Dual Metalloprotease Inhibitors. 6. Incorporation of Bicyclic and Substituted Monocyclic Azepinones as Dipeptide Surrogates in Angiotensin-Converting Enzyme/Neutral Endopeptidase Inhibitors

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A series of substituted monocyclic and bicyclic azepinones were incorporated as dipeptide surrogates in mercaptoacetyl dipeptides with the desire to generate a single compound which would potently inhibit both angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP). Many of these compounds displayed excellent potency against both enzymes. Two of the most potent compounds, monocyclic azepinone **2n** and bicyclic azepinone **3q**, demonstrated a high level of activity versus ACE and NEP both *in vitro* and *in vivo*.

Introduction

Angiotensin-converting enzyme (ACE), a zinc-containing carboxydipeptidase, is largely responsible for the conversion of the inactive decapeptide angiotensin I (AI) to the biologically active octapeptide angiotensin II (AII). AII, a main component of the renin−angiotensin−aldosterone system (RAS), serves to raise blood pressure by both increasing vascular resistance and triggering the production of the sodium-retaining steroid aldosterone. Thus, inhibition of ACE has been an actively pursued target in the drug industry. Over 2 decades of research has established the benefits of ACE inhibitors in the treatment of essential human hypertension and congestive heart failure.

Neutral endopeptidase (E.C. 3.4.24.11, NEP), a zinc-containing endopeptidase found in high concentration within the brush border region of the kidney, is in large part responsible for the degradation and inactivation of atrial natriuretic peptide (ANP). ANP is a 28-amino acid peptide hormone produced by the heart in response to atrial distention and plays a role in modulating cardiovascular homeostasis. Through interaction with its biological receptor, ANP upregulates cGMP production, which in turn leads to vasodilatation, natriuresis, diuresis, and inhibition of aldosterone formation. Because the hormonal actions of AII and ANP are functionally opposed, it is expected that attenuation of AII with concurent potentiation of ANP will lead to a beneficial synergistic effect by lowering vascular resistance and inhibiting the RAS. Indeed, recent studies have shown that coadministration of selective ACE and NEP inhibitors is more effective in animal models of hypertension and congestive heart failure than treatment with either enzyme inhibitor given alone.[1] On the basis of this premise, a number of groups, including our own, have been active in the pursuit of a dual-acting ACE/NEP inhibitor.[2]

In a recent series of articles, we outlined the rationale, design, and synthesis of a class of sulfhydryl-containing compounds which inhibited ACE and NEP both *in vitro* and *in vivo*.[3] In our initial disclosure, we identified mercaptoacyl dipeptides **1a,b** as early leads.[3a] Compound **1a**, originally developed as an ACE inhibitor (IC_{50} = 30 nM vs ACE), was found to be marginally active versus NEP *in vitro* (IC_{50} = 400 nM). The related mercaptopropanoyl analog **1b** possessed excellent *in vitro* potency against both enzymes but, unlike its mercaptoacetyl analog **1a**, was poorly active *in vivo*.[4] In an effort to increase both the *in vitro* and *in vivo* potency of **1** toward ACE and NEP, the Ala-Pro portion of the molecule was replaced with a variety of conformationally restricted dipeptide mimetic surrogates.[3b−e] One of the most potent of these compounds was benzazepinone-based mercaptoacetyl **4** (BMS-182657). This compound possessed excellent activity versus ACE and NEP *in vitro* and in addition demonstrated potent inhibition of both metalloproteases *in vivo*.[3b,5]

In this present study, we examined the effect of conformational restraint on the Ala-Pro portion of compound **1a** utilizing a series of monocyclic and bicyclic dipeptide surrogates, leading to inhibitors represented by **2** and **3**, respectively. Conceptually, bicyclic compounds of type **3** arise via incorporation of an ethyl linker between the alanine methyl group and the 5-position of the proline ring. Combining this cyclization with a ring-opened proline gives the monocyclic derivatives **2**. Although both types of dipeptide surrogates (mono- and bicyclic azepinones) had been previously exploited for use as conformationally restricted surrogates of Ala-Pro in the ACE inhibitor enalapril,[6] past synthetic methodologies allowed access to only a few simple dipeptide surrogates. Recently, we have developed new synthetic methods for the synthesis of both the 7,5-fused (m = 0) and the 7,6-fused (m = 1) bicyclic azepinone nuclei[7] found in **3** as well as methods for the generation of a wide variety of substituted monocyclic azepinones[8] (as found in **2**) in homochiral form. Incorporation of these azepinones as conformationally restricted mimetics of mercaptoacetyl dipeptides and the biological evaluation of these compounds versus both ACE and NEP are the basis of this report.

