Heterocyclic Lactam Derivatives as Dual Angiotensin Converting Enzyme and Neutral Endopeptidase 24.11 Inhibitors

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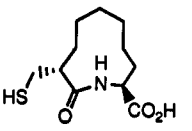
A series of 13- and 14-membered ring lactam derivatives **9a,b**, **10**, **11**, and **12a-c** was prepared from L-cysteine. Compounds **9a,b** and **12a,b** were tested *in vitro* for inhibition of neutral endopeptidase 24.11 (NEP) and angiotensin converting enzyme (ACE) inhibition. The structure–activity profile of the series is discussed. Compound **9b**, a 13-membered ring macrocyclic lactam, had an NEP IC_{50} of 18 nM and an ACE IC_{50} of 12 nM *in vitro* and showed dual plasma inhibition after intravenous or oral administration.

Atrial natriuretic peptide (ANP), a 28-amino acid hormone synthesized primarily in atrial myocytes, shows potent natriuretic, diuretic, and vasorelaxant properties *in vivo*.[1] The rapid clearance of bioactive ANP from plasma appears to be regulated by both clearance receptors[2] and enzymatic degradation.[3-5] Neutral endopeptidase 24.11 (EC 3.4.24.11), also known as enkephalinase, has been shown to inactivate ANP by cleavage between Cys^7 and Phe^8.[3-8] Since neutral endopeptidase (NEP) inhibitors have been shown to enhance the biological activity of ANP,[4,5,9-13] we initiated a program to identify novel NEP inhibitors as potential therapeutic agents for the treatment of hypertension and congestive heart failure.

During the course of these studies we also became interested in exploring the potential of incorporating angiotensin converting enzyme (ACE) inhibitory properties into our NEP inhibitors. This goal was attractive since ACE inhibitors are well-known antihypertensive agents[14] and have been shown to produce synergistic antihypertensive effects in combination with NEP inhibitors.[15] The goal seemed feasible since both enzymes are zinc metalloproteases and nonspecific inhibitors are known.[16] Herein we report our results on some of our efforts in the area of macrocyclic lactam derivatives.

For the design of compounds as potential NEP inhibitors, computer modeling studies based on the active site of thermolysin complexed with inhibitors were carried out.[17] This work had previously led to a series of carbocyclic lactams joining the P_1' and P_2' binding subsites, such as **1**, which were prepared and were shown to be potent inhibitors of NEP.[18] Extending these results to ACE inhibitors, modeling studies were carried out based on a template constructed from the superimposition of several energy-minimized rigid ACE inhibitors (**2a–c**).[19,20] This work indicated that energy-minimized 12–15-membered ring macrocycles provided a qualitatively good fit in both the NEP and ACE models, with good overlap of the hydrophobic regions and alignment of key hydrogen bonds.[21] Therefore it was decided to prepare 13- and 14-membered ring derivatives. Furthermore it was planned to prepare macrocycles incorporating L-Cys, which has the advantage of providing direct access to compounds with the desired absolute configuration at one of the asymmetric centers.



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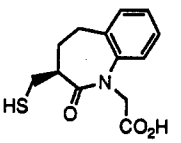

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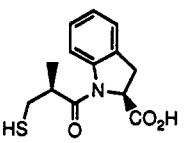

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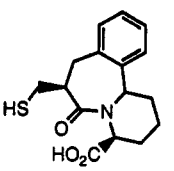

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Chemistry

The synthesis of the target compounds is illustrated in Scheme I for the 13-membered ring lactams. S-Alkylation of L-cysteine methyl ester with the alkyl iodide prepared by alkylation of *tert*-butyl methyl malonate with 1,7-dibromoheptane followed by treatment with sodium iodide gave **3a**. Acid-catalyzed removal of the *tert*-butyl group followed by cyclization at 5 mM using pentafluorophenol and *N*-(3-(dimethylamino)propyl)-*N'*-ethylcarbodiimide[22] generated lactam **4** in 41% yield. More concentrated solutions resulted in lower yields, with increased formation of polar byproducts. Using diphenyl phosphorazidate as coupling agent, the lactam was formed in only 15% yield. Alkaline hydrolysis of diester **4** generated diacid **5**, which underwent methylenation with formaldehyde[23] to give **6a** in 83% yield. Conjugate addition with thiolacetic acid produced **7** as an approximately 1:1 mixture of diastereomers by NMR and TLC. Esterification with diazomethane led to **8**, which allowed separation of the diastereomers by fractional crystallization or flash chromatography. Alkaline hydrolysis of each isomer of **8** generated the thiols **9a** and **9b**. Although the optical purity of these diastereomers is unknown, preparation of several batches gave essentially the same optical rotations.

