26}H_{25}NO_5$	C, H, N
26a		4-Bzl-O-Ph	PhCO	Me	2	89-91	*i*-Pr_2O	75	$C_{27}H_{27}NO_5$	C, H, N
27	11	3,4-$(MeO)_2$-Ph	PhCO	H	2	146-148	EtOAc	54	$C_{21}H_{23}NO_6$	C, H, N
27a		3,4-$(MeO)_2$-Ph	PhCO	Me	2	95-100	Et_2O	45	$C_{22}H_{25}NO_6$	C, H, N
28	12, 13	3-pyridyl	PhCO	H	2	190-192	*i*-Pr_2O	22	$C_{18}H_{18}N_2O_4$	C, H, N
28a		3-pyridyl	PhCO	Me	2	gum		68	not isolated	
29	14, 15	Ph	BzlOCO	H	2	116-117	EtOAc	94	$C_{20}H_{21}NO_5$	C, H, N
29a		Ph	BzlOCO	Me	2	64-65	hexane	71	$C_{21}H_{23}NO_5$	C, H, N
30	16	Ph	CH_3CO	H	2	130-131	EtOAc	65	$C_{14}H_{17}NO_4$	C, H, N
31	17, 18	Ph		H	2	144-145	Et_2O	79	$C_{17}H_{17}NO_5$	C, H, N
31a		Ph		Me	2	91-92	*i*-PrOH	36	$C_{18}H_{19}NO_5$	C, H, N
32	19, 20	Ph	2-MePhCO	H	2	173-175	EtOH	91	$C_{20}H_{21}NO_4$[a]	C, H, N
32a		Ph	2-MePhCO	Me	2	84-85	*i*-Pr_2O	80	$C_{21}H_{23}NO_4$	C, H, N
33	10	H	PhCO	H	2	125-127	EtOAc	74	$C_{13}H_{15}NO_4$	C, H, N
33a		H	PhCO	Me	2	195-200 (0.45)		46	$C_{14}H_{17}NO_4$	C, H, N
34	14-16	Ph	H, HCl	H	2	130-132	CH_3CN	89	$C_{12}H_{16}NO_3Cl$	C, H, N
35	21	Ph	PhCO	H	3	132-133	aq *i*-PrOH	73	$C_{20}H_{21}NO_4$	C, H, N
35a		Ph	PhCO	Me	3	92-94	*i*-Pr_2O	79	$C_{21}N_{23}NO_4$	C, H, N

[a] $0.25H_2O$.

Table III. Summary of the Effects of ACE Inhibitors against Angiotensin I in Conscious, Normotensive Rats

compd	dose, mg/kg	route[a]	no. tested	antagonism of max inhibn, %	AI[b] recovery time,[c] min
1	30	po	2	48	49
	3	iv		57	34
6	30	po	2	46	24
	3	iv		82	34
11	30	po	2	33	5
	3	iv		74	24
17	30	po	2	32	84
	3	iv		84	52
18	30	po	2	24	10
	3	iv		64	
captopril	0.03	po	5	16	80
	0.3	po	5	69	170
	3	po	5	95	>240

[a] Vehicle employed for (1) po: 4% gum acacia in 2 mL of distilled H_2O/kg of body weight. (2) iv: phosphate buffer. [b] Angiotensin I (0.32 μg/kg iv) was administered 2 or 3 times before and every 5-10 min after each drug treatment. The postdrug AI responses were compared to the average of the predrug AI responses. [c] Recovery time = time in minutes required for the AI response to return to 70% of the pretreatment control response.

Since 1 appeared to have little oral activity and limited intravenous activity of short duration, infusion experiments were carried out to determine the maximum ACE inhibitory effect that could be achieved. In these experiments, 1 was infused at a rate of 0.1 or 0.2 mg/min [approximately 0.3 or 0.6 (mg/kg)/min] for 60 min, interrupted only every 10 min for AI challenges. In these experiments, 1 at 0.1 mg/min produced 92% inhibition (virtually complete) of the AI response 10 min after the onset of the infusion. The AI responses were inhibited as long as the infusions were continued. Upon termination of the infusion of 1, the inhibition quickly waned and the AI responses returned to their pretreatment values after 50 min.