Chemistry

Construction of mercaptoacetyl-containing inhibitors **2** and **3** is depicted in Scheme 1. BOP reagent-mediated coupling of the requisite amine dipeptide surrogates **5a−q** (where R^2 and R^3 could be joined to form an additional ring) with (*S*)-α-(acetylthio)-2-benzenepro-

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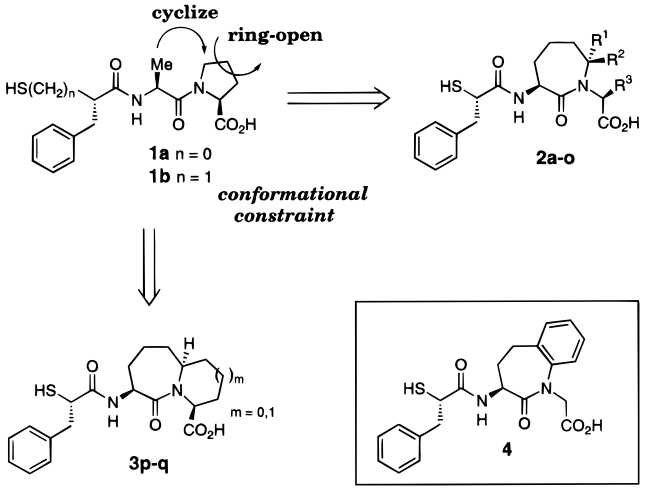
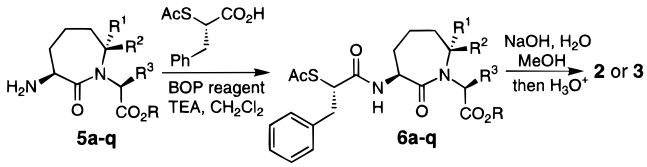


Figure 1.

Scheme 1



panoic acid[9] gave **6a**−**q** in generally good yield and excellent diastereomeric purity. Saponification of the thioacetate and alkyl ester groups under strictly oxygen-free conditions followed by acidification afforded the desired inhibitors **2** or **3**.

Amines **5a**−**q** were generated from the corresponding N-protected azepinone esters **7a**−**q** (Table 1) by treatment with either hydrazine monohydrate in methanol (protecting group (PG) = phthalimido) or anhydrous hydrogen chloride in dioxane (PG = Boc). Azepinones **7a**−**d,i,n** were synthesized using methodology previously developed in this laboratory.[8a] Generation of the methyl- and propyl-substituted azepinones **7e**−**h** followed our recent literature procedures[8b] as did the synthesis of bicyclic azepinones **7p,q**.[7] The remaining surrogates were constructed following the chemistry outlined in Schemes 2−5.

Compound **7o** was synthesized utilizing methodology similar to that developed for the generation of **7n**. Thus, (*S*)-6-hydroxy-2-phthalimidohexanoic acid (**8**) was coupled to (*S*)-2-aminobutyric acid (**9**) to give an intermediate dipeptidyl alcohol (Scheme 2). Subsequent Swern oxidation afforded aldehyde **10** which was subjected to acid-catalyzed cyclization to give bicyclic lactam **11** in 72% yield and 94% diastereomeric purity. The assigned stereochemistry at the newly formed stereocenter was confirmed by the observance of a strong NOE signal between the bridgehead hydrogen and the hydrogen α to the phthalimido-protected nitrogen. Treatment of diastereomerically pure **11** with allyltrimethylsilane in the presence of the Lewis acid catalyst $TiCl_4$ gave crude acid **12a** which was esterified to give **12b** as a single diastereomer. Subsequent hydrogenation of **12b** over palladium cleanly afforded the highly substituted azepinone **7o**. In close analogy, 3-(trimethylsilyl)cyclopentene was added to bicyclic lactam **13**[8a] in the presence of $SnCl_4$ to give, after esterification, compound **14** as a 2:1 mixture (atom labeled with an asterisk in structure **14**) of diastereomers (Scheme 3). Catalytic hydrogenation of the mixture provided the corresponding C-7 cyclopentyl-substituted azepinone **7m** as a single diastereomer, confirming the stereochemical homogeneity of the lactam ring at the C-7 center.