Several potential prodrugs esters were also prepared. Esterification of **6a** with ethanolic HCl followed by conjugate addition of thiolacetic acid led to **10**. Alkylation of **7** with benzyl bromide and cesium carbonate produced **11**. Although each of these reactions led to a mixture of diastereomers, the desired isomer, as determined by relative R_f and NMR chemical shifts in comparison with

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Scheme I

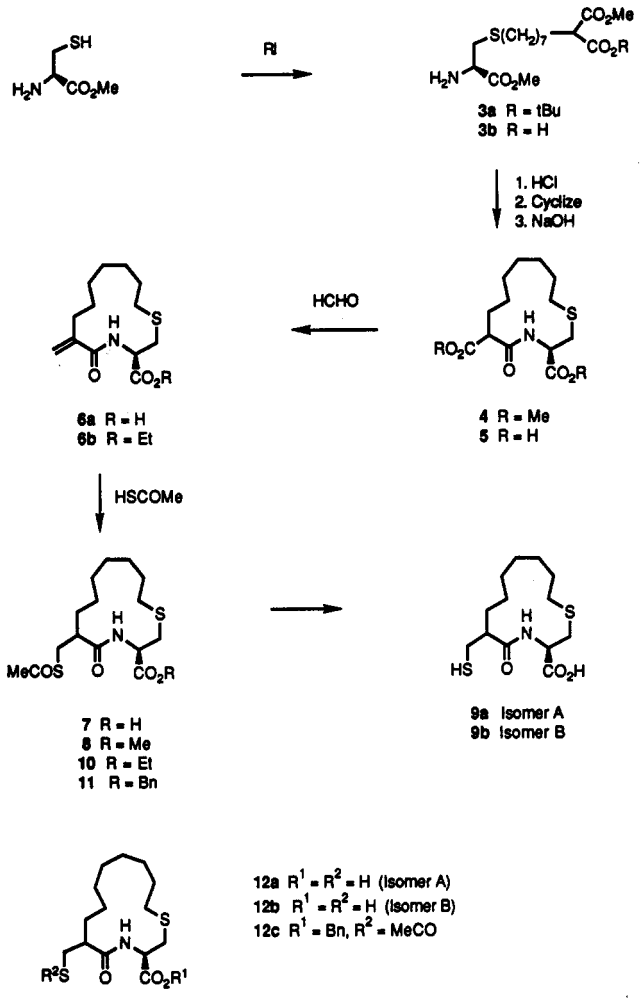

Table I. Characterization and *In Vitro* NEP and ACE Inhibition of **9a,b**, **10**, **11**, and **12a–c**

no.	formula[a]	mp (°C)	IC_{50}, nM NEP[b]	IC_{50}, nM ACE[c]
9a	$C_{13}H_{23}NO_3S_2$	181–184	190	72
9b	$C_{13}H_{23}NO_3S_2$	169–173	18	12
12a	$C_{14}H_{25}NO_3S_2$	165–168	1300	4600
12b	$C_{14}H_{25}NO_3S_2$	200–203	167	17
10	$C_{17}H_{29}NO_4S_2$	193–196		
11	$C_{22}H_{31}NO_4S_2$	192–193		
12c	$C_{23}H_{33}NO_4S_2$	174–178		
thiorphan[d]			6.0	
benazeprilat[e]				2.0

[a] Each compound had NMR consistent with structure and expected M + H ion seen in MS. [b] Assays were carried out as described in ref 7. [c] Assays were carried out as described in ref 24. [d] Literature[25] IC_{50} = 3.5 nM. [e] Literature[20] IC_{50} = 2.2 nM.

the isomer of **8** which led to **9b**, was obtained by flash chromatography. The corresponding 14-membered ring lactams **12a–c** were prepared analogously.