The results with the infusion experiments suggested that 1 was metabolized very rapidly. However, the point of such a rapid metabolic attack was not apparent. The terminal proline amide bond is apparently stable in the case of related compounds.[10] Infusion experiments were carried out with **20**, since it was assumed that if the attack was on the 5-(benzoylamino) group the increased steric hindrance would slow up the metabolic degradation. Compound **20** was less active than 1, inhibiting AI less than 50% during a 0.2 mg/min infusion, and when the infusion was stopped, the rate of loss of inhibition was very similar to that of 1. The limitation of drug response by rapid plasma clearance is also possible but has not been examined in the present study.

The oral antihypertensive efficacy of 1 and a number of its analogues was evaluated in the conscious renal (2 kidney/1 clip) hypertensive rat. Compound 1 at 30 mg/kg

(10) B. Rubin and M. J. Antonaccio, in "Pharmacology of Antihypertensive Drugs", A. Scriabine, Ed., Raven Press, New York, 1980, p 34.

Table IV. Summary of the Effects of Certain ACE Inhibitors on Blood Pressure in the Conscious Renal (1 Clip/2 Kidney) Hypertensive Rat

compd	dose,[a] mg/kg po	no. tested	mean aortic blood pressure,[b] mmHg: base line	max effect	change
1	30	4	190 ± 6	178 ± 6	−12 (at 8 h)
2	30	4	153 ± 3	142 ± 2[c]	−11 (at 10 h)
2	3	4	199 ± 10	165 ± 3[c]	−34 (at 2 h)
5	30	3	188 ± 10	168 ± 10	−20 (at 4 h)
6	30	3	195 ± 9	172 ± 12	−23 (at 5 h)
7	30	3	200 ± 11	195 ± 11	−5 (at 8 h)
11	30	4	179 ± 7	174 ± 6	−5 (at 2 h)
12	30	3	191 ± 1	159 ± 10[c]	−32 (at 8 h)
13	30	3	204 ± 8	184 ± 3	−20 (at 6 h)
15	30	3	197 ± 7	175 ± 4	−22 (at 10 h)
16	30	3	202 ± 8	176 ± 10	−26 (at 8 h)
17	30	3	185 ± 13	184 ± 13	−1 (at 1 h)
18	30	4	188 ± 7	163 ± 5[c]	−23 (at 8 h)
19	10	3	195 ± 9	175 ± 12	−20 (at 6 h)
20	30	3	203 ± 5	188 ± 2[c]	−15 (at 5 h)
22	30	3	187 ± 5	182 ± 8	−5 (at 6 h)
captopril	0.3	4	189 ± 3	170 ± 7[c]	−19 (at 10 h)
	3.0	4	192 ± 7	99 ± 7[c]	−93 (at 6 h)
	30	4	186 ± 4	85 ± 1[c]	−101 (at 6 h)

[a] Vehicle employed: 4% gum acacia in 2 mL of distilled H_2O/kg of body weight. [b] All values are the mean ± 1 SEM. [c] Values significantly different from the comparable base-line value $p < 0.05$ (two-tailed probability, 4–6 df).

po produced only a small reduction in mean aortic blood pressure (Table IV). Compounds **2**, **12**, **18** and **20** produced somewhat greater responses, but the reductions in pressures were limited, although statistically significant ($p < 0.05$). By comparison, captopril produced a marked, dose-related decrease in blood pressure at doses less than 3.0 mg/kg. These results show that the oral efficacy of compound 1 previously reported[2] is limited both in magnitude and duration.