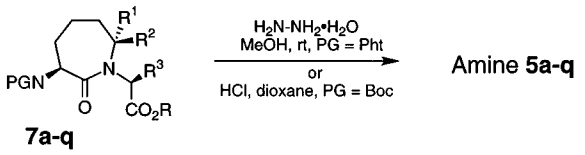
The allyl functionality at the C-7 position of the azepinone nucleus of **7i** provided an excellent handle for subsequent alkyl group modification. Palladium-catalyzed cyclopropanation[10] of **7i** provided the corresponding methylenecyclopropyl analog **7k** in nearly quantitative yield (Scheme 4). Hydrazinolysis of **7k** provided **5k** cleanly. Hydrogenation of **5k** under forcing conditions occurred regioselectively at the least hindered face of the cyclopropyl group[11] to give the C-7 isobutyl analog **5l**. In this case removal of the protecting group prior to hydrogenation was necessary in order to prevent reduction of the phthalimido group. Additionally, reduction of the C-7 allyl substituent cleanly afforded the propyl-substituted azepinone **7g**. This method for the generation of **7g** (where PG is phthalimido and R is methyl) is complimentary to the previously described procedure for the synthesis of this C-7-substituted azepinone (where PG is Boc and R is ethyl).[8b] The hydroxyl isostere of **7g**, azepinone **7j**, was generated by the procedure outlined in Scheme 5. Due to the relatively base labile nature of the phthalimido group, compound **7i** was first treated with hydrazine and the intermediate amine **5i** was reprotected as its Boc derivative. Oxidative cleavage of **15** with $NaIO_4$ in the presence of catalytic OsO_4 afforded aldehyde **16** which cleanly underwent reduction to the corresponding alcohol **7j**. Subsequent removal of the Boc group with HCl in dioxane gave amine **5j**.

Discussion

Compounds **2** and **3** were tested for their ability to inhibit both ACE and NEP *in vitro*, and the data are listed in Table 2. Inhibition of ACE activity *in vivo* was determined from plots of percent maximal inhibition of the AI-induced pressor response versus dose after intravenous (iv) administration in the conscious normotensive rat. The ED_{50} values correspond to the dose required to effect 50% inhibition of the pressor response. For comparative purposes, data for compounds **1a** and **4** are also listed in Table 2.

Compound **2a**, the simplest of the conformationally restricted ACE/NEP inhibitors ($R^1 = R^2 = R^3 = H$), exhibited a 5-fold gain in *in vitro* inhibitory potency versus NEP as compared to the Ala-Pro analog **1a**. Unfortunately this modification led to a 14-fold diminution in ACE activity *in vivo* despite similar potency versus ACE *in vitro*. In addition a dramatic loss in duration of activity for **2a** was observed in the AI pressor assay ($t_{1/2} \approx$ <10 min for **2a** versus $t_{1/2} \approx 45$ min for **1a** at a comparative dose of 0.5 μmol/kg iv). Introduction of a methyl substituent on the acetic acid side chain of **2a** to give **2b** resulted in essentially no change in activity versus ACE or NEP both *in vitro* and *in vivo*. Larger substituents at this position (benzyl analog **2c** and isopropyl analog **2d**) afforded compounds with greatly diminished activity against one or both enzymes. In contrast, the presence of substituents at the C-7 position of the monocyclic azepinone ring resulted in a subset of inhibitors with greater affinity for the target enzymes. Introduction of a methyl (**2e**) or propyl (**2g**) group in the *S* configuration at the C-7