Results and Discussions

The diastereomeric pairs **9a,b** and **12a,b** were tested *in vitro* for both NEP and ACE inhibition, with **9b** and **12b** showing approximately 10-fold superior potency compared to the other diastereomers (**9a** and **12a**, respectively) (Table I). The 13-membered ring thiol **9b** was approximately 10 times more potent as an NEP inhibitor than the 14-membered ring compound **12b**. Both **9b** and **12b** showed good activity as ACE inhibitors. The absolute stereochemistry of the diastereomeric pairs **9a,b** and **12a,b** at the methine adjacent to the thiol was tentatively assigned as *R* for **9a** and **12a** and *S* for **9b** and **12b**. This assignment is based on a comparison of the relative ^1H NMR chemical shifts of the methine protons (**9a**, δ 4.48; **9b**, δ 4.09; and **12a**, δ 4.51; **12b**, δ 4.10) to the corresponding chemical shifts of a related 10-membered ring macrocycle, the *S*-acetyl ethyl ester of **1** (*R*-isomer, δ 4.70; *S*-isomer, δ 4.55), whose structure was determined by X-ray crystallography.[18a] The assignment is further supported by the ACE inhibitory data, in which the *S*-isomer has invariably been shown to be more potent than the corresponding *R*-isomer.[14]

Table II. Plasma Inhibition of NEP and ACE by **9b**, **10**, **11**, **12b**, and **12c**

no.	dose (mg/kg po)	plasma concentration (μM) based on *ex vivo* NEP inhibition[a] 0.5 h	1 h	2 h	3 h	% inhibition AI pressor response[b] 0.5 h	1 h	2 h
9b	10	1.81	0.32	0.17	0.06	71 ± 5	17 ± 1	0
10	30	ND[c]	ND	ND	ND			
11	30	ND	ND	ND	ND			
12c	30	0.36	0.42	ND	ND			
acetophan[d]	30	0.95	0.34	0.38	0.11			
benazeprilat	10					100	86 ± 3	77 ± 6

[a] Tests were carried out as described in the Experimental Section. The data indicate the plasma concentration of active compound in DOCA-salt rats. [b] Tests were carried out as described in the Experimental Section. The data indicate the percent inhibition of angiotensin I pressor response in normotensive rats after a test dose of 10 mg/kg iv. [c] ND = not detected. [d] See ref 26. [e] See ref 20.

In vivo, the parent inhibitor **9b** as well as the potential prodrugs **10**, **11**, and **12c** were tested for *ex vivo* plasma NEP inhibition after oral administration at 10 or 30 mg/kg (Table II). Disappointingly, the prodrugs **10** and **11** showed no inhibition of plasma NEP activity, and the activity of **12c** disappeared after 1 h, indicating poor absorption or a short duration of action. Thiol **9b** initially produced good levels of inhibition but showed significant decrease in activity over the 3-h test period. The duration of plasma inhibition of **9b** was inferior to the reference inhibitor acetorphan.[26]

Thiol **9b** was also tested at 10 mg/kg iv for inhibition of angiotensin I (AI) pressor response. Consistent with the findings for plasma NEP inhibition, **9b** produced the desired inhibitory activity, but the duration of action was relatively short (Table II).

In summary, the 13-membered ring thiol **9b**, designed as a dual NEP/ACE inhibitor, showed the desired *in vitro* profile with NEP IC_{50} of 18 nM and ACE IC_{50} of 12 nM. The compound also was active after intravenous or oral administration, but the duration of action was less than 1 h. These macrocyclic lactam thiols represent a new class of dual NEP/ACE inhibitors. On the basis of these results, additional heteroatom-containing macrocyclic lactam derivatives are under investigation.