Compound 1 and its analogues, like captopril, exert their maximum AI antagonistic effects in the normotensive rat within 30 min following oral dosing, whereas in renal hypertensive rats the antihypertensive effects do not fully develop until 2 to 8 h posttreatment. Unlike captopril, however, these new compounds produced only limited effects, even at 30 mg/kg po. It is possible that this limited effect is not related to ACE inhibition. Compounds 1 and **16** were administered to spontaneously hypertensive rats at 100 mg/kg po. Compound 1 was without effect, but **16** produced a limited but significant ($p < 0.05$) blood pressure lowering (172 ± 3 mmHg base line to 151 ± 6 mmHg after 6 h). Since captopril has been shown to produce similar effects in the SHR, an additional mechanism of action for these compounds is not supported.

Experimental Section

Melting points were determined in a Thomas-Hoover capillary melting point apparatus or a Mel-Temp apparatus. Infrared (IR) data were recorded on a Beckman IR-9 or IR-7 prism grating instrument on a Digilab FTS-14 interferometer. Nuclear magnetic resonance measurements (NMR) were made on a Bruker WH-90 pulsed Fourier transform instrument. IR and NMR were compatible with the assigned structures. Homogeneity of the products was determined by ascending thin-layer chromatography (TLC) on precoated TLC sheets (silica gel 60 F 254, Merck), using principally the solvent system HOAc–MeCN–toluene (1:9:10). The TLC of the compounds described in Table I were homogeneous single spots when visualized with UV and/or I_2 vapors. Microanalytical results that are reported by symbols of the elements were within ±0.4% of theory unless otherwise indicated.

Methyl δ-(Benzoylamino)-γ-oxobenzenehexanoate (25a). Triethylamine (75 mL, 0.55 mol) was added dropwise with stirring and cooling in an ice bath to a solution of 2-phenyl-4-(phenylmethyl)-5(4*H*)-oxazolone[4] (125 g, 0.5 mol) and 3-carbomethoxypropionyl chloride (75 g, 0.5 mol) in THF (1000 mL). After 2 h the ice bath was removed and the suspension was allowed to stand overnight. The filtrate was evaporated at reduced pressure at <50 °C to give a quantitative yield of acylated oxazolone [TLC (*i*-Pr_2O) R_f 0.51]. The crude product was dissolved in pyridine (600 mL), warmed to 80 °C, and HOAc (450 mL) was added in one lot (CO_2 evolution). The solution was heated on a steam bath with stirring for 1 h and was evaporated at reduced pressure to give 184 g of crude ester **25a**, which crystallized on standing. The resulting solid was slurried in *i*-Pr_2O (500 mL) and washed (2 × 50 mL of *i*-Pr_2O) to yield 132.5 g (78%) of **25a**, mp 90–95 °C, sufficiently pure for the next step. An analytical sample from *i*-Pr_2O had mp 102–103 °C; TLC (*i*-Pr_2O) R_f 0.13. Anal. ($C_{20}H_{21}NO_4$) C, H, N.

δ-(Benzoylamino)-γ-oxobenzenehexanoic Acid (25). A solution of the crude **25a** (132.5 g, 0.391 mol) in 1000 mL of THF and 900 mL of aqueous NaOH (0.5 N) was warmed to 40 °C and then allowed to stand for 20 h at ambient temperature. THF was evaporated at reduced pressure at <40 °C, and the solution was acidified to pH 4 with dilute HCl. The product was collected by filtration, washed with H_2O, and recrystallized from EtOH to give 96.4 g (76%) of analytically pure **25**, mp 182–184 °C. Anal. ($C_{19}H_{19}NO_4$) C, H, N.

(*S*)-1-[5-(Benzoylamino)-1,4-dioxo-6-phenylhexyl]-L-proline (1). To a slurry of **25** (65 g, 0.2 mol) in 1000 mL of THF was added at 20 °C 1,1′-carbonyldiimidazole (40 g, 0.247 mol). The reaction mixture was warmed for 1 h at 40 °C, the solution was cooled in ice, and *N,O*-bis(trimethylsilyl)-L-proline[8] (60 g, 0.23 mol) was added and refluxed for 5 h. After the solvent was evaporated, the residue was partitioned between $CHCl_3$ and H_2O and then acidified to pH 4 with dilute HCl. The organic layer was washed with H_2O and evaporated, and the product crystallized 3 times from EtOAc to give optically pure product: yield 16.9 g (20%); mp 155–157 °C. Anal. ($C_{24}H_{26}N_2O_5$) C, H, N.