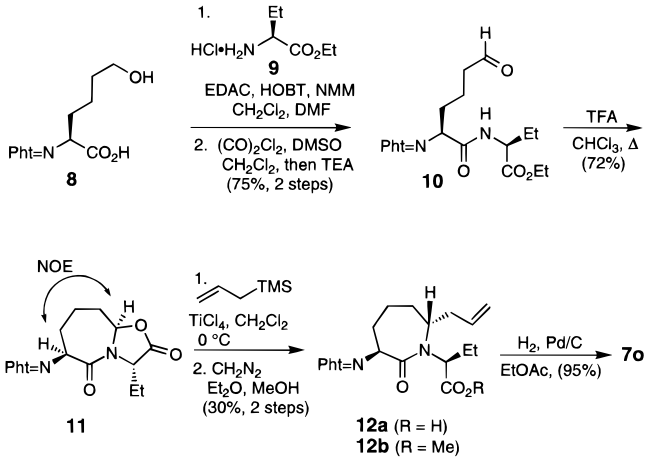
Table 1. Protected Monocyclic and Bicyclic Azepinones **7** Utilized in the Synthesis of Amines **5**



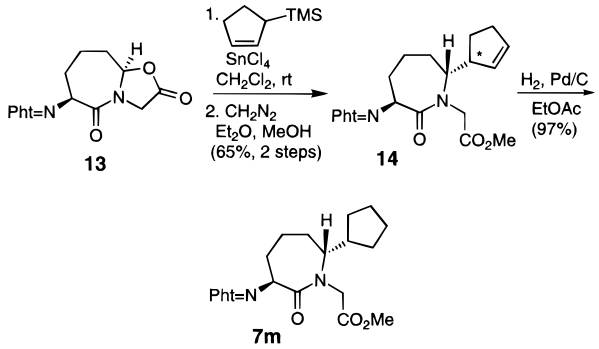
protected azepinones	R	R^1	R^2	R^3	PG removed
7a	Et	H	H	H	Boc[a]
7b	Me	H	H	methyl	Pht[b]
7c	Me	H	H	benzyl	Pht
7d	Me	H	H	isopropyl	Pht
7e	Et	methyl	H	H	Boc
7f	Et	H	methyl	H	Boc
7g	Me, Et	propyl	H	H	Pht, Boc
7h	Et	H	propyl	H	Boc
7i	Me	allyl	H	H	Pht
7j	Me	hydroxyethyl	H	H	Boc
7k	Me	methylcyclopropyl	H	H	Pht
7l	Me	isobutyl	H	H	
7m	Me	cyclopentyl	H	H	Pht
7n	Me	propyl	H	methyl	Pht
7o	Me	propyl	H	ethyl	Pht
7p	Me	H	$-CH_2CH_2-$		Pht
7q	Me	H	$-CH_2CH_2CH_2-$		Pht

[a] Boc = (*tert*-butyloxy)carbonyl. [b] Pht = phthalimido.

Scheme 2

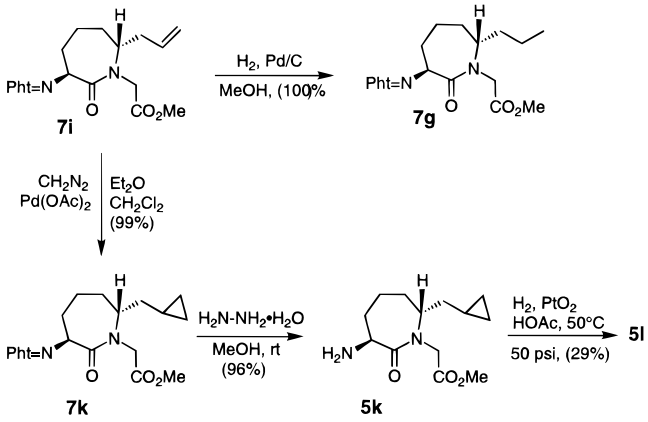


Scheme 3



position resulted in an additional 2-fold enhancement in both ACE and NEP activity *in vitro* relative to **2a**. More importantly, the activities of these compounds in the AI pressor assay were dramatically enhanced (8–14-fold) by this modification. The reason for this boost in *in vivo* activity is unclear. It may be that the presence of alkyl substituents at this position of the molecule confers greater metabolic stability to the inhibitors. A comparison of **2e** with **2f** and of **2g** with

Scheme 4



2h indicates that the preferred stereochemistry at C-7 with respect to NEP is *S*. The corresponding *R* isomers of **2e,g** were 3- and 11-fold less potent versus NEP *in vitro*. Surprisingly ACE seemed relatively insensitive to both the nature and stereochemical orientation of the substituent at this position.

