Mercaptoacyl Dipeptides as Orally Active Dual Inhibitors of Angiotensin-Converting Enzyme and Neutral Endopeptidase

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Dual inhibitors of the two zinc metallopeptidases, neutral endopeptidase (NEP, EC 3.4.24.11) and angiotensin-I-converting enzyme (ACE, EC 2.4.15.1), have been the focus of much clinical interest for the treatment of hypertension and congestive heart failure. We have previously reported that compound **2** (*N*-[[1-[(2(*S*)-mercapto-3-methyl-1-oxobutyl)amino]-1-cyclopentyl]-carbonyl]-L-tyrosine) was a potent dual inhibitor *in vitro* (IC_{50}(ACE) = 7.0 nM, IC_{50}(NEP) = 1.5 nM) (Fink *et al. J. Med. Chem.* **1995**, *38*, 5023−5030). This compound was found to have oral activity; however, its duration of effect was short. A series of thioacetate carboxylic acid ester analogs of compound **2** was prepared. Modifications were also made to the tyrosine phenol. These compounds were evaluated for their ability to inhibit plasma ACE activity when administered orally to conscious normotensive rats. Most of the compounds prepared were found to be orally active with longer durations of effect than compound **2**. Compound **38** (*N*-[[1-[(2(*S*)-(acetylthio)-3-methyl-1-oxobutyl)amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine ethyl ester), administered at 11.7 mg/kg po, was found to be more efficacious than captopril at 10 mg/kg po. This compound was also found to inhibit plasma NEP activity following oral administration to conscious rats and was more efficacious than acetorphan. Compound **38** was found to lower blood pressure in the aorta-ligated rat and the spontaneously hypertensive rat when administered orally. The synthesis and biological activity of these dual inhibitors are discussed.

Introduction

The development of a single agent which possesses the ability to inhibit both of the membrane-bound zinc metalloproteases, angiotensin converting enzyme (EC 2.4.15.1, ACE) and neutral endopeptidase (EC 3.4.24.11, NEP), has been the focus of recent drug discovery.[1−17] Both of these ectoenzymes are intimately involved in regulatory systems which modulate blood pressure and fluid volume homeostasis. ACE is a dipeptidyl carboxypeptidase which is part of the renin−angiotensin−aldosterone system. This enzyme catalyzes the conversion of the biologically inactive decapeptide angiotensin-I (AI) to the octapeptide angiotensin-II. Angiotensin-II is a potent vasoconstrictor, which also promotes the release of aldosterone, leading to sodium and fluid retention.

NEP is the major enzyme involved in the metabolic inactivation of atrial natriuretic factor (ANF).[18−20] ANF is a 28-amino acid polypeptide which is synthesized in the atrial myocyte and secreted into the circulation in response to changes in intracardiac pressure. Through interaction with its receptor, ANF upregulates cGMP production and elicits a number of biological responses including diuresis, natriuresis, vasodilation, and reduction of plasma aldosterone levels.[21,22]

ACE inhibitors have gained wide acceptance clinically and are commonly prescribed for the treatment of hypertension and congestive heart failure (CHF).[23] The magnitude of blood pressure lowering that can be achieved with ACE inhibitor therapy is dependent upon several factors such as the severity of hypertension, the plasma renin activity, and the sodium status of the patient.[24] ACE monotherapy is effective in only 40−50% of the essential hypertensive population and most efficacious in patients with high renin activity.[25,26] Furthermore, the rate of responsiveness for this class of therapeutic agent can be increased by coadministration of a diuretic agent.[27] Diuretics, however, can evoke a number of undesired side effects such as activation of pressor systems, hypokalemia, hyperglycemia, and elevation of plasma lipids.[28] Selective inhibitors of NEP have been studied in animal models as well as humans and have been found to produce significant diuretic and natriuretic effects without kaliuresis.[29] NEP inhibitors are effective in low-renin animal models of hypertension in contrast to ACE inhibitors which are ineffective.

Several studies have shown that coadministration of selective ACE and NEP inhibitors in animal models of hypertension and CHF has a more beneficial effect over the administration of the single agents separately.[30,31] In recent reports, it has also been demonstrated that single molecules which possess dual ACE and NEP inhibitory activity also exhibit these synergistic properties.[32−34] The antihypertensive response that is achieved with the combination or the single-molecule dual inhibitor is independent of the plasma renin activity or the sodium status of the animal model chosen. These molecules represent a new class of cardiovascular agents that should be more effective in a broader range of hypertensive patients and in CHF.

We initially reported our results on a series of α-mercapto dipeptides which possessed dual ACE and NEP inhibitory activity.[16] One of the best compounds in this series was the biphenyl analog **1**. This compound was found to be a potent dual inhibitor (ACE, IC_{50} = 62 nM; NEP, IC_{50} = 28 nM). Unfortunately, it was

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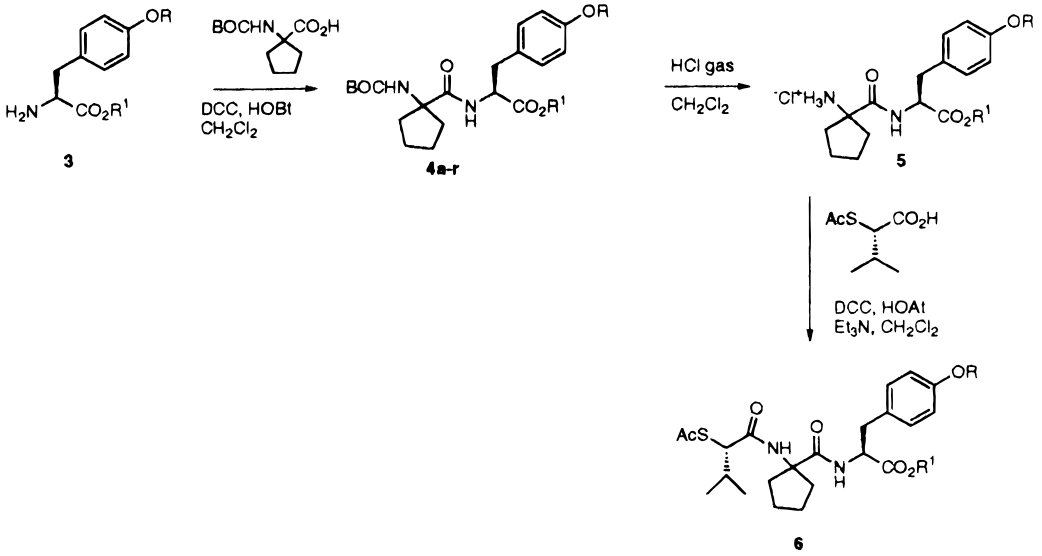
Scheme 1



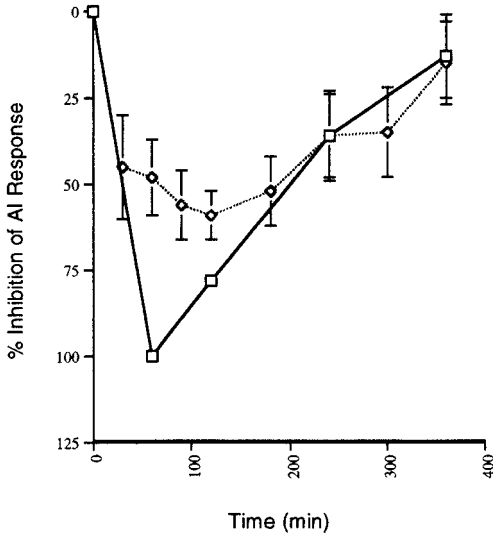
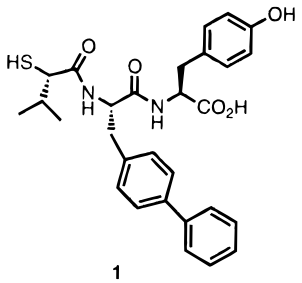
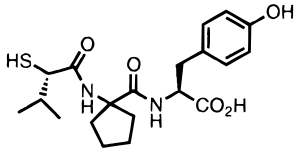


Figure 1. Inhibition of AI pressor response in conscious rats treated with captopril (□) and compound **2** (◇) at 10 mg/kg po.



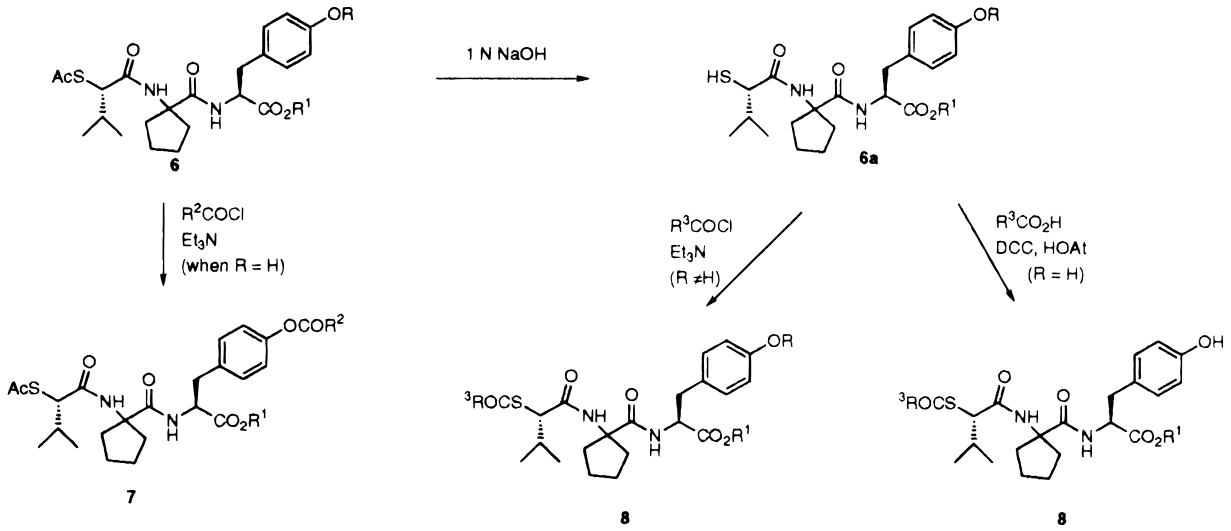
short acting *in vivo* and inhibited the AI-induced pressor response in normotensive conscious rats by 50% for only 1 h after intravenous administration. Further modification of this compound was undertaken in order to improve its duration of action. We replaced the amino acids in this molecule with non-natural amino acids that would not impair the *in vitro* potency of the compound but might improve the metabolic stability and thus its efficacy. Replacement of the central amino acid with spiroalkyl amino acids improved the *in vivo* activity of these analogs. In a recent communication, we reported that the cycloleucine analog compound **2** was a potent dual inhibitor (ACE, IC_{50} = 7.0 nM; NEP, IC_{50} = 1.5 nM).[17] It inhibited the AI pressor response in normotensive conscious rats at greater than 50% for more than 6 h after intravenous administration. When administered to conscious rats at 10 mg/kg po, **2** was found to have oral activity with a similar duration of effect as captopril (Figure 1). The maximum response achieved, however, was less than that of captopril. To further improve the efficacy of compound **2**, we prepared a number of carboxylic acid ester−thioester prodrug derivatives and studied their resulting *in vivo* activity. Modifications were also made to the tyrosine residue. Herein we report our results on a series of mercaptoacyl spirocyclopentyl-containing dipeptides.