Experimental Section

Proton NMR spectra were determined on a Varian XL-300 spectrometer with Me_4Si as the internal standard. Infrared spectra were recorded on a Nicolet 5SXFT spectrophotometer. Optical rotations were measured with a JASCO DIP370 polarimeter. Melting points were taken on a Haake Buchler melting point apparatus and are uncorrected. Mass spectra were recorded on a Hewlett-Packard GCMS 5985 spectrometer. Flash chromatography was performed with silica gel (Bodman 230–400 mesh). All compounds were prepared by methods analogous to those described below. Intermediate products were used directly without further purification. Unless indicated below, the ex-

perimental details for the biological tests have been described previously.[7,24]

***tert*-Butyl Methyl (7-Bromoheptyl)malonate.** NaH (6.08 g of a 50% oil dispersion, 127 mmol) was suspended in DMF (300 mL) and cooled to 0 °C. *tert*-Butyl methyl malonate (20.1 g, 115 mmol) was added dropwise slowly, and the reaction mixture was warmed to room temperature. 1,7-Dibromoheptane (29.8 g, 115 mmol) was added dropwise, and the mixture was stirred for 3 h. The mixture was partitioned between diethyl ether (500 mL) and H_2O (1000 mL). The organic layer was washed with H_2O (3 × 500 mL) and brine (1 × 500 mL), dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography (10% ethyl acetate/hexane) to give the product as an oil (11.9 g, 29%), used directly in the following reaction.

***tert*-Butyl Methyl (7-Iodoheptyl)malonate.** *tert*-Butyl methyl (7-bromoheptyl)malonate (8.81 g, 25.1 mmol) was dissolved in acetone (50 mL). NaI (3.76g, 25,1 mmol) was added, and the mixture was refluxed for 2 h. The reaction mixture was filtered, and the filtrate was concentrated to dryness. The residue was dissolved in CH_2Cl_2 and filtered again. The filtrate was concentrated to dryness to give the product as an oil (9.24 g, 92%), used directly in the following reaction.

Dimethyl (2*R*)-2-Amino-12-(*tert*-butoxycarbonyl)-4-thia-1,13-tridecanedicarboxylate (3a). L-Cysteine methyl ester hydrochloride (9.05 g, 52.7 mmol) was dissolved in N_2-degassed MeOH (300 mL), and NaOMe (6.11 g, 113 mmol) was added. The reaction mixture was stirred 15 min at room temperature and t-butyl methyl 7-iodo-heptylmalonate (15.0 g, 37.7 mmol) was added. The mixture was stirred overnight at room temperature and partitioned between diethyl ether (500 mL) and H_2O (1000 mL). The organic layer was washed with brine (300 mL), and dried ($MgSO_4$), and the solvent was evaporated. The product was purified by flash chromatography (5% MeOH in CH_2Cl_2) to give **3a** (7.42 g, 49%) as an oil: ^1H NMR ($CDCl_3$) δ 1.30 (m, 8H), 1.44 (s, 9H), 1.56 (m, 2H), 1.76 (s, 2H), 1.83 (m, 2H), 2.51 (t, 2H, J = 7 Hz), 2.75 (dd, 1H, J = 6, 14 Hz), 2.91 (dd, 1H, J = 4, 15 Hz), 3.22 (t, 1H, J = 7 Hz), 3.64 (m, 1H), 3.72 (s, 3H), 3.74 (s, 3H); $[\alpha]_D$ = +13.7° (c = 1, MeOH); IR (neat) 2929, 1744, 1732, 1368, 1147 cm^{-1}; DCI MS m/e 406 (M + H)$^+$, 350.

Dimethyl (2*R*)-2-Amino-12-carboxy-4-thia-1,13-tridecanedicarboxylate (3b) Hydrochloride. Diester **3a** (3.39 g, 8.36 mmol) was dissolved in CH_2Cl_2 (50 mL), and HCl gas was bubbled through the solution for 5 min. The reaction mixture was stirred 2 h at room temperature. The solvent was evaporated to give **3b** as an oil (3.23 g, 100%): ^1H NMR ($CDCl_3$) δ 1.25–1.47 (m, 8H), 1.58 (m, 2H), 1.88 (m, 2H), 2.60 (m, 2H), 3.24 (m, 2H), 3.38 (t, 1H, J = 7 Hz), 3.72 (d, 3H), J = 5 Hz), 3.84 (s, 3H), 4.44 (m, 1H); IR (CH_2Cl_2) 2987, 2932, 1743, 1720 cm^{-1}.