(*S*)-1-[5-(Benzoylamino)-4-(hydroxyimino)-1-oxo-6-phenylhexyl]-L-proline (4). A solution of 1 (6.3 g, 0.015 mol) and H_2NOH·HCl (1.2 g, 0.0173 mol) in pyridine (50 mL) was allowed to stand at room temperaure for 20 h, warmed for 0.5 h on a steam bath, and evaporated. The product was taken up in $CHCl_3$ and washed with H_2O. The crude product, 5.7 g, was crystallized from EtOAc/*i*-Pr_2O to give 3.9 g (60%) of white powder, mp ~100 °C. Anal. ($C_{24}H_{27}N_3O_5$) C, H, N.

δ-Amino-γ-oxobenzenehexanoic Acid Hydrochloride (34). A solution of **25** (32.5 g, 0.1 mol) in HOAc (500 mL), concentrated HCl (500 mL), and H_2O (250 mL) was refluxed for 6 h. After the solvent was evaporated, the hydrochloride was crystallized from MeCN to give 22.8 g (89%) of **34**, mp 130–132 °C. Anal. ($C_{12}H_{16}NO_3Cl$) C, H, N.

δ-(Acetylamino)-γ-oxobenzenehexanoic Acid (32). To a solution of **34** (12.8 g, 0.05 mol) in H_2O (100 mL) and HOAc (500

mL) was added NaOAc (5 g, 0.06 mol) followed by the slow addition of Ac_2O (25 mL, 0.266 mol) at ice-bath temperature. After 2 h the reaction mixture was allowed to warm to room temperature and allowed to stand for 60 h. The residue after evaporation of solvent was taken up in H_2O and acidified to pH 4 with aqueous $KHSO_4$. The product solidified on scratching; it was washed with H_2O and recrystallized from EtOAc to give 8.5 g (65%) of **32**, mp 130–131 °C. Anal. ($C_{14}H_{17}NO_4$) C, H, N.

Methyl δ-[[(Phenylmethoxy)carbonyl]amino]-γ-oxobenzenehexanoate (29a). A solution of **34** (1 g, 3.9 mmol) in MeOH (30 mL) was saturated with gaseous HCl, allowed to stand for 18 h, and evaporated. The residue was again dissolved in MeOH and treated with HCl. Evaporation gave an oil. The crude ester was dissolved in H_2O (30 mL) and benzyl chloroformate (1.5 g, 8.8 mmol) was added. The pH of the solution was brought to 6.5 with Na_2CO_3 solution. The reaction proceeded rapidly at this pH and was maintained there by adding Na_2CO_3 solution. At the end of 1 h the precipitated gum was taken up in Et_2O and dried ($MgSO_4$), and the Et_2O was evaporated. The residue was recrystallized from hexane/petroleum ether to give white needles of **29a**: yield 1 g (71%); mp 64–65 °C. Anal. ($C_{21}H_{23}NO_5$) C, H, N.

δ-[[(Phenylmethoxy)carbonyl]amino]-γ-oxobenzenehexanoic Acid (29). A solution of **29a** (0.5 g, 1.25 mmol) in MeOH (10 mL) and 2 N NaOH (1 mL, 2 mmol) was allowed to stand at 25 °C for 10 min and was then diluted with H_2O (10 mL) and acidified with 2 N HCl (1 mL). MeOH was evaporated at reduced pressure; the product was collected, washed with H_2O, and recrystallized from EtOAc to give 0.45 g (94%) of **29**, mp 116–117 °C. Anal. ($C_{20}H_{21}NO_5$) C, H, N.