Chemistry

The target α-mercapto spirocyclopentyl-containing dipeptides were prepared as outlined in Schemes 1 and 2. Synthesis of the dipeptide was initiated by coupling various α-amino acid esters **3** to Boc-protected cycloleucine by the classical 1,3-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBt) method.[35,36] Deprotection of the Boc-protected dipeptides **4a**−**r** with anhydrous HCl gas in methylene chloride afforded the amine hydrochloride salts **5**. Coupling of the amine hydrochloride **5** with (*S*)-2-(acetylthio)-3-methylbutanoic acid, DCC, 1-hydroxy-7-azabenzotriazole (HOAt), and triethylamine gave the mercaptoacyl dipeptides **6**.[37,38] Treatment of the dipeptide **6** (when R = H) with 1 equiv of an acid chloride or a chloroformate with triethylamine yielded the phenolic ester or carbonate **7**, respectively. Treatment of dipeptide **6** with 1 equiv of 1 N sodium hydroxide in degassed methanol afforded the free thiol **6a**. The thiol **6a** (R ≠ H) was then reacted with a variety of acid chlorides and triethylamine to give new thioesters **8**. When R = H, the free thiol **6a** can also be treated with an acid, DCC, HOAt, and triethylamine

Scheme 2



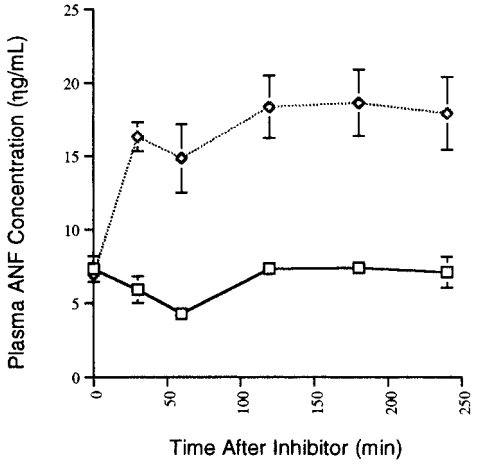


Figure 2. Potentiation of infused ANF in conscious rats by compound **38** at 11.7 mg/kg po (◇); vehicle, 20% EtOH/80% PEG at 0.5 mL/kg po (□).

to yield thioesters **8**. A small amount of the phenol-coupled product is observed which is easily separated by flash chromatography. The mercaptoacyl dipeptides (compounds **9**−**52**) prepared are shown in Table 1.

Results and Discussion

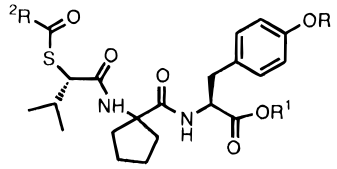
***In Vivo* ACE and NEP Inhibition.** The thioester carboxylic acid esters (compounds **9**−**52**) prepared were tested for their ability to inhibit the AI pressor response in normal conscious rats. We studied the effect of these compounds only after oral administration, since this is the desired route of administration for an antihypertensive agent. The *in vivo* effects of these compounds were compared to those of captopril and benazepril, two clinically prescribed ACE inhibitors. Several of these compounds were found to be more efficacious than compound **2**, captopril, and benazepril as can be seen in Table 2. By simply preparing the thioester carboxylic acid esters of compound **2**, compounds **9**−**33**, significant improvement in oral activity was observed in some instances. Among the best compounds were **13**, **15**, and **27**. In an attempt to improve the water solubility of these derivatives, heteroatoms were added to the acyl group on the sulfur (compounds **21**−**24**). Unfortunately, these analogs were not among the longest acting. We also prepared compounds where modifications were made to the tyrosine phenol. We prepared simple *O*-alkyl ethers (compounds **34**−**42**), esters (compounds **43**−**51**), and a carbonate (compound **52**). In comparing the tyrosine ethyl ester analog compound **10** to the O-alkylated tyrosine ethyl ester compounds **35**, **36**, and **38**, it appears that simple alkylation of the phenol groups provides an enhancement in oral activity. The esters in general with the exception of compound **47** were not as good as the *O*-alkyl ethers. Carbonate **52** was less active than compound **10**, the tyrosine analog.

We believe that the thioester and carboxylic acid ester groups are metabolized *in vivo* to give the free thiocarboxylic acids and are thus behaving as prodrugs of compound **2**. For a compound to function as an inhibitor of ACE, it must contain a functional group that chelates to the zinc atom which resides in the active site of the enzyme and a C-terminal carboxylic acid.[39] For NEP inhibition, a zinc-chelating group is also essential. A C-terminal carboxylic acid is advantageous to NEP inhibition; however, its exact placement is not as critical as it is for ACE inhibition.[40] To confirm this, several of the thioester carboxylic acid esters were evaluated for their *in vitro* activity against ACE and NEP and found to have little (1×10^{-6} M) or no activity against either enzyme.[41] The *O*-alkyl ether group on the tyrosine may also be metabolically removed, but this is not required for activity as we have previously reported that the free thiol carboxylic acid of compound **38** itself is a potent dual inhibitor (IC_{50} = 19 nM (ACE), IC_{50} = 2.2 nM (NEP)).[17]

A sampling of the different prodrugs prepared was also investigated for the ability to potentiate exogenous ANF. The effects of these compounds were studied in conscious rats after oral administration (Table 3). All of the compounds evaluated were found to be more efficacious than acetorphan, the thioacetate analog of thiorphan, a known NEP inhibitor. Of the compounds tested in the *in vivo* ACE and NEP assays, compounds **34** and **38** performed well in both tests. Since we were concerned with the release of methanol from compound **34** by hydrolysis of the methyl ester *in vivo*, compound **38**, the ethyl ester analog, was selected for further biological evaluation.

As can be seen from Figure 2, compound **38** at 11.7 mg/kg po was found to significantly increase exogenously administered ANF plasma concentrations, ap-

Table 1. Physicochemical Properties of α-Mercapto Dipeptides



compd	R	R^1	R^2	mp, °C	formula
9	H	Me	Me	157−158	$C_{23}H_{32}N_2O_6S$
10	H	Et	Me	148−149	$C_{24}H_{34}N_2O_6S$
11	H	Pr	Me	119−120	$C_{25}H_{36}N_2O_6S$
12	H	allyl	Me	119−120	$C_{25}H_{34}N_2O_6S$
13	H	Bu	Me	85−86	$C_{26}H_{38}N_2O_6S$
14	H	hexyl	Me	65−66	$C_{28}H_{42}N_2O_6S$
15	H	$CH(Me)_2$	Me	155−156	$C_{25}H_{36}N_2O_6S$
16	H	$CH_2CH(Me)_2$	Me	148−149	$C_{26}H_{38}N_2O_6S$
17	H	$(CH_2)_2CH(Me)_2$	Me	94−95	$C_{27}H_{40}N_2O_6S$
18	H	cyclopentyl	Me	133−135	$C_{27}H_{38}N_2O_6S$
19	H	benzyl	Me	66−67	$C_{29}H_{36}N_2O_6S$
20	H	Et	tBu	150−151	$C_{27}H_{40}N_2O_6S$
21	H	Et	$CH_2N(CH_2)_4O$	147−148	$C_{28}H_{41}N_3O_7S$
22	H	Et	CH_2OMe	129−130	$C_{25}H_{36}N_2O_7S$
23	H	Et	$CH_2N(Me)_2$	136−137	$C_{26}H_{39}N_3O_6S$
24	H	Et	2-pyridyl	154−155	$C_{28}H_{35}N_3O_6S$
25	H	Et	cyclopentyl	130−131	$C_{28}H_{40}N_2O_6S$
26	H	Bu	Et	102	$C_{27}H_{40}N_2O_6S$
27	H	Bu	Pr	105	$C_{28}H_{42}N_2O_6S$
28	H	Bu	$CH(Me)_2$	125	$C_{28}H_{42}N_2O_6S$
29	H	Bu	cyclopentyl	140	$C_{30}H_{44}N_2O_6S$
30	H	Bu	cyclohexyl	110	$C_{31}H_{46}N_2O_6S$
31	H	Bu	$CH_2N(CH_2)_4O$	143−144	$C_{30}H_{45}N_3O_7S$
32	H	Bu	$CH_2N(CH_2)_5$	130−131	$C_{31}H_{47}N_3O_6S$
33	H	Bu	CH_2OMe	110−111	$C_{27}H_{40}N_2O_7S$
34	Me	Me	Me	118−120	$C_{24}H_{34}N_2O_6S$
35	Et	Et	Me	102−103	$C_{26}H_{38}N_2O_6S$
36	Pr	Et	Me	116	$C_{27}H_{40}N_2O_6S$
37	Me	Et	CH_2OMe	95−100	$C_{26}H_{38}N_2O_7S$
38	Me	Et	Me	102−103	$C_{25}H_{36}N_2O_6S$
39	Me	Bu	Me	114−115	$C_{27}H_{40}N_2O_6S$
40	Me	$CH_2CON(Et)_2$	Me	106−107	$C_{29}H_{43}N_3O_7S$
41	Me	benzyl	Me	110−111	$C_{30}H_{38}N_2O_6S$
42	Me	CH_2(3-pyridyl)	Me	107−109	$C_{29}H_{37}N_3O_6S$
43	COMe	Et	Me	94−95	$C_{26}H_{36}N_2O_7S$
44	COEt	Et	Me	102−103	$C_{27}H_{38}N_2O_7S$
45	$COCH(Me)_2$	Et	Me	118−119	$C_{28}H_{40}N_2O_7S$
46	$COCH_2OMe$	Et	Me	80−81	$C_{27}H_{38}N_2O_8S$
47	$COCH_2CO_2Et$	Et	Me	71−72	$C_{29}H_{40}N_2O_9S$
48	CO(phenyl)	Et	Me	121−122	$C_{31}H_{38}N_2O_7S$
49	CO(2-thienyl)	Et	Me	125−126	$C_{29}H_{36}N_2O_7S_2$
50	CO(3-pyridyl)	Et	Me	67−68	$C_{30}H_{37}N_3O_7S$
51	CO(4-pyridyl)	Et	Me	85−86	$C_{30}H_{37}N_3O_7S$
52	CO_2Et	Et	Me	94−95	$C_{27}H_{38}N_2O_8S$

proximately 250% over controls. We further explored the *in vivo* inhibition of ACE and NEP after oral administration at lower doses as shown in Figures 3 and 4, respectively. Compound **38** exhibits good dose−response behavior for both the plasma NEP inhibition and the AI pressor response assays. As can be seen in Figure 3 compound **38** is approximately equal in its effect at 3 mg/kg po as captopril (36% inhibition at 10 mg/kg po, 4 h; Table 2) and benazepril (39% inhibition at 10 mg/kg po, 4 h) given at 10 mg/kg po.