Methyl (3*R*)-6-Carbomethoxy-5-oxo-1-thia-4-azacyclotridecane-3-carboxylate (4). Amino acid **3b**·HCl (6.87 g, 17.8 mmol) was dissolved in CH_2Cl_2 (3.5 L), and Et_3N (9.00 g, 89 mmol) was added. Pentafluorophenol (9.83 g, 53.4 mmol) was added followed by *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (17.1 g, 89 mmol). The reaction was stirred overnight at room temperature and then concentrated to 500 mL. The organic solution was washed with H_2O (500 mL), 1 N KOH (2 × 500 mL), and brine (500 mL), dried ($MgSO_4$), and concentrated to dryness. The residue was purified by flash chromatography (40% ethyl acetate/hexane) to give **4** as a diastereomeric mixture (2.41 g, 41%): mp 156-164 °C; ^1H NMR ($CDCl_3$) δ 1.29–1.62 (m, 10H), 1.82–2.14 (m, 2H), 2.40–2.54 (m, 2H), 3.00–3.40 (m, 3H), 3.70–3.81 (4 overlapping singlets, 6H, methyl esters), 4.89 (m, 1H), 6.76, 8.21 (2 doublets, 1H, J = 6 Hz, NH); DCI MS m/e = 332 (M + H)$^+$. Anal. ($C_{15}H_{25}NO_5S$) C, H, N.

(3*R*)-6-Carboxy-5-oxo-1-thia-4-azacyclotridecane-3-carboxylic Acid (5). Diester **4** (2.39 g, 7.21 mmol) was suspended in MeOH (75 mL), and 1 N NaOH (22.0 mL, 22.0 mmol) was added. The mixture was stirred for 4 h at room temperature. The solvent was evaporated, and the residue was dissolved in H_2O (150 mL). The aqueous solution was adjusted to pH 1 by addition of 1 N HCl and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$), and evaporated to give **5** as a diastereomeric mixture (2.10 g, 96%): mp 167–170 °C; ^1H NMR (DMSO-d_6) δ 1.20–1.52 (m, 10H), 1.62–2.02 (m, 2H), 2.35–2.98 (m, 3H), 3.05–3.53 (m, 2H), 4.33, 4.50 (2 multiplets, 1H, methine), 8.10, 8.33 (2 doublets, 1H, J = 6 Hz, NH); DCI MS m/e 304 (M + H)$^+$, 260. Anal. ($C_{13}H_{21}NO_5S$) C, H, N.

(3*R*)-6-Methylidene-5-oxo-1-thia-4-azacyclotridecane-3-carboxylic Acid (6a). Diacid **5** (1.71g, 5.64 mmol) was dissolved in pyridine (20 mL), and piperidine (96 mg, 1.13 mmol) was added followed by paraformaldehyde (250 mg, 8.46 mmol). The mixture was stirred for 2 h at 65 °C. The reaction mixture was cooled to room temperature, poured into 6 N HCl (100 mL), and extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$), and evaporated to give **6a** (1.21 g, 83%): mp 184–186 °C; ^1H NMR ($CDCl_3$) δ 1.15–1.52 (m, 10H), 2.20 (m, 2H), 2.55 (m, 2H), 2.99 (dd, 1H, J = 4, 14 Hz), 3.12 (dd, 1H, J = 5, 14 Hz), 4.70 (m, 1H), 5.25 (s, 1H), 5.64 (s, 1H), 6.84 (d, 1H, J = 6 Hz); $[\alpha]_D$ = +22.7° (c = 0.69, MeOH). Anal. ($C_{13}H_{21}NO_3S$) C, H, N.