Biological Methods. The in vitro ACE inhibitory activity was determined by a radioassay procedure reported previously.[11] Activity is reported as the IC_{50}, which is the approximate molar concentration of test compound causing a 50% inhibition of the control converting-enzyme activity.

The test solutions were prepared by dissolving 2–5 mg of test compound in 1 mL of Me_2SO and diluting to the desired concentration with a pH 8 buffer of 0.05 mol of Hepes (Calbiochem), 0.1 mol NaCl, and 0.6 mol of Na_2SO_4 in H_2O.

AI Challenge Test in the Conscious Rat. The oral and intravenous efficacy of test compounds to inhibit the conversion of AI to AII was evaluated in conscious normotensive rats. For this test, male albino rats (CD strain; Charles River; Wilmington, MA) weighing 300 to 387 g were surgically prepared with an aortic cannula (for blood pressure monitoring) and a vena caval cannula (for intravenous drug injection) as described below. AI (0.32 μg/kg iv) was administered before and at 5- to 10-min intervals following intravenous or oral test drug administration. ACE inhibitors block the pressor effect of AI by interfering with its conversion to AII, which is the active pressor agent.

At the time of testing, the rats in their individual cages were transferred to the test room and their aortic cannulae were connected to pressure transducers (P23Gb or P23De; Gould Statham, Hato Ray, Puerto Rico). Systolic, diastolic, and mean aortic blood pressures and heart rate were obtained from the pressure signal (Gould Brush couplers) and displayed on a strip chart recorder (Model 260, Gould Brush; Cleveland, OH).

Blood Pressure and Heart Rate Test in the Conscious Rat. Hypertension of renal origin was produced in rats by placing a silver clip (0.2-mm gap) around the left renal artery near the aorta and leaving the contralateral kidney intact. Four-week-old male albino rats (CD strain, Charles River; Wilmington, MA) were clipped soon after arrival, and the hypertension was allowed to develop for 3–4 weeks. The rats were then cannulated for blood pressure monitoring as described below. Only rats with pulsatile mean aortic blood pressures of >160 mmHg were used. At the time of cannulation the rats weighed 280 to 320 g. The rats were given free access to a standard lab chow (5012, Purina; Richmond, IN) and tap water and were maintained on a 12-h dark/12-h light cycle.

Two to four days prior to testing, rats were surgically implanted with chronic polyethylene cannulae. Each rat was anesthetized intramuscularly with 20 mg/kg of Telazol (tiletamine hydrochloride/zolazepam hydrochloride, 1:1), and the descending aorta and vena cava were exposed via a midline incision. For blood pressure monitoring, cannulae consisting of a PE 100 (0.86-mm i.d.) body and a PE 50 (0.58-mm i.d.) tip were inserted into an undersized puncture hole below the renal arteries. The cannulae are anchored to the psoas muscle, passed subcutaneously along the midline of the back, and externalized between the scapulae. Following surgery, each rat was given 30 000 units of penicillin subcutaneously (Penicillin G Procaine Sterile Suspension; Parke-Davis, Detroit, MI). The rats were then fitted with a harness–spring–swivel assembly designed to protect the cannula and to provide the rat relative freedom of movement. The aortic cannula of each rat was connected to a pressure transducer (P23Gb, Statham; Hato Rey, Puerto Rico) and an infusion pump (Sage Model 234-7, Orion Research, Cambridge, MA) by means of PE 100 tubing. While on test, each animal received a continuous slow infusion of heparinized saline solution (approximately 400 μL or 40 units of heparin per 24-h period) to prevent clot formation in the blood pressure monitoring cannula. As required, a PE 20 (0.38-mm i.d.) cannula was inserted directly into the vena cava and externalized as described for the aortic cannula. The intravenous cannulae are plugged when not being used.

One-minute running average values of heart rate and aortic blood pressure (mean, systolic, and diastolic) for each rat were recorded every 30th min by means of a computer-assisted data capture scheme as previously described.[12]

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