Blood Pressure-Lowering Studies of Compound 38. The blood pressure-lowering effects of **38** were further studied in two animal models of hypertension, a renin-dependent model and a genetic model. In the aorta-ligated rat, oral administration (3 mg/kg) of compound **38** significantly lowered mean arterial pressure. At 2 and 4 h, a 15 ($p < 0.05$) and 17 ($p < 0.025$) mmHg lowering of blood pressure was observed.

In the conscious telemetered spontaneously hypertensive rat (SHR), compound **38** given orally at 1, 3, and 10 mg/kg bid lowered mean arterial pressure in a dose-dependent manner by 16, 21, and 33 mmHg. Benazepril at the same doses decreased blood pressure by 7, 16, and 27 mmHg, respectively (Figures 5−7).

In summary, significant enhancement of *in vivo* activity was seen for the α-mercapto dipeptides by preparing the thioester−carboxylic acid esters. These compounds are most likely acting as prodrugs since several of these compounds were found to have weak or no activity *in vitro* as inhibitors of ACE and NEP. Compound **38** was identified as a long acting dual ACE and NEP inhibitor *in vivo*. This compound was found to lower blood pressure in two animal models of hypertension. A more detailed pharmacological evaluation of compound **38** will be presented in the near future.

Experimental Section

Chemistry. Melting points (mp) were determined on a Thomas-Hoover or Melt-Temp melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a

Table 2. Plasma Inhibition of ACE Activity in the Conscious Rat

compd	dose, mg/kg	inhibition of AI pressor response, % 1 h	2 h	4 h	6 h
2	10 (iv)	90 ± 1	91 ± 4	83 ± 1	68 ± 9
2	10 (po)	48 ± 11	59 ± 7	36 ± 13	15 ± 12
9	10 (po)	76 ± 2	72 ± 3	25 ± 4	14 ± 1
10	10 (po)	86 ± 3	81 ± 2	83 ± 2	47 ± 16
11	12.1 (po)	74 ± 7	69 ± 4	37 ± 2	19 ± 7
12	12.0 (po)	68 ± 3	75 ± 8	67 ± 8	59 ± 10
13	12.4 (po)	76 ± 5	78 ± 3	70 ± 7	72 ± 6
14	13.1 (po)	64 ± 12	78 ± 1	61 ± 8	16 ± 8
15	12.1 (po)	67 ± 14	85 ± 8	81 ± 6	78 ± 2
16	12.4 (po)	76 ± 10	77 ± 2	69 ± 17	20 ± 6
17	12.7 (po)	83 ± 4	77 ± 2	77 ± 4	60 ± 7
18	12.7(po)	58 ± 16	51 ± 15	33 ± 21	29 ± 15
19	13.1 (po)	72 ± 2	74 ± 2	54 ± 12	28 ± 5
20	12.8 (po)	8 ± 7	17 ± 9	14 ± 11	19 ± 13
21	13.8 (po)	54 ± 6	60 ± 12	40 ± 7	28 ± 12
22	12.5 (po)	81 ± 2	77 ± 3	70 ± 7	34 ± 12
23	12.8 (po)	70 ± 1	63 ± 5	35 ± 10	18 ± 4
24	13.3 (po)	10 ± 6	11 ± 9	8 ± 5	12 ± 6
25	13.0 (po)	34 ± 25	33 ± 25	55 ± 13	38 ± 11
26	12.8 (po)	47 ± 15	71 ± 4	56 ± 12	39 ± 14
27	13.1 (po)	82 ± 6	85 ± 4	76 ± 6	69 ± 18
28	13.1 (po)	27 ± 15	16 ± 13	25 ± 10	
29	13.7 (po)	57 ± 9	49 ± 9	38 ± 11	27 ± 14
30	14.1 (po)	−3 ± 11	8 ± 6		
31	14.5 (po)	57 ± 7	53 ± 11	34 ± 3	12 ± 5
32	14.4 (po)	65 ± 7	55 ± 6	50 ± 5	26 ± 3
33	13.1 (po)	76 ± 15	75 ± 11	58 ± 17	54 ± 23
34	11.7 (po)	97 ± 3	90 ± 2	87 ± 3	78 ± 5
35	12.4 (po)	88 ± 2	76 ± 1	73 ± 2	70 ± 2
36	12.3 (po)	90 ± 2	80 ± 3	72 ± 3	68 ± 3
37	11.7 (po)	81 ± 1	91 ± 0	76 ± 10	52 ± 15
38	11.7 (po)	73 ± 4	81 ± 4	79 ± 5	77 ± 4
39	12.8 (po)	75 ± 6	70 ± 5	65 ± 4	58 ± 7
40	13.7 (po)	88 ± 3	86 ± 7	73 ± 2	64 ± 12
41	13.1(po)	85 ± 3	84 ± 2	81 ± 2	64 ± 12
42	13.2(po)	99 ± 1	97 ± 2	87 ± 6	63 ± 5
43	12.8 (po)	64 ± 4	52 ± 7	31 ± 6	16 ± 5
44	13.1 (po)	50 ± 9	35 ± 11	23 ± 13	4 ± 9
45	13.4 (po)	73 ± 6	75 ± 3	63 ± 12	46 ± 22
46	13.5 (po)	79 ± 6	79 ± 5	70 ± 5	61 ± 12
47	14.5 (po)	88 ± 3	78 ± 6	82 ± 1	73 ± 7
48	14.3 (po)	45 ± 8	30 ± 13	36 ± 6	10 ± 5
49	14.4 (po)	29 ± 14	37 ± 12	36 ± 9	26 ± 10
50	14.3 (po)	78 ± 6	75 ± 6	67 ± 6	60 ± 6
51	14.3 (po)	79 ± 4	75 ± 6	70 ± 5	55 ± 9
52	13.5 (po)	24 ± 3	28 ± 3	25 ± 10	21 ± 4
captopril	10 (po)	100 ± 0	78 ± 1	36 ± 12	13 ± 12
benazepril	10 (po)	100 ± 0	77 ± 11	39 ± 11	16 ± 12

Nicolet 5SXB FTIR spectrometer and are reported in cm^{-1}. Proton NMR spectra were recorded on a Bruker AC-250, Varian XL-300, or Varian XL-400 spectrometer with tetramethylsilane as the internal standard. The chemical shifts (δ) are reported in parts per million (ppm). Mass spectra were recorded on a Hewlett-Packard GC/MS 5985 spectrometer using chemical ionization (DCI) or on a Vestec 201 spectrometer using thermospray technology (TSP). Microanalyses were carried out at Robertson Laboratory Inc., Madison, NJ. Flash chromatography under a nitrogen atmosphere on silica gel 60 (0.04−0.06 mm) (Baker) was used for compound purification. The amino acids used were commercially available or prepared according to literature procedures. All organic solvents used were of anhydrous grade. The chemical yields are not optimized.

General Procedure for the Preparation of Compounds 4. To a stirred solution of the amino acid ester **3** (1 equiv) and Boc-cycloleucine (1 equiv) in dichloromethane were added 1,3-dicyclohexylcarbodiimide (1 equiv) and 1-hydroxybenzotriazole (1 equiv). The mixture was stirred for 16−24 h, and then the solid precipitate was removed by filtration. The organic phase was washed with a saturated solution of sodium bicarbonate and brine and dried over magnesium sulfate. The mixture was then filtered and concentrated under reduced pressure to give a white foam. The foam was purified by flash chromatography on silica gel, eluting with hexane−ethyl acetate. The pure fractions were combined and concentrated.

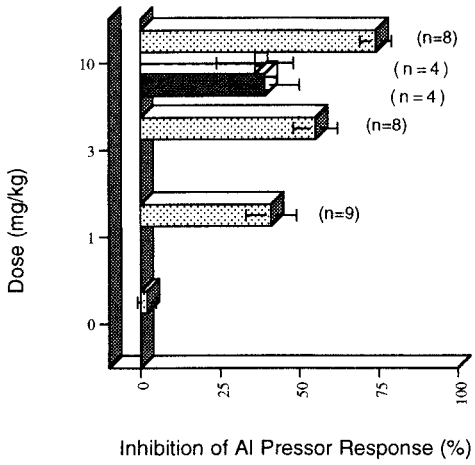


Figure 3. Dose−response inhibition of AI pressor response 4 h after oral administration of **38** (dotted bar), captopril (open bar), and benazepril (solid bar).

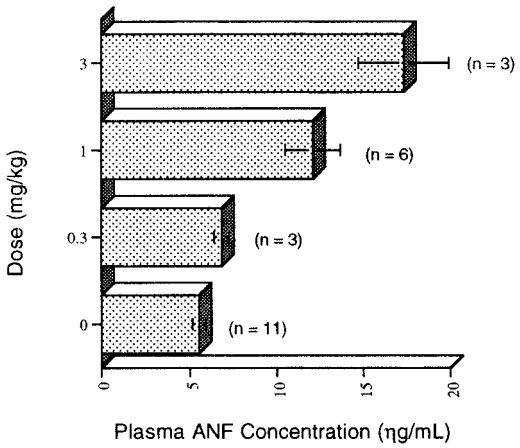


Figure 4. Plasma NEP inhibition at 4 h after oral administration of **38**.