(3*R*)-6-[(Acetylthio)methyl]-5-oxo-1-thia-4-azacyclotridecane-3-carboxylic Acid (7). Lactam **6a** (1.21 g, 4.70 mmol) was suspended in thiolacetic acid (40 mL). The mixture was stirred overnight at room temperature. The solvent was evaporated at 40 °C, and the residue was triturated from diethyl ether and hexane (1:1). The resulting solid was filtered and dried to give both diastereomers of **7** (1.41 g, 86%): mp 125–130 °C; ^1H NMR (DMSO) δ 1.05–1.62 (m, 12H), 2.30 (s, 3H), 2.40–3.19 (m, 8H), 4.00 (t, $^1/_2$H, J = 4 Hz), 4.36 (t, $^1/_2$H, J = 4 Hz), 8.38 (d, $^1/_2$H, J = 6 Hz), 8.45 (d, $^1/_2$H, J = 6 Hz). Anal. ($C_{15}H_{25}NO_4S_2$) C, H, N.

Methyl (3*R*)-6-[(Acetylthio)methyl]-5-oxo-1-thia-4-azacyclotridecane-3-carboxylate (8). Acid **7** (1.40 g, 4.03 mmol) was partially dissolved in MeOH (50 mL), and freshly prepared etheral CH_2N_2 was added until a yellow color persisted. The reaction was quenched with glacial acetic acid, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (100 mL), washed with saturated $NaHCO_3$ (100 mL) and brine (100 mL), dried (Na_2SO_4), and evaporated to give 1.41 g (97%) of crude diastereomeric methyl esters. The crude product was purified by flash chromatography (30% ethyl acetate/hexane) to give two separate diastereomers of **8**. Isomer A (less polar, 0.62 g, 43%), mp 156–161 °C; ^1H NMR ($CDCl_3$) δ 1.24–1.68 (m, 12H), 2.33 (s, 3H), 2.37–2.52 (m, 3H), 2.95–3.16 (m, 4H), 3.80 (s, 3H), 4.89 (m, 1H), 6.49 (d, 1H, J = 6 Hz); $[\alpha]^{25}_D$ = +16.03° (c = 0.8, MeOH); IR (KBr) 3332, 2932, 1740, 1691, 1638, 1528 cm^{-1}; DCI MS m/e 362 (M + H)$^+$, 320.

Isomer B (more polar, 0.22 g, 15%); mp 199–201 °C; ^1H NMR ($CDCl_3$) δ 1.36–- 1.68 (m, 12H), 2.35 (s, 3H), 2.39–2.62 (m, 3H), 3.01–3.19 (m, 4H), 3.77 (s, 3H), 4.60 (m, 1H), 6.49 (d, 1H, J = 5 Hz); IR (KBr) 3332, 2932, 1740, 1691, 1638, 1528 cm^{-1}; DCI MS m/e = 362 (M + H)$^+$, 320. Anal. ($C_{16}H_{27}NO_4S_2$) C, H, N.

(3*R*)-6-(Mercaptomethyl)-5-oxo-1-thia-4-azacyclotridecane-3-carboxylic Acid (Isomer A) (9a). Thiol **9a** was prepared from **8** (isomer A) in a manner analogous to **9b** to give the product (110 mg, 93%): mp 181–184 °C; ^1H NMR ($CDCl_3$) δ 1.14–1.65 (m, 13H), 2.35–2.76 (m, 7H), 3.07 (m, 1H), 4.48 (m, 1H), 8.13 (d, 1H, J = 6Hz); $[\alpha]^{25}_D$ = −16.07° (c = 0.7, MeOH); DCI MS m/e 306 (M + H)$^+$. Anal. ($C_{13}H_{23}NO_3S_2$) C, H, N.