We next explored a series of monocyclic based inhibitors which varied with respect to steric bulk at the R^1 position. Allyl-substituted azepinone **2i** was slightly less potent than its dihydro analog **2g** versus NEP, whereas the hydroxyethyl analog **2j** was somewhat more potent. Both the allyl and the hydroxyethyl analogs displayed activity comparable to **2g** against ACE both *in vitro* and *in vivo*. NEP proved to be exquisitely sensitive to the steric nature of the R^1 substituent. The addition of a single methylene group in **2g**, giving **2k**, resulted in a 3.5-fold loss in NEP inhibitory potency. More surprisingly, replacement of the propyl group in **2g** with isobutyl, a one-carbon addition, decreased the inhibitors affinity for NEP by 40-fold. Introduction of the sterically demanding cyclopentyl group in **2m** had a similar effect on NEP inhibitory potency. In sharp contrast, *in vitro* activity versus ACE did not vary more than 3-fold among the C-7-substituted azepinones stud-

Scheme 5

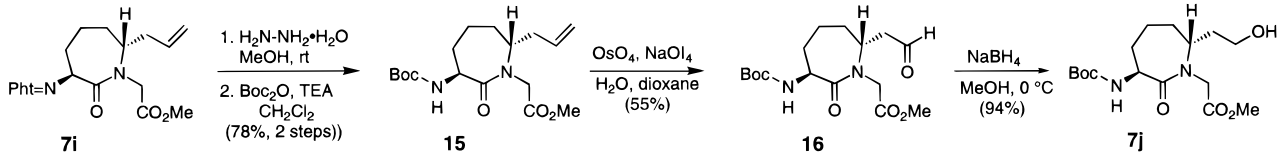
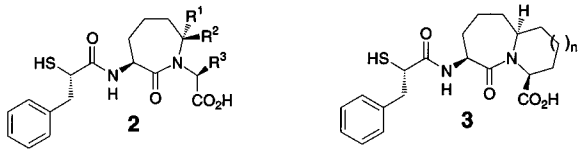