4a (R = H, R^1 = Me): white foam (61%); ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H), 1.72 (m, 6H), 2.22 (m, 2H), 3.04 (d, 2H), 3.76 (s, 3H), 4.72 (bs, 1H), 4.80 (q, 1H), 6.78 (d, 2H), 7.02 (d, 2H).

4b (R = H, R^1 = Et): white foam (62%); ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H), 1.43 (s, 9H), 1.69 (m, 6H), 2.17 (m, 2H), 3.00 (d, 2H), 4.11 (q, 2H), 4.70 (m, 2H), 6.69 (d, 2H), 6.95 (d, 2H).

4c (R = H, R^1 = Pr): white foam (91%); ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H), 1.39 (s, 9H), 1.61 (m, 8H), 2.20 (m, 2H), 3.01 (d, 2H), 4.07 (q, 2H), 4.70 (m, 2H), 6.71 (d, 2H), 7.02 (d, 2H).

4d (R = H, R^1 = allyl): white foam (47%); ^{1}H NMR (DMSO) δ 1.37 (s, 9H), 1.55 (m, 6H), 1.95 (m, 2H), 2.87 (d, 2H), 4.50 (m, 3H), 5.22 (dd, 2H), 5.80 (dt, 1H), 6.60 (d, 2H), 6.90 (d, 2H), 7.00 (bs, 1H), 7.47 (d, 1H), 9.20 (bs, 1H).

4e (R = H, R^1 = butyl): white foam (52%); ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H), 1.15 (m, 2H), 1.35 (s, 9H), 1.70 (m, 2H), 1.78 (m, 6H), 2.14 (m, 2H), 3.01 (d, 2H), 4.08 (q, 2H), 4.75 (m, 2H), 6.23 (bs, 1H), 6.72 (d, 2H), 6.95 (d, 2H), 7.02 (bs, 1H).

4f (R = H, R^1 = hexyl): white foam (68%); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.21 (m, 6H), 1.33 (s, 9H), 1.73 (m, 8H), 2.16 (m, 2H), 3.00 (d, 2H), 4.02 (q, 2H), 4.75 (m, 2H), 6.23 (bs, 1H), 6.72 (d, 2H), 6.99 (d, 2H), 7.02 (bs, 1H).

4g (R = H, R^1 = isopropyl): white foam (55%); ^{1}H NMR ($CDCl_3$) δ 1.23 (dd, 6H), 1.41 (s, 9H), 1.70 (m, 6H), 2.19 (m, 2H), 3.00 (d, 2H), 4.71 (q, 1H), 4.83 (bs, 1H), 4.95 (m, 1H), 6.49 (bs, 1H), 6.69 (d, 2H), 6.98 (d, 2H).

4h (R = H, R^1= $CH_2CH(Me)_2$): white foam (56%); ^{1}H NMR ($CDCl_3$) δ 0.91 (d, 6H), 1.39 (s, 9H), 1.64 (m, 7H), 2.22 (m, 2H), 3.02 (d, 2H), 4.11 (q, 2H), 4.74 (q, 1H), 5.19 (bs, 1H), 6.69 (d, 2H), 7.01 (d, 2H).

Table 3. Plasma ANF Concentration in the Conscious Rat (Percent of Control)

		percent of control			
compd	dose, mg/kg	0.5 h	1 h	2 h	4 h
13	12.4(po)	131 ± 7	133 ± 15	178 ± 11	185 ± 14
17	12.7 (po)	178 ± 31	235 ± 74	236 ± 58	251 ± 60
34	11.7 (po)	279 ± 30	337 ± 17	263 ± 19	342 ± 16
38	11.7 (po)	275 ± 17	346 ± 54	250 ± 29	251 ± 35
40	13.7 (po)	159 ± 11	187 ± 5	215 ± 9	264 ± 12
41	13.1 (po)	107 ± 7	111 ± 9	143 ± 12	181 ± 11
42	13.2 (po)	223 ± 8	267 ± 15	260 ± 19	332 ± 29
46	13.5 (po)	203 ± 7	213 ± 18	252 ± 23	223 ± 24
50	14.3 (po)	246 ± 6	234 ± 4	246 ± 4	277 ± 7
51	14.3 (po)	165 ± 15	193 ± 26	183 ± 21	159 ± 10
acetorphan	10 (po)	209 ± 9	191 ± 9	173 ± 1	106 ± 9

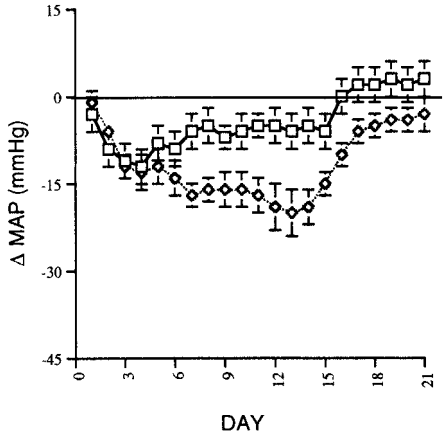


Figure 5. Benazepril (□) and compound **38** (◇) in the SHR at 1 mg/kg po × bid.

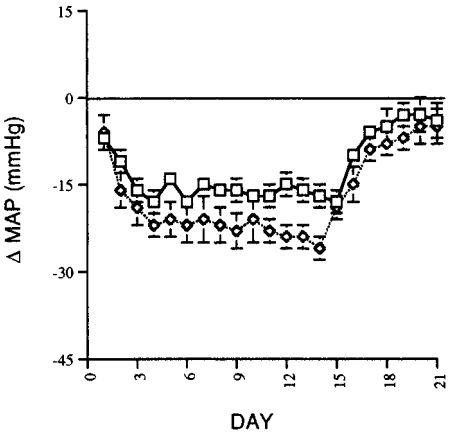


Figure 6. Benazepril (□) and compound **38** (◇) in the SHR at 3 mg/kg po × bid.

4i (R = H, R^1= $(CH_2)_2CH(Me)_2$): white foam (64%); ^{1}H NMR ($CDCl_3$) δ 0.87 (d, 6H), 1.39 (s, 9H), 1.70 (m, 9H), 2.19 (m, 2H), 3.00 (d, 2H), 4.09 (q, 2H), 4.72 (q, 1H), 5.12 (bs, 1H), 6.71 (d, 2H), 6.97 (d, 2H).

4j (R = H, R^1 = cyclopentyl): white foam (51%); ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H), 1.70 (m, 14H), 2.19 (m, 2H), 2.98 (d, 2H), 4.69 (q, 1H), 4.89 (bs, 1H), 5.12 (m, 1H), 6.69 (d, 2H), 6.97 (d, 2H).

4k (R = H, R^1 = benzyl): white foam (48%); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H), 1.68 (m, 6H), 2.22 (m, 2H), 3.00 (d, 2H), 4.81 (q, 1H), 5.11 (dd, 2H), 6.68 (d, 2H), 6.85 (d, 2H), 7.32 (m, 5H).

4l (R = Me, R^1 = Me): white foam (66%); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H), 1.73 (m, 6H), 2.14 (m, 2H), 3.02 (d, 2H), 3.70 (s, 3H), 3.79 (s, 3H), 4.80 (m, 2H), 6.80 (d, 2H), 7.02 (d, 2H).

4m (R = Pr, R^1= Et): white foam (78%); ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H), 1.15 (t, 3H), 1.35 (s, 9H), 1.70 (m, 8H), 2.15 (m, 2H), 2.98 (d, 2H), 3.31 (t, 2H), 4.05 (q, 2H), 4.70 (m, 2H), 6.72 (d, 2H), 6.95 (d, 2H).

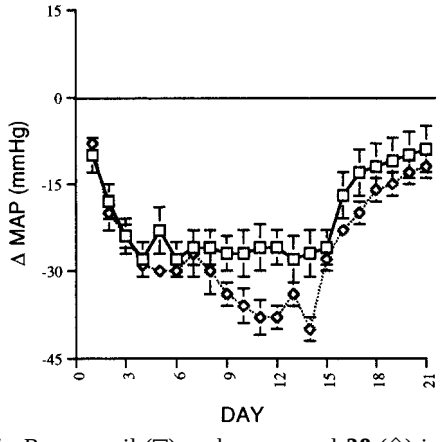


Figure 7. Benazepril (□) and compound **38** (◇) in the SHR at 10 mg/kg po × bid.

4n (R = Me, R^1 = Et): white foam (70%); ^{1}H NMR ($CDCl_3$) δ 1.11 (t, 3H), 1.39 (s, 9H), 1.69 (m, 6H), 2.19 (m, 2H), 3.03 (d, 2H), 3.73 (s, 3H), 4.11 (q, 2H), 4.73 (m, 2H), 6.72 (d, 2H), 7.04 (d, 2H).

4o (R = Me, R^1 = Bu): white foam (64%); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H), 1.31 (m, 2H), 1.46 (s, 9H), 1.60 (m, 2H), 1.73 (m, 6H), 2.21 (m, 2H), 3.05 (d, 2H), 3.75 (s, 3H), 4.05 (q, 2H), 4.75 (q, 1H), 6.79 (d, 2H), 7.07 (d, 2H).

4p (R = Me, R^1 = $CH_2CON(Et)_2$): white foam (81%); ^{1}H NMR ($CDCl_3$) δ 1.12 (t, 3H), 1.18 (t, 3H), 1.38 (s, 9H), 1.70 (m, 6H), 2.20 (m, 2H), 3.15 (m, 2H), 3.21 (q, 2H), 3.38 (q, 2H), 3.75 (s, 3H), 4.71 (m, 1H), 4.74 (d, 1H), 4.87 (d, 1H), 6.79 (d, 2H), 7.04 (bs, 1H), 7.18 (d, 2H).

4q (R = Me, R^1 = benzyl): white foam (66%); ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H), 1.70 (m, 6H), 2.20 (m, 2H), 3.02 (d, 2H), 3.75 (s, 3H), 4.70 (bs, 1H), 4.82 (q, 1H), 5.12 (d, 2H), 6.71 (d, 2H), 6.95 (d, 2H), 7.05 (bs, 1H), 7.32 (m, 5H).

4r (R = Me, R^1 = CH_2(3-pyridyl)): white foam (60%); ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H), 1.78 (m, 6H), 2.20 (m, 2H), 3.02 (d, 2H), 3.77 (s, 3H), 4.78 (bs, 1H), 4.81 (q, 1H), 5.12 (q, 2H), 6.74 (d, 2H), 6.97 (d, 2H), 7.19 (bs, 1H), 7.33 (dd, 1H), 7.63 (d, 1H), 8.56 (d, 1H), 8.60 (dd, 1H).