(3*R*)-6-(Mercaptomethyl)-5-oxo-1-thia-4-azacyclotridecane-3-carboxylic Acid (Isomer B) (9b). Isomer B of **8** (0.16 g, 0.44 mmol) was partially dissolved in N_2-degassed THF (10 mL) and H_2O (5 mL). LiOH·H_2O (56 mg, 1.33 mmol) was added, and the mixture was stirred at room temperature for 3 h. The mixture was poured into 1 N HCl and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), and evaporated to give **9b** (0.14 g, 100%): mp 169–173 °C; ^1H NMR (DMSO) δ 1.20–1.66 (m, 13H), 2.29–2.60 (m, 5H), 2.70–2.86 (m, 2H), 3.12 (m, 1H), 4.09 (m, 1H), 8.00 (d, 1H, J = 6 Hz); $[\alpha]^{25}_D$ = −16.73° (c = 0.7, MeOH); IR (KBr) 1711, 1643, 1540 cm^{-1}; DCI MS m/e 306 (M + H)$^+$. Anal. ($C_{13}H_{23}NO_3S_2$) C, H, N.

Ethyl (3*R*)-6-Methylidene-5-oxo-1-thia-4-azacyclotridecane-3-carboxylate (6b). Acid **6a** (0.16 g, 0.62 mmol) was dissolved in EtOH (5 mL), and HCl gas was bubbled through the solution for 5 min. The solution was stirred overnight at room temperature and evaporated to give **6b** (0.11 g, 59%): ^1H NMR ($CDCl_3$) δ 1.25–1.54 (m, 13H), 2.19 (m, 1H), 2.34 (m, 1H), 2.52 (m, 1H), 2.70 (m, 1H), 3.03 (dd, 1H, J = 4, 14 Hz), 3.26 (dd, 1H,

J = 5, 14 Hz), 4.25 (m, 2H), 4.83 (m, 1H), 5.33 (s, 1H), 5.72 (s, 1H), 6.83 (d, 1H, J = 6 Hz). Anal. ($C_{15}H_{25}NO_3S$) C, H, N.

Ethyl (3*R*)-6-[(Acetylthio)methyl]-5-oxo-1-thia-4-azacyclotridecane-3-carboxylate (10). Lactam **6b** (0.11 g, 0.37 mmol) was suspended was thiolacetic acid (5 mL), and the reaction mixture was stirred for 48 h at room temperature. The solvent was evaporated, and the residue was triturated from diethyl ether/hexane (1:1). The desired more polar diastereomer was obtained by flash chromatography (10% ethyl acetate/hexane) to give **10** (20 mg, 15%): mp 193–196 °C; ^{1}H NMR ($CDCl_3$) δ 1.22–1.70 (m, 16H), 2.35 (s, 3H), 2.39–2.61 (m, 2H), 3.00–3.19 (m, 4H), 4.24 (q, 2H, J = 6 Hz), 4.59 (m, 1H), 6.47 (d, 1H, J = 6 Hz). Anal. ($C_{17}H_{29}NO_4S_2$) C, H, N.

Benzyl (3*R*)-6-[(Acetylthio)methyl]-5-oxo-1-thia-4-azacyclotridecane-3-carboxylate (11). Acid **7** (0.62 g, 1.78 mmol) was dissolved in DMF (8 mL), and Cs_2CO_3 (0.64 g, 1.96 mmol) was added, followed by benzyl bromide (0.31g, 1.78 mmol). The mixture was stirred overnight at room temperature, poured into H_2O (300 mL), and extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with H_2O (2 × 100 mL) and brine (1 × 200 mL), dried ($MgSO_4$), and evaporated. The desired more polar diastereomer was obtained by flash chromatography (5% ethyl acetate/hexane) to give **11** (0.12 g, 16%): mp 192–193 °C; ^{1}H NMR ($CDCl_3$) δ 1.25–1.69 (m, 12H), 2.31 (s, 3H), 2.36–2.61 (m, 3H), 2.98–3.19 (m, 4H), 4.60 (m, 1H), 5.19 (s, 2H), 6.48 (d, 1H, J = 6 Hz), 7.28–7.42 (m, 5H). Anal. ($C_{22}H_{31}NO_4S_2$) C, H, N.