Table 2. Inhibition of ACE and NEP for Compounds **2** and **3**



no.[a]	R^1	R^2	R^3	IC_{50} (nM) NEP[b]	IC_{50} (nM) ACE[c]	ACE AI pressor[d] ED_{50} (μmol/kg iv)	yield from **7** (%)	$[\alpha]_D$ (deg) (*c*, solvent)	formula[e]
1a				400	30	0.06			
4				6	12	0.02			
2a	H	H	H	82	22	0.84	83	+8.0 (0.6, CH_3OH)	$C_{17}H_{22}N_2O_4S$
2b	H	H	methyl	92	26	1.22	67	−39.1 (0.6, $CHCl_3$)	$C_{18}H_{24}N_2O_4S$[f]
2c	H	H	benzyl	32	479	NT[g]	65	−64.2 (0.5, $CHCl_3$)	$C_{24}H_{28}N_2O_4S·0.3H_2O$
2d	H	H	isopropyl	1000	907	NT	75	−90.4 (0.8, CH_3OH)	$C_{20}H_{28}N_2O_4S$
2e	methyl	H	H	49	8	0.11	37	−28.7 (0.3, CH_3OH)	$C_{18}H_{24}N_2O_4S$
2f	H	methyl	H	149	16	NT	37	+16.8 (0.3, CH_3OH)	$C_{18}H_{24}N_2O_4S·0.1H_2O$
2g	propyl	H	H	40	11	0.06	50	−51.2 (0.7, $CHCl_3$)	$C_{20}H_{28}N_2O_4S·0.3H_2O$[h]
2h	H	propyl	H	466	14	NT	75	+7.6 (0.8, $CHCl_3$)	$C_{20}H_{28}N_2O_4S·0.1H_2O$
2i	allyl	H	H	64	7	0.04	55	−48.5 (0.6, $CHCl_3$)	$C_{20}H_{26}N_2O_4S·0.2H_2O$
2j	hydroxyethyl	H	H	31	25	0.08	18	−41.9 (1.0, $CDCl_3$)	$C_{19}H_{26}N_2O_5S·0.5H_2O$
2k	methylcyclopropyl	H	H	140	23	NT	66	−78.9 (1.0, $CDCl_3$)	$C_{21}H_{28}N_2O_4S·0.5H_2O$
2l	isobutyl	H	H	1655	10	NT	28	−40.1 (1.0, $CDCl_3$)	$C_{21}H_{30}N_2O_4S$
2m	cyclopentyl	H	H	1653	17	NT	36	−78.1 (1.0, $CDCl_3$)	$C_{22}H_{30}N_2O_4S$
2n	propyl	H	methyl	17	5	0.12	30	−51.6 (0.5, CH_2Cl_2)	$C_{21}H_{30}N_2O_4S$
2o	propyl	H	ethyl	86	25	NT	31	−57.7 (0.4, CH_2Cl_2)	$C_{22}H_{32}N_2O_4S·0.2EtOAc$
3p ($n = 0$)				18	5	0.06	76	−107.9 (0.6, $CHCl_3$)	$C_{19}H_{24}N_2O_4S$
3q ($n = 1$)				25	11	0.12	80	−31.0 (0.8, $CHCl_3$)	$C_{20}H_{26}N_2O_4S·0.45H_2O$[i]

[a] All spectral data were consistent with the assigned structures. [b] Compounds were assayed against purified rat kidney neutral endopeptidase using a fluorometric assay with dansyl-Gly-Phe-Arg as the substrate. [c] Compounds were assayed against angiotensin-converting enzyme isolated from rabbit lung extract using hippuryl-L-histidyl-L-leucine (HHL) as the substrate. [d] Represents dose required for 50% inhibition of the Al pressor response in normotensive rats. See the Experimental Section for a description of this assay. [e] Analyzed for C, H, N, and S. Results were within ±0.4% of theory unless otherwise noted. [f] Anal. Calcd: S, 8.80. Found: S, 8.36. [g] Not tested. [h] Anal. Calcd: S, 8.06. Found: S, 7.63. [i] Anal. Calcd: N, 7.03. Found: N, 6.57.

ied. In combination with other studies we have done with mercaptoacetyl-containing inhibitors, these results underscore our belief that NEP is much more sensitive than ACE with respect to the nature of the ligands within their binding subsites.

In the case of **2a**, introduction of a methyl group on the acetic acid side chain to give **2b** had little effect on inhibitory potency versus either enzyme. In contrast, the C-7 propyl, α-methyl side chain analog **2n** exhibited 2 times the potency against both ACE and NEP *in vitro* as compared to its desmethyl counterpart **2g**. Inhibitor **2n** was found to be the most potent of the monocyclic based ACE/NEP inhibitors in this study, and its *in vitro* activity compared favorably with that of the previous lead benzazepinone **4**. Although the ED_{50} of **2n** is 6-fold less than that of **4**, its duration of activity in this assay is longer ($t_{1/2} \approx 50$ min for **2n** versus $t_{1/2} \approx 20$ min for **4**) at a comparable dose of 0.5 μmol/kg iv. The ethyl analog of **2n**, azepinone **2o**, was 5-fold less active with respect to both ACE and NEP *in vitro*.