General Procedure for the Preparation of Compounds 6. The purified Boc-protected dipeptide **4** was dissolved in dichloromethane, and dry HCl gas was bubbled through the solution for 15 min. The solvent was removed under reduced pressure to yield a white foam. The compound was used without further purification. To a solution of the dipeptide hydrochloride **5** (1 equiv) in dichloromethane was added triethylamine (1 equiv). The mixture was stirred for 5 min, and then the (*S*)-2-(acetylthio)-3-methylbutanoic acid (1 equiv), 1,3-dicyclohexylcarbodiimide (1 equiv), and 1-hydroxy-7-azabenzotriazole (1 equiv) were added. The mixture was stirred for 16 h, and then the solid precipitate was removed by filtration. The organic phase was washed with a saturated solution of sodium bicarbonate and brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give a white foam. The foam was purified by flash chromatography on silica gel, eluting with hexane−ethyl

acetate. The pure fractions were combined and concentrated under reduced pressure.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine methyl ester (9):** white solid (55%); mp 157−158 °C; IR (KBr) 3373, 2958, 1748, 1679, 1657, 1614, 1516, 1227 cm^{-1}; ^1H NMR δ 1.01 (dd, 6H), 1.70 (m, 4H), 1.90 (m, 2H), 2.20 (m, 3H), 2.31 (s, 3H), 3.00 (dd, 2H), 3.62 (d, 1H), 4.69 (q, 1H), 6.36 (bs, 1H), 6.68 (d, 2H), 6.95 (d, 2H), 7.06 (d, 1H); MS (CDI, CH_4) *m*/*z* 465 (MH^+). Anal. ($C_{23}H_{32}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine ethyl ester (10):** white solid (45%); mp 148−149 °C; IR (KBr) 3380, 2963, 1741, 1682, 1649, 1615, 1515, 1223, 1201, 1173, 1122 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (dd, 6H), 1.15 (t, 3H), 1.68 (m, 4H), 1.93 (m, 2H), 2.17 (m, 3H), 2.32 (s, 3H), 3.00 (dd, 2H), 3.64 (d, 1H), 4.11 (q, 2H), 4.69 (q, 1H), 6.29 (s, 1H), 6.37 (s, 1H), 6.70 (d, 2H), 6.96 (d, 2H), 7.11 (d, 1H); MS (CDI, CH_4) *m*/*z* 479 (MH^+). Anal. ($C_{24}H_{34}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine propyl ester (11):** white solid (48%); mp 119−120 °C; IR (KBr) 3261, 2966, 1741, 1682, 1669, 1650, 1515, 1215, 1200 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H), 1.00 (dd, 6H), 1.68 (m, 6H), 1.93 (m, 2H), 2.22 (m, 3H), 2.32 (s, 3H), 2.99 (dd, 2H), 3.64 (d, 1H), 4.01 (q, 2H), 4.72 (q, 1H), 6.39 (bs, 1H), 6.47 (s, 1H), 6.68 (d, 2H), 6.92 (d, 2H), 7.05 (d, 1H); MS (CDI, CH_4) *m*/*z* 493 (MH^+). Anal. ($C_{25}H_{36}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine allyl ester (12):** white solid (36%); mp 119−120 °C; IR (KBr) 3361, 2969, 1749, 1675, 1637, 1514, 1229, 1188 cm^{-1}; ^1H NMR δ 1.00 (dd, 6H), 1.69 (m, 4H), 1.92 (m, 2H), 2.22 (m, 3H), 2.32 (s, 3H), 3.00 (dd, 2H), 3.62 (d, 1H), 4.55 (d, 2H), 4.71 (q, 1H), 5.21 (dd, 2H), 5.81 (dt, 1H), 6.32 (bs, 1H), 6.70 (d, 2H), 6.97 (d, 2H), 7.11 (d, 1H); MS (CDI, CH_4) *m*/*z* 491 (MH^+). Anal. ($C_{25}H_{34}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine butyl ester (13):** white solid (45%); mp 85−86 °C; IR (KBr) 3334, 2963, 1739, 1682, 1649, 1616, 1515, 1225 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H), 1.00 (dd, 6H), 1.33 (m, 2H), 1.60 (m, 6H), 1.90 (m, 2H), 2.20 (m, 3H), 2.32 (s, 3H), 3.00 (dd, 2H), 3.62 (d, 1H), 4.00 (dd, 2H), 4.69 (q, 1H), 6.30 (bs, 2H), 6.70 (d, 2H), 6.94 (d, 2H), 7.08 (d, 1H); MS (CDI, CH_4) *m*/*z* 507 (MH^+). Anal. ($C_{26}H_{38}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine hexyl ester (14):** white solid (67%); mp 65−66 °C; IR (KBr) 3379, 2960, 1674, 1653, 1615, 1515, 1268, 1224, 1197 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (t, 3H), 1.00 (dd, 6H), 1.30 (m, 6H), 1.70 (m, 6H), 1.92 (m, 2H), 2.23 (m, 3H), 2.32 (s, 3H), 3.00 (dd, 2H), 3.61 (d, 1H), 4.03 (q, 2H), 4.72 (q, 1H), 5.62 (s, 1H), 6.36 (s, 1H), 6.69 (d, 2H), 6.91 (d, 2H), 7.11 (d, 1H); MS (CDI, CH_4) *m*/*z* 535 (MH^+). Anal. ($C_{28}H_{42}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine isopropyl ester (15):** white solid (36%); mp 155−156 °C; IR (KBr) 3382, 3253, 1736, 1516, 1448, 1373, 1218, 1109 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (dd, 6H), 1.15 (dd, 6H), 1.70 (m, 4H), 1.90 (m, 2H), 2.20 (m, 3H), 2.37 (s, 3H), 3.00 (dd, 2H), 3.55 (d, 1H), 4.69 (q, 1H), 4.95 (pentet, 1H), 5.80 (s, 1H), 6.36 (s, 1H), 6.72 (d, 2H), 6.96 (d, 2H), 7.04 (d, 1H); MS (CDI, CH_4) *m*/*z* 493 (MH^+). Anal. ($C_{25}H_{36}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine isobutyl ester (16):** white solid (43%); mp 148−149 °C; IR (KBr) 3297, 2963, 1740, 1615, 1653, 1447, 1200, 1175 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (d, 6H), 0.95 (dd, 6H), 1.68 (m, 4H), 1.95 (m, 3H), 2.18 (m, 3H), 2.35 (s, 3H), 2.96 (dd, 2H), 3.62 (d, 1H), 3.82 (dd, 2H), 4.72 (q, 1H), 6.35 (s, 1H), 6.67 (m, 3H), 6.95 (d, 2H), 7.06 (d, 1H); MS (CDI, CH_4) *m*/*z* 507 (MH^+). Anal. ($C_{26}H_{38}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine 3-methylbutyl ester (17):** white solid (57%); mp 94−95 °C; IR (KBr) 3413, 2963, 1735, 1689, 1515, 1200, 1172 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (d, 6H), 0.98 (dd, 6H), 1.65 (m, 7H), 1.94 (m, 2H), 2.18 (m, 3H), 2.32 (s, 3H), 2.96 (dd, 2H), 3.62 (d, 1H), 4.05 (q, 2H), 4.77 (q, 1H), 6.11 (bs, 1H), 6.30 (s, 1H), 6.69 (d, 2H), 6.97 (d, 2H), 7.09 (d, 1H); MS (CDI, CH_4) *m*/*z* 521 (MH^+). Anal. ($C_{27}H_{40}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine cyclopentyl ester (18):** white solid (44%); mp 133−135 °C; IR (KBr) 3316, 2963, 1736, 1681, 1653, 1615, 1515, 1268, 1217, 1163 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (dd, 6H), 1.70 (m, 12H), 1.92 (m, 2H), 2.23 (m, 3H), 2.39 (s, 3H), 2.98 (dd, 2H), 3.65 (d, 1H), 4.68 (q, 1H), 5.11 (m, 1H), 6.40 (s, 1H), 6.70 (d, 2H), 6.99 (d, 2H), 7.07 (d, 1H); MS (CDI, CH_4) *m*/*z* 519 (MH^+). Anal. ($C_{27}H_{38}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine benzyl ester (19):** white solid (57%); mp 66−67 °C; IR (KBr) 3372, 2962, 1742, 1675, 1655, 1515, 1225 cm^{-1}; ^1H NMR δ 0.89 (dd, 6H), 1.65 (m, 4H), 1.89 (m, 2H), 2.21 (m, 3H), 2.39 (s, 3H), 2.98 (dd, 2H), 3.62 (d, 1H), 4.73 (q, 1H), 5.05 (dd, 2H), 6.41 (s, 1H), 6.62 (d, 2H), 6.88 (d, 2H), 7.11 (d, 1H), 7.31 (m, 5H); MS (CDI, CH_4) *m*/*z* 541 (MH^+). Anal. ($C_{29}H_{36}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine methyl ester (34):** white solid (58%); mp 118−120 °C; IR (KBr) 1756, 1743, 1697, 1688, 1658, 1650, 1512, 1247 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 (d, 3H), 1.02 (d, 3H), 1.68 (m, 4H), 1.92 (m, 3H), 2.22 (m, 2H), 2.36 (s, 3H), 3.04 (m, 2H), 3.62 (d, 1H), 3.67 (s, 3H), 3.78 (s, 3H), 4.75 (q, 1H), 6.30 (s, 1H), 6.80 (d, 2H), 7.06 (d, 2H), 7.12 (d, 1H); MS (CDI, CH_4) *m*/*z* 479 (MH^+). Anal. ($C_{24}H_{34}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-ethyl-L-tyrosine ethyl ester (35):** white solid (61%); mp 102−103 °C; IR (KBr) 3290, 1731, 1673, 1650, 1512, 1246, 1197 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (dd, 6H), 1.10 (t, 3H), 1.30 (t, 3H), 1.60 (m, 4H), 1.92 (m, 2H), 2.19 (m, 3H), 2.30 (s, 3H), 3.04 (dd, 2H), 3.59 (d, 1H), 3.89 (q, 2H), 4.05 (q, 2H), 4.62 (q, 1H), 6.22 (s, 1H), 6.70 (d, 2H), 6.99 (m, 3H); MS (CDI, CH_4) *m*/*z* 507 (MH^+). Anal. ($C_{26}H_{38}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-propyl-L-tyrosine ethyl ester (36):** white solid (17%); mp 116 °C; IR (KBr) 3300, 2963, 1743, 1695, 1659, 1640, 1610, 1528, 1512, 1243, 1186 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (m, 9H), 1.19 (t, 3H), 1.75 (m, 8H), 2.22 (m, 3H), 2.34 (s, 3H), 3.02 (m, 2H), 3.64 (d, 1H), 3.85 (t, 2H), 4.10 (q, 2H), 4.70 (q, 1H), 6.30 (s, 1H), 6.78 (d, 2H), 7.04 (m, 3H); MS (CDI, CH_4) *m*/*z* 521 (MH^+). Anal. ($C_{27}H_{40}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine ethyl ester (38):** white solid (42%); mp 102−103 °C; IR (KBr) 2962, 1742, 1695, 1675, 1652, 1513, 1247, 1102 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (dd, 6H), 1.21 (t, 3H), 1.70 (m, 4H), 1.90 (m, 2H), 2.25 (m, 3H), 2.36 (s, 3H), 3.02 (m, 2H), 3.62 (d, 1H), 3.80 (s, 3H), 4.08 (q, 2H), 4.72 (q, 1H), 6.30 (bs, 1H), 6.79 (d, 2H), 7.04 (m, 3H); MS (CDI, CH_4) *m*/*z* 493 (MH^+). Anal. ($C_{25}H_{36}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine butyl ester (39):** white solid (36%); mp 114−115 °C; IR (KBr) 1738, 1696, 1657, 1640, 1528, 1245, 1202 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, 3H), 1.00 (dd, 6H), 1.30 (m, 2H), 1.64 (m, 6H), 1.95 (m, 2H), 2.20 (m, 3H), 3.00 (dd, 2H), 3.60 (d, 1H), 3.75 (s, 3H), 4.05 (q, 2H), 4.71 (q, 1H), 6.30 (bs, 1H), 6.67 (d, 2H), 7.04 (d, 3H); MS (CDI, CH_4) *m*/*z* 521 (MH^+). Anal. ($C_{27}H_{40}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine 2-(diethylamino)-2-oxoethyl ester (40):** white solid (19%); mp 106−107 °C; IR (KBr) 3394, 1768, 1673, 1646, 1573, 1534, 1247, 1148 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (dd, 6H), 1.15 (t, 3H), 1.20 (t, 3H), 1.67 (m, 4H), 1.91 (m, 2H), 2.18 (m, 3H), 2.33 (s, 3H), 3.13 (m, 2H), 3.22 (q, 2H), 3.39 (q, 2H), 3.65 (d, 1H), 3.76 (s, 3H), 4.72 (dd, 2H), 4.82 (m, 1H), 6.34 (s, 1H), 6.78 (d, 2H), 7.06 (d, 1H), 7.15 (d, 2H); MS (CDI, CH_4) *m*/*z* 578 (MH^+). Anal. ($C_{29}H_{43}N_3O_7S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine benzyl ester (41):** white solid (19%); mp 110−111 °C; IR (KBr) 3297, 1741, 1695, 1660, 1640, 1527, 1513, 1248, 1168 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (dd, 6H), 1.68 (m, 4H), 1.94 (m, 2H), 2.20 (m, 3H), 2.33 (s, 3H), 3.00 (d, 2H), 3.60 (d, 1H), 3.75 (s, 3H), 4.77 (q, 1H), 5.07 (m, 2H), 6.28 (s, 1H), 6.72 (d, 2H), 6.94 (d, 2H), 7.08 (d, 1H), 7.24 (m, 2H), 7.33 (m, 3H); MS (CDI, CH_4) *m*/*z* 555 (MH^+). Anal. ($C_{30}H_{38}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine 3-pyridinylmethyl ester (42):** white solid (11%); mp 107−109 °C; IR (KBr) 3340, 1741, 1691, 1674, 1657, 1603, 1513, 1251, 1170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (dd, 6H), 1.68 (m, 4H), 1.90 (m, 3H), 2.20 (m, 2H), 2.35 (s, 3H), 2.99 (d, 2H), 3.67 (d, 1H), 3.76 (s, 3H), 4.74 (q, 1H), 5.05 (m, 2H), 6.34 (s, 1H), 6.73 (d, 2H), 6.98 (d, 2H), 7.20 (d, 1H), 7.30 (m, 1H), 7.56 (d, 1H), 8.58 (m, 2H); MS (CDI, CH_4) *m*/*z* 556 (MH^+). Anal. ($C_{29}H_{37}N_3O_6S$) C, H, N.