(3*R*)-6-(Mercaptomethyl)-5-oxo-1-thia-4-azacyclotetradecane-3-carboxylic Acid (Isomer A) (12a). The reaction sequence used to prepare **9a** was applied to give **12a** (57 mg, 71%): mp 165–168 °C; ^{1}H NMR (DMSO) δ 1.08–1.60 (m, 15H), 2.32–2.72 (m, 7H), 3.01 (m, 1H), 4.51 (m, 1H), 8.36 (d, 1H, J = 6 Hz). Anal. ($C_{14}H_{25}NO_3S_2 \cdot 0.25H_2O$) C, H, N.

(3*R*)-6-(Mercaptomethyl)-5-oxo-1-thia-4-azacyclotetradecane-3-carboxylic Acid (Isomer B) (12b). The reaction sequence used to prepare **9b** was applied to give **12b** (55 mg, 69%): mp 200–203 °C; ^{1}H NMR (DMSO) δ 1.05–1.62 (m, 15H), 2.23–2.73 (m, 6H), 2.76–3.10 (m, 2H), 4.10 (m, 1H), 8.09 (m, 1H). Anal. ($C_{14}H_{25}NO_3S_2$) C, H, N.

Benzyl (3*R*)-6-[(Acetylthio)methyl]-5-oxo-1-thia-4-azacyclotetradecane-3-carboxylate (12c). The reaction sequence used to prepare **11** was applied to give **12c** (0.20 g, 63%): mp 174–178 °C; ^{1}H NMR ($CDCl_3$) δ 1.20–1.68 (m, 14H), 2.32 (s, 3H), 2.85 (m, 3H), 2.94–3.16 (m, 4H), 4.60 (q, 1H, J = 6 Hz) 5.17 (AB q, 2H, J = 12 Hz), 6.64 (d, 1H, J = 6 Hz), 7.37 (br s, 5H). Anal. ($C_{23}H_{33}NO_4S_2$) C, H, N.

***Ex Vivo* Pharmacokinetics in Conscious Rats.** DOCA-salt hypertensive rats (308–380 g) were prepared by the standard methods. Sprague–Dawley rats (Taconic Farms, Germantown, NY) underwent a unilateral nephrectomy and 1 week later were implanted with silastic pellets containing 100 mg/kg of DOCA. The rats were maintained on 1% NaCl/0.2% KCl drinking water for 3–5 weeks until sustained hypertension was established. The rats were anesthetized with methoxyflurane and instrumented with a catheter in the femoral artery to obtain blood samples. The catheter was threaded through a swivel system which enabled the rats to move freely after regaining consciousness. Rats were allowed to recover from this surgical procedure for 24 h and were then studied in the conscious, unrestrained state. Blood samples for determining plasma NEP inhibitor concentrations were obtained 1, 2, 3, 4, 5, and 6 h after drug administration at 30 mg/kg po. An equivalent volume of saline was given to the animal after each blood sample (0.25 mL) was taken. The blood was collected in EDTA and centrifuged for 1 min in a microfuge. Protein-bound NEP inhibitor was separated from the plasma by centrifugation through an ultrafiltration membrane. The concentration of unbound NEP inhibitor in the plasma ultrafiltrate was then determined (*ex vivo*) in the standard *in vitro* NEP inhibition assay. It should be emphasized that this assay measures NEP inhibitor activity in the plasma but does not directly detect these compounds.

Angiotensin I Pressor Test

The *in vivo* ACE inhibitory activities of **9b** and benazeprilat were determined in conscious, unrestrained Sprague–Dawley rats (270–400 g). Rats were anesthetized with ketamine (100 mg/kg)/acepromazine (1 mg/kg) im and instrumented with catheters in the femoral artery and vein to measure mean arterial pressure and administer compounds, respectively. The catheters were tunneled subcutaneously to exit from the lower back through a stainless-steel spring and swivel system. On the following day, angiotensin I (Ang I, 30 ng/kg iv) was administered four times at 15-min intervals to establish a reproducible control pressor response. Test compounds (10 mg/kg) were given iv before rechallenging with Ang I during the subsequent 4-h period. Pressor responses produced by Ang I prior to and after compound administration were compared; the data are expressed as percent inhibition of the control response.

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