The potent activity observed by the fused bicyclic lactams **3p,q** underscores our previous findings[3d] that greater *in vitro* inhibitory potency versus both metalloproteases, especially NEP, can be more easily attained with conformationally constrained *bicyclic* mimetics as opposed to their *monocyclic* counterparts. The 7,6-fused bicyclic lactam **3q** was found to be comparable in potency to **2n** both *in vitro* and *in vivo* (iv administration). The related 7,5-fused analog **3p** was 2-fold more active than either **3q** or **2n** as an ACE inhibitor *in vivo*, but this increase in activity was not accompanied by an improved duration of activity in the AI pressor assay ($t_{1/2} \approx 30$ min for **3p** versus $t_{1/2} \approx 60$ min for **3q** at a comparable dose of 0.5 μmol/kg iv). We have previously found that in various bicyclic fused inhibitors, an α-configured hydrogen at the bridgehead center is optimal for good inhibitory potency versus NEP.[12] The excellent *in vitro* potency of **3p,q** against both NEP and ACE supports this statement. In contrast, optimal activity versus NEP in the monocyclic class of compounds could only be obtained when the related hydrogen at the C-7 position was in the β-configuration. It is suspected that the presence of the substituent at the C-7 position serves to effect the overall conformation of the monocyclic azepinone ring, positioning the C-3 amine, the C-2 carbonyl group, and the acetic acid side chain in an optimum orientation for binding similar to that attained by the bicyclic class of inhibitors. Thus when the alkyl substituent in **2** is α (hydrogen is β), the monocyclic seven-membered lactam ring is believed to adopt a conformation similar to that of the highly active 7,6(5)-fused bicyclic class of inhibitors **3**.

Chart 1. ACE/NEP Inhibitors in the 1K DOCA Rats at 100 μmol/kg iv

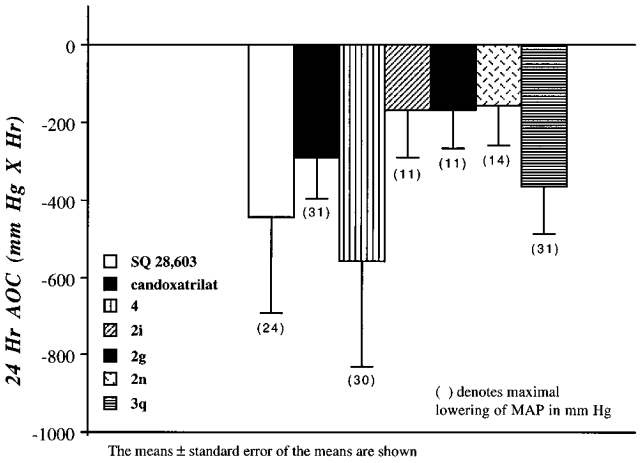
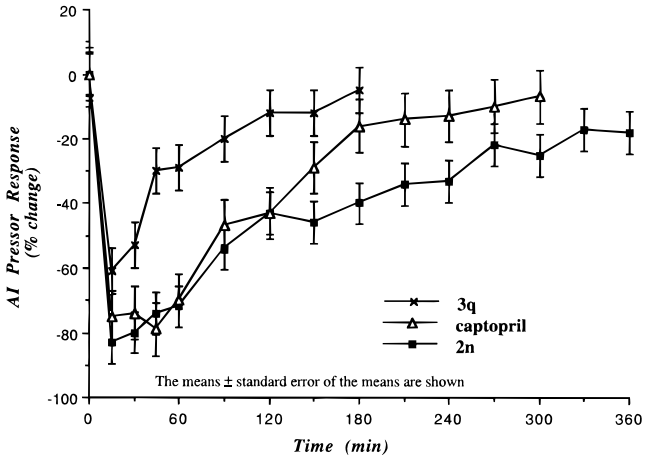


Chart 2. Inhibition of the AI Pressor Response after Oral Administration at 5 μmol/kg in Rats



Compounds which displayed acceptable iv activity in the AI pressor assay and possessed reasonable potency versus NEP *in vitro* were tested in the 1K DOCA salt hypertensive rat assay. Selective NEP inhibitors have been shown to lower mean arterial pressure in this assay, whereas selective ACE inhibitors (i.e., captopril) are usually ineffective. Compounds were given as bolus injections at 100 μmol/kg (≈40 mpk) iv, and mean arterial pressure (MAP) was measured over a 24 h period. The data in Chart 1 represent the compiled area-over-the-curve (AOC) during this time period. Dual inhibitor **3q** was essentially equipotent to the selective NEP inhibitors SQ 28,603[13] and candoxatrilat.[14] Monocyclic based inhibitors **2i,g,n** were somewhat less effective but still showed a significant antihypertensive effect in this assay. Benzazepinone-based inhibitor **4** was the most potent compound in this comparison, displaying activity only slightly better than that of SQ 28,603 and **3q**. Since the antihypertensive activity of selective NEP inhibitors is not potentiated by the addition of ACE inhibitors in this assay,[1a] the ability of the dual-acting inhibitors to lower MAP is attributed to their intrinsic ability to singularly inhibit NEP *in vivo*.

Based on their potent activity against ACE and NEP both *in vitro* and *in vivo*, compounds **2n** and **3q** were evaluated orally in the AI pressor assay at a comparative dose of 5 μmol/kg (≈2 mpk) (Chart 2). Bicyclic lactam **3q** was less effective than captopril with respect to both potency and duration of action. In contrast, monocyclic azepinone **2n** exhibited activity in this assay which was slightly superior to that of captopril.

Conclusion

In previous studies we have demonstrated that replacement of the dipeptide portion of mercaptoacetyl dipeptides with suitably constrained peptidomimetics can dramatically enhance the ability of these compounds to act as dual ACE and NEP inhibitors both *in vitro* and *in vivo*. In this work we have concentrated on the utilization of non-benzo-fused azepinones as conformationally restrained mimetics of Ala-Pro, applying new chemical methods developed in this laboratory for their preparation. Structure−activity relationships within these classes of compounds clearly demonstrate that potent *in vitro* activity versus both ACE and NEP can be obtained with 7,5- or 7,6-fused bicyclic azepinones or with appropriately substituted monocyclic azepinones. Both the bicyclic inhibitor **3q** and the monocyclic inhibitor **2n** exhibited high levels of activity *in vitro* and *in vivo* versus ACE and NEP. In the AI pressor assay, compound **2n** also demonstrated oral activity that was slightly superior to that of captopril, a clinically proven ACE inhibitor. A more detailed pharmacological evaluation of these conformationally restrained monocyclic and bicyclic mercaptoacetyl dipeptides and their related analogs will be presented in the near future.

Experimental Section

All reactions were carried out under a static atmosphere of argon, and the mixtures were stirred magnetically unless otherwise noted. All reagents used were of commercial quality and obtained from Aldrich Chemical Co. or Sigma Chemical Co. DMF was obtained from American Burdick and Jackson and used without purification. Dry CH_2Cl_2 was obtained by distillation from CaH_2 under nitrogen. 3-(Trimethylsilyl)-cyclopentene was purchased from Pfaltz & Bauer, Inc. Melting points were obtained on a Hoover Unimelt melting point apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Sirius 100-FTIR spectrophotometer. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JEOL GSX400 spectrometer using Me_4Si as an internal standard. Optical rotations were measured in a 1 dm cell on a Perkin-Elmer 241 polarimeter, and *c* is expressed in g/100 mL. All flash chromatographic separations were performed using E. Merck silica gel 60 (particle size, 0.040−0.063 mm). Reactions were monitored by TLC using 0.25 mm E. Merck silica gel plates (60 F_{254}) and were visualized with UV light or 5% phosphomolybdic acid in 95% EtOH.

General Procedure for the Conversion of Phthalimido-Protected Azepinones 7 to Their Corresponding Amines 5. (3*S*)-*trans*-3-Amino-7-(cyclopropylmethyl)-hexahydro-2-oxo-1*H*-azepine-1-acetic Acid, Methyl Ester (5k). A solution of phthalimido-protected azepinone **7k** (697 mg, 1.81 mmol) in methanol (8 mL) at room temperature was treated with hydrazine monohydrate (92 μL, 95 mg, 1.90 mmol). After 2 days of stirring, the reaction mixture was filtered to remove the phthalhydrazide byproduct, and the solid was washed well with methanol. The filtrate was concentrated, and the residue was triturated with CH_2Cl_2 to precipitate out additional byproduct. Filtration followed by removal of the solvent provided pure amine **5k** (441 mg, 96% of theory) as a white foam which was used without further purification: TLC R_f 0.25 (1:9 MeOH:CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.09 (m, 2H), 0.49 (m, 2H), 0.64 (m, 1H), 1.50 (m, 2H), 1.