General Procedure for the Preparation of Compounds 7 (R ≠ H). To a stirred solution of the dipeptide **6** (1 equiv) in dichloromethane were added an acid chloride or a chloroformate (1 equiv) and triethylamine (1 equiv). The mixture was stirred for 5 h and then washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give a white foam. The foam was purified by flash chromatography on silica gel, eluting with hexane−ethyl acetate. The pure fractions were combined and concentrated under reduced pressure.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-acetyl-L-tyrosine ethyl ester (43):** white solid (68%); mp 94−95 °C; IR (KBr) 3296, 2964, 1762, 1733, 1673, 1651, 1525, 1508, 1197 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (dd, 6H), 1.15 (t, 3H), 1.68 (m, 4H), 1.89 (m, 3H), 2.20 (m, 2H), 2.35 (s, 3H), 2.40 (s, 3H), 3.03 (d, 2H), 3.62 (d, 1H), 4.12 (q, 2H), 4.72 (q, 1H), 6.31 (bs, 1H), 6.91 (d, 2H), 7.12 (m, 3H); MS (CDI, CH_4) *m*/*z* 521 (MH^+). Anal. ($C_{26}H_{36}N_2O_7S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-(1-oxopropyl)-L-tyrosine ethyl ester (44):** white solid (50%); mp 102−103 °C; IR (KBr) 3255, 1760, 1744, 1695, 1659, 1527, 1201, 1141 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (dd, 6H), 1.19 (t, 3H), 1.29 (t, 3H), 1.70 (m, 4H), 1.89 (m, 2H), 2.22 (m, 3H), 2.32 (s, 3H), 2.58 (q, 2H), 3.05 (dd, 2H), 3.62 (d, 1H), 4.10 (q, 2H), 4.70 (q, 1H), 6.30 (bs, 1H), 6.94 (d, 2H), 7.05 (d, 1H), 7.10 (d, 2H); MS (CDI, CH_4) *m*/*z* 535 (MH^+). Anal. ($C_{27}H_{38}N_2O_7S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-(1-oxo-2-methylpropyl)-L-tyrosine ethyl ester (45):** white solid (21%); mp 118−119 °C; IR (KBr) 3293, 1757, 1749, 1697, 1660, 1528, 1135 cm^{-1}; ^1H NMR (DMSO) δ 0.89 (dd, 6H), 1.05 (t, 3H), 1.19 (d, 6H), 1.60 (m, 4H), 1.83 (m, 3H), 2.00 (m, 2H), 2.20 (s, 3H), 2.70 (pentet, 1H), 2.91 (d, 2H), 3.96 (m, 3H), 4.43 (q, 1H), 6.99 (d, 2H), 7.19 (d, 2H), 7.36 (d, 1H), 8.32 (s, 1H); MS (CDI, CH_4) *m*/*z* 549 (MH^+). Anal. ($C_{28}H_{40}N_2O_7S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-(2-methoxy-1-oxoethyl)-L-tyrosine ethyl ester (46):** white solid (45%); mp 80−81 °C; IR (KBr) 3399, 3296, 2962, 1777, 1732, 1673, 1650, 1518, 1197 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (dd, 6H), 1.21 (t, 3H), 1.29 (t, 3H), 1.67 (m, 4H), 1.89 (m, 2H), 2.19 (m, 3H), 2.30 (s, 3H), 3.05 (dd, 2H), 3.51 (s, 3H), 3.63 (d, 1H), 4.11 (q, 2H), 4.22 (s, 2H), 4.72 (q, 1H), 6.31 (bs, 1H), 6.97 (d, 2H), 7.08 (d, 1H), 7.13 (d, 2H); MS (CDI, CH_4) *m*/*z* 551 (MH^+). Anal. ($C_{27}H_{38}N_2O_8S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-(3-ethoxy-1,3-dioxopropyl)-L-tyrosine ethyl ester (47):** white solid (15%); mp 71−72 °C; IR (KBr) 3291, 1771, 1741, 1697, 1658, 1530, 1188, 1136 cm^{-1}; ^1H NMR (DMSO) δ 0.79 (t, 6H), 1.05 (t, 3H), 1.21 (t, 3H), 1.70 (m, 4H), 1.87 (m, 3H), 2.12 (m, 2H), 2.25 (s, 3H), 2.94 (d, 2H), 3.71 (s, 2H), 3.96 (m, 3H), 4.20 (q, 2H), 4.40 (q, 1H), 7.00 (d, 2H), 7.22 (d, 2H), 7.39 (d, 1H), 8.31 (s, 1H); MS (CDI, CH_4) *m*/*z* 593 (MH^+). Anal. ($C_{29}H_{40}N_2O_9S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-benzoyl-L-tyrosine ethyl ester (48):** white solid (53%); mp 121−122 °C; IR (KBr) 3308, 2976, 1744, 1736, 1695, 1679, 1638, 1591, 1535, 1273, 1195, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (dd, 6H), 1.09 (t, 3H), 1.69 (m, 4H), 1.80 (m, 2H), 2.20 (m, 3H), 2.33 (s, 3H), 3.07 (d, 2H), 3.65 (d, 1H), 4.12 (q, 2H), 4.73 (q, 1H), 6.32 (bs, 1H), 7.10 (d, 2H), 7.20 (m, 3H), 7.49 (t, 2H), 7.60 (t, 1H), 8.19 (d, 2H); MS (CDI, CH_4) *m*/*z* 583 (MH^+). Anal. ($C_{31}H_{38}N_2O_7S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-(2-thienylcarbonyl)-L-tyrosine ethyl ester (49):** white solid (52%); mp 125−126 °C; IR (KBr) 3308, 2960, 1730, 1694, 1674, 1643, 1505, 1273, 1196 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (dd, 6H), 1.21 (t, 3H), 1.69 (m, 4H), 1.92 (m, 2H), 2.21 (m, 3H), 2.31 (s, 3H), 3.08 (dd, 2H), 3.62 (d, 1H), 4.10 (q, 2H), 4.72 (q, 1H), 6.32 (bs, 1H), 7.15 (m, 5H), 7.62 (d, 1H), 7.92 (d, 1H); MS (CDI, CH_4) *m*/*z* 589 (MH^+). Anal. ($C_{29}H_{36}N_2O_7S_2$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-(3-pyridinylcarbonyl)-L-tyrosine ethyl ester (50):** white solid (40%); mp 67−68 °C; IR (KBr) 3397, 3298, 2964, 1744, 1672, 1652, 1590, 1508, 1276, 1197 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (dd, 6H), 1.17 (t, 3H), 1.69 (m, 4H), 1.87 (m, 2H), 2.20 (m, 3H), 2.32 (s, 3H), 3.10 (d, 2H), 3.65 (d, 1H), 4.12 (q, 2H), 4.75 (q, 1H), 6.31 (bs, 1H), 7.08 (d, 2H), 7.21 (m, 3H), 7.41 (dd, 1H), 8.42 (dt, 1H), 8.81 (d, 1H), 9.39 (s, 1H); MS (CDI, CH_4) *m*/*z* 584 (MH^+). Anal. ($C_{30}H_{37}N_3O_7S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-(4-pyridinylcarbonyl)-L-tyrosine ethyl ester (51):** white solid (48%); mp 85−86 °C; IR (KBr) 2920, 1744, 1672, 1508, 1272, 1196 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (dd, 6H), 1.19 (t, 3H), 1.68 (m, 4H), 1.89 (m, 2H), 2.20 (m, 3H), 2.32 (s, 3H), 3.09 (dd, 2H), 3.62 (d, 1H), 4.11 (q, 2H), 4.73 (q, 1H), 6.32 (bs, 1H), 7.09 (d, 2H), 7.15 (d, 3H), 8.00 (d, 2H), 8.69 (d, 2H); MS (CDI, CH_4) *m*/*z* 584 (MH^+). Anal. ($C_{30}H_{37}N_3O_7S$) C, H, N.

***N*-[[1-[[2(*S*)-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-(ethoxycarbonyl)-L-tyrosine ethyl ester (52):** white solid (68%); mp 94−95 °C; IR (KBr) 3420, 3271, 1749, 1697, 1673, 1647, 1505, 1259, 1216 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (dd, 6H), 1.20 (t, 3H), 1.30 (t, 3H), 1.70 (m, 4H), 1.87 (m, 2H), 2.20 (m, 3H), 2.30 (s, 3H), 3.07 (dd, 2H), 3.60 (d, 1H), 4.10 (q, 2H), 4.25 (q, 2H), 4.74 (q, 1H), 6.32 (bs, 1H), 7.01 (d, 2H), 7.11 (d, 2H); MS (CDI, CH_4) *m*/*z* 551 (MH^+). Anal. ($C_{27}H_{38}N_2O_8S$) C, H, N.

General Procedure of the Preparation of Compounds 8. To a stirred solution of the α-thioacetate dipeptide **6** (1 equiv) in degassed methanol was added 1N sodium hydroxide (1 equiv). The mixture was stirred for 45 min and then concentrated. The residue was dissolved in ethyl acetate, washed with brine, dried over magnesium sulfate, and filtered, and the solvent was removed to give the free thiol **6a** which was used without further purification.

When R = H. The residue was dissolved in dichloromethane, and a carboxylic acid (1 equiv), 1,3-dicyclohexylcarbodiimide (1 equiv), 1-hydroxy-7-azabenzotriazole (1 equiv), and triethylamine (1 equiv) were added. The mixture was stirred overnight, filtered, washed with brine, and dried over magnesium sulfate. The solution was filtered and concentrated under vacuum to give a foam. The foam was purified by flash chromatography on silica gel, eluting with hexane−ethyl acetate. The pure fractions were combined and concentrated under reduced pressure.

***N*-[[1-[[2(*S*)-(1,1,1-Trimethylacetyl)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine ethyl ester (20):** white solid (31%); mp 150−151 °C; IR (KBr) 3409, 3262, 2968, 1745, 1660, 1647, 1516, 1225 cm^{-1}; ^1H NMR (DMSO) δ 0.93 (dd, 6H), 1.08 (t, 3H), 1.28 (s, 9H), 1.70 (m, 4H), 1.85 (m, 3H), 2.11 (m, 2H), 2.73 (d, 2H), 3.97 (m, 3H), 4.35 (q, 1H), 6.62 (d, 2H), 6.88 (d, 2H), 6.98 (d, 2H), 7.23 (d, 1H), 8.27 (bs, 1H), 9.21 (bs, 1H); MS (CDI, CH_4) *m*/*z* 521 (MH^+). Anal. ($C_{27}H_{40}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-[(4-Morpholinoacetyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine ethyl ester (21):** white solid (24%); mp 147−148 °C; IR (KBr) 3327,

1745, 1666, 1649, 1515, 1243, 1116 cm^{-1}: ^1H NMR ($CDCl_3$) δ 1.00 (dd, 6H), 1.18 (t, 3H), 1.66 (m, 6H), 2.20 (m, 3H), 2.55 (m, 4H), 3.00 (dd, 2H), 3.31 (d, 2H), 3.62 (d, 1H), 3.73 (m, 4H), 4.11 (q, 2H), 4.71 (q, 1H), 5.73 (s, 1H), 6.31 (s, 1H), 6.71 (d, 2H), 6.99 (d, 2H), 7.11 (d, 1H); MS (CDI, CH_4) *m/z* 564 (MH^+). Anal. ($C_{28}H_{41}N_3O_7S$) C, H, N.

***N*-[[1-[[2(*S*)-[(Methoxyacetyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine ethyl ester (22):** white solid (30%); mp 129−130 °C; IR (KBr) 3396, 3259, 1746, 1665, 1648, 1615, 1517, 1200 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (dd, 6H), 1.20 (t, 3H), 1.64 (m, 4H), 1.95 (m, 2H), 2.25 (m, 3H), 3.00 (dd, 2H), 3.49 (s, 3H), 3.68 (d, 1H), 4.05 (m, 4H), 4.70 (q, 1H), 5.55 (s, 1H), 6.32 (bs, 1H), 6.66 (d, 2H), 6.97 (d, 2H), 7.10 (d, 1H); MS (CDI, CH_4) *m/z* 509 (MH^+). Anal. ($C_{25}H_{36}N_2O_7S$) C, H, N.

***N*-[[1-[[2(*S*)-[(Dimethylacetyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine ethyl ester (23):** white solid (20%); mp 136−137 °C; IR (KBr) 3326, 1746, 1667, 1643, 1515, 1222, 1195 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (dd, 6H), 1.20 (t, 3H), 1.70 (m, 4H), 1.90 (m, 2H), 2.22 (m, 3H), 2.30 (s, 6H), 3.01 (dd, 2H), 3.20 (s, 2H), 3.60 (d, 1H), 4.12 (q, 2H), 4.70 (q, 1H), 6.30 (bs, 1H), 6.35 (bs, 1H), 6.68 (d, 2H), 6.95 (d, 2H), 7.10 (d, 1H); MS (CDI, CH_4) *m/z* 522 (MH^+). Anal. ($C_{26}H_{39}N_3O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-[(2-Pyridinylcarbonyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine ethyl ester (24):** white solid (70%); mp 154−155 °C; IR (KBr) 3399, 2969, 1750, 1667, 1633, 1612, 1540, 1225 cm^{-1}; ^1H NMR (DMSO) δ 0.99 (m, 9H), 1.60 (m, 4H), 1.87 (m, 3H), 2.12 (m, 2H), 2.72 (d, 2H), 3.97 (q, 2H), 4.10 (d, 1H), 4.45 (q, 1H), 6.59 (d, 2H), 6.82 (d, 2H), 7.25 (d, 1H), 7.70 (dd, 1H), 7.79 (d, 1H), 8.05 (t, 1H), 8.47 (bs, 1H), 8.72 (d, 1H), 9.19 (bs, 1H); MS (CDI, CH_4) *m/z* 542 (MH^+). Anal. ($C_{28}H_{35}N_3O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-[(Methoxyacetyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine ethyl ester (25):** white solid (32%); mp 130−131 °C; IR (KBr) 1746, 1647, 1549, 1516, 1222 cm^{-1}; ^1H NMR (DMSO) δ 0.90 (dd, 6H), 1.20 (t, 3H), 1.73 (bm, 17H), 2.80 (d, 2H), 3.01 (pent, 1H), 3.99 (m, 3H), 4.35 (q, 1H), 6.60 (d, 2H), 6.90 (d, 2H), 7.25 (d, 1H), 8.23 (bs, 1H), 9.21 (bs, 1H); MS (CDI, CH_4) *m/z* 533 (MH^+). Anal. ($C_{28}H_{40}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-[(Ethylcarbonyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine butyl ester (26):** white solid (13%); mp 102 °C; IR (KBr) 3252, 2962, 1749, 1673, 1648, 1615, 1515, 1224, 1196 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H), 1.00 (dd, 6H), 1.15 (t, 3H), 1.30 (m, 2H), 1.64 (m, 6H), 1.95 (m, 2H), 2.24 (m, 3H), 2.60 (q, 2H), 3.00 (dd, 2H), 3.65 (d, 1H), 4.05 (m, 2H), 4.75 (q, 1H), 5.51 (bs, 1H), 6.35 (bs, 1H), 6.70 (d, 2H), 7.00 (d, 2H), 7.13 (d, 1H); MS (CDI, CH_4) *m/z* 521 (MH^+). Anal. ($C_{27}H_{40}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-[(Propylcarbonyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine butyl ester (27):** white solid (43%); mp 105 °C; IR (KBr) 3256, 2962, 1747, 1647, 1614, 1515, 1222, 1196 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (m, 12H), 1.25 (m, 2H), 1.60 (m, 8H), 1.86 (m, 2H), 2.17 (m, 3H), 2.51 (t, 2H), 2.96 (dd, 2H), 3.63 (d, 1H), 4.00 (m, 2H), 4.68 (q, 1H), 5.60 (bs, 1H), 6.30 (bs, 1H), 6.65 (d, 2H), 6.92 (d, 2H), 7.10 (d, 1H); MS (CDI, CH_4) *m/z* 535 (MH^+). Anal. ($C_{28}H_{42}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-[(Isopropylcarbonyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine butyl ester (28):** white solid (33%); mp 125 °C; IR (KBr) 3404, 3255, 2962, 1747, 1647, 1611, 1515, 1225, 1195 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (t, 3H), 0.94 (dd, 6H), 1.12 (m, 6H), 1.28 (m, 2H), 1.63 (m, 6H), 1.86 (m, 2H), 2.17 (m, 3H), 2.73 (sept, 1H), 2.98 (dd, 2H), 3.60 (d, 1H), 4.02 (m, 2H), 4.66 (q, 1H), 5.75 (bs, 1H), 6.35 (bs, 1H), 6.35 (s, 2H), 6.65 (d, 2H), 6.92 (d, 2H), 7.10 (d, 1H); MS (CDI, CH_4) *m/z* 535 (MH^+). Anal. ($C_{28}H_{42}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-[(Cyclopentylcarbonyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine butyl ester (29):** white solid (18%); mp 140 °C; IR (KBr) 3260, 2960, 1746, 1646, 1614, 1615, 1515, 1220, 1194 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H), 0.99 (dd, 6H), 1.26 (m, 3H), 1.70 (m, 16H), 2.21 (m, 3H), 2.98 (dd, 3H), 3.65 (d, 1H), 4.04 (m, 2H), 4.70 (q, 1H), 5.30 (bs, 1H), 6.45 (bs, 1H), 6.72 (d, 2H), 7.00 (d, 2H), 7.18 (d, 1H); MS (CDI, CH_4) *m/z* 561 (MH^+). Anal. ($C_{27}H_{40}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-[(Cyclohexylcarbonyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine butyl ester (30):** white solid (13%); mp 110 °C; IR (KBr) 3257, 2932, 1744, 1657, 1644, 1515, 1196 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H), 0.98 (dd, 6H), 1.25 (m, 8H), 1.64 (bm, 23H), 2.45 (m, 1H), 2.95 (dd, 2H), 3.64 (d, 1H), 4.05 (t, 2H), 4.74 (q, 1H), 5.54 (bs, 1H), 6.40 (bs, 1H), 6.60 (d, 2H), 6.95 (d, 2H), 7.15 (d, 1H); MS (CDI, CH_4) *m/z* 575 (MH^+). Anal. ($C_{31}H_{46}N_2O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-[(4-Morpholinoacetyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine butyl ester (31):** white solid (39%); mp 143−144 °C; IR (KBr) 3393, 2961, 1745, 1646, 1515, 1343, 1193 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, 3H), 1.00 (dd, 6H), 1.20 (m, 2H), 1.64 (m, 6H), 1.95 (m, 2H), 2.29 (m, 3H), 2.51 (m, 4H), 2.95 (dd, 2H), 3.21 (d, 2H), 3.59 (d, 1H), 3.69 (m, 4H), 4.02 (m, 2H), 4.70 (q, 1H), 6.30 (bs, 1H), 6.65 (d, 2H), 6.97 (m, 3H), 7.10 (d, 1H); MS (CDI, CH_4) *m/z* 592 (MH^+). Anal. ($C_{30}H_{45}N_3O_7S$) C, H, N.

***N*-[[1-[[2(*S*)-[(Piperdinoacetyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine butyl ester (32):** white solid (71%); mp 130−131 °C; IR (KBr) 3396, 1744, 1672, 1647, 1628, 1605, 1514, 1213, 1194 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (t, 3H), 0.99 (dd, 6H), 1.30 (m, 2H), 1.65 (m, 12H), 1.95 (m, 2H), 2.24 (m, 3H), 2.50 (t, 4H), 3.01 (dd, 2H), 3.21 (s, 2H), 3.60 (d, 1H), 4.07 (m, 2H), 4.72 (q, 1H), 5.50 (bs, 1H), 6.30 (bs, 1H), 6.70 (d, 2H), 6.97 (d, 2H), 7.10 (d, 1H); MS (CDI, CH_4) *m/z* 590 (MH^+). Anal. ($C_{31}H_{47}N_3O_6S$) C, H, N.

***N*-[[1-[[2(*S*)-[(Methoxyacetyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine butyl ester (33):** white solid (63%); mp 110−111 °C; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H), 1.02 (dd, 6H), 1.30 (m, 2H), 1.64 (m, 6H), 1.99 (m, 2H), 2.27 (m, 3H), 3.00 (dd, 2H), 3.47 (s, 3H), 3.65 (d, 1H), 4.05 (m, 2H), 4.08 (s, 2H), 4.73 (q, 1H), 5.81 (s, 1H), 6.30 (bs, 1H), 6.69 (d, 2H), 6.93 (d, 2H), 7.07 (d, 1H); MS (CDI, CH_4) *m/z* 537 (MH^+). Anal. ($C_{27}H_{40}N_2O_7S$) C, H, N.

When R ≠ H. The residue was dissolved in dichloromethane and triethylamine (1 equiv) and an acid chloride (1 equiv) were added. The mixture was stirred for 3 h and then washed with brine and dried over magnesium sulfate. The solution was filtered and concentrated under vacuum to give a foam. The foam was purified by flash chromatography on silica gel, eluting with hexane−ethyl acetate. The pure fractions were combined and concentrated under reduced pressure.

***N*-[[1-[[2(*S*)-[(Methoxyacetyl)thio]-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine ethyl ester (37):** white solid (70%); mp 95−100 °C; IR (KBr) 3252, 2962, 1740, 1659, 1639, 1605, 1248, 1148 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (d, 3H), 1.04 (d, 3H), 1.20 (t, 3H), 1.70 (m, 4H), 1.93 (m, 3H), 2.35 (m, 2H), 3.00 (dd, 2H), 3.44 (s, 3H), 3.67 (d, 1H), 3.76 (s, 3H), 4.10 (m, 4H), 4.70 (q, 1H), 6.27 (bs, 1H), 6.80 (d, 2H), 7.05 (m, 3H); MS (CDI, CH_4) *m/z* 523 (MH^+). Anal. ($C_{26}H_{38}N_2O_7S$) C, H, N.

Biological Tests. *In Vivo* Angiotensin-I-Converting Enzyme Inhibition. The *in vivo* tests were performed in male, conscious normotensive Sprague−Dawley rats (275−390 g). The rats were anesthetized with methohexital sodium (75 mg/kg, ip) and instrumented with femoral arterial and venous catheters for direct blood pressure measurements and iv administration of compounds, respectively. On the following day, the pressor responses to three challenges of angiotensin-I (300 ng/kg, iv) were obtained. Test compounds were then administered iv or po, and the rats were rechallenged with angiotensin-I at scheduled times thereafter. All responses obtained after the administration of test compound were compared to the average of the initial three responses. Any observed decrease of the pressor response is an indication of angiotensin-I-converting enzyme inhibition.

***In Vivo* Neutral Endopeptidase Inhibition.** Male Sprague−Dawley rats (275−390 g) were anesthetized with ketamine (150 mg/kg)/acepromazine (10%) and instrumented with catheters in the femoral artery and vein to obtain blood samples and infuse ratANF(99-126), respectively. The rats were tethered with a swivel system and allowed to recover for 24 h before being studied in the conscious, unrestrained state.

Plasma ANF levels were determined in the presence and absence of NEP inhibition. On the day of study, all rats were infused continuously with ANF at 450 ng/kg/min iv for the entire 5 h of the experiment. Sixty minutes after beginning the infusion, blood samples for base-line ANF measurements were obtained (time 0), and the rats were then randomly divided into groups to be treated with the test compound or vehicle. Additional blood samples were taken at 30, 60, 120, 180, and 240 min after administration of the test compound.

Plasma ANF concentrations were determined by a specific radioimmunoassay. The plasma was diluted (50-, 100-, and 200-fold) in buffer containing 19 mM sodium phosphate monobasic and 81 mM sodium phosphate dibasic (pH 7.4), 50 mM NaCl, 0.1% BSA, 0.1% Triton X-100, and 0.1% NaN_3.

One hundred microliters of standard [rANF(99-126)] or sample was added to 100 μL of rabbit anti-rANF serum and incubated at 4 °C for 16 h. Ten thousand cpm of [^{125}I]rANF were then added to the reaction mixture which was incubated at 4 °C for an additional 24 h. Goat anti-rabbit IgG serum coupled to paramagnetic particles was added to the reaction mixture, and bound [^{125}I]rANF was pelleted by exposing the mixture to an attracting magnetic rack. The supernatant was decanted, and the pellets were counted in a gamma counter. All determinations were performed in duplicate.

Blood Pressure Determinations. Aortic-Ligated Rat. Male Sprague−Dawley rats (150−200 g) were housed in pending cages and given tap water and regular pelleted food ad libitum. They were maintained in an isolated room with a constant temperature of 74 °F with cycles of light and darkness lasting for 12 h. A complete aortic ligation between the renal arteries was performed according to the method of Rojo-Ortega and Genest.[42] Sham-operated animals underwent a similar surgical procedure by which the peritoneal cavity was opened but the aorta remained untouched. After surgery, each animal received a subcutaneous injection of 200 000 U of wycillin (sterile penicillin procaine suspension; Wyeth Laboratories Inc., Philadelphia, PA). The elevation in blood pressure reached a plateau 12 days after aortic ligation. In this time period, plasma renin activity was significantly increased. Direct blood pressure measurements were performed in conscious rats, mildly restrained in plastic cylinders, instrumented with an indwelling catheter (PE-50) implanted in the right carotid artery and connected to a Gould Statham physiological pressure transducer. This was coupled to a transducer amplifer (Gould, model 13-4615-50), and the blood pressure waves were displayed on a Gould 2400 S recorder. After a period of stabilization, the compounds were administered orally, and the changes in blood pressure were monitored for a 4 h period.

Spontaneously Hypertensive Rat. Male spontaneously hypertensive rats (SHR), 16−18 weeks of age (Tac: N(SHR)-fBr), were anesthetized with Amytal (sodium amobarbital, 100 mg/kg ip) and implanted with radiotelemetric devices (Data Sciences International, Minneapolis, MN) to enable continuous mean arterial pressure and heart rate determinations in conscious unrestrained rats. After a 10−14 day recovery period, the chronic effects of compound **38** were examined and compared to those of the ACE inhibitor benazepril. Separate groups of rats received twice daily oral administration (gavage) of either compound **38** or benazepril at doses of 1, 3, and 10 mg/kg ($n = 7$/group). Additional groups of control rats received an identical volume of 3% cornstarch (1 mL/kg of body weight) administered twice daily by oral gavage. Rats received either drug or vehicle at 12 h intervals at 6:00 a.m. and 6:00 p.m. for 14 consecutive days.

Base-line measurements were determined by monitoring blood pressure continuously over a 3 day period immediately prior to drug administration. Comparative hemodynamic effects of compound **38** and benazepril were then evaluated over the ensuing 14 days. Blood pressure was recorded during a 1 week recovery period following the 2 week dosing interval. Data are reported as the group average for the change in mean arterial pressure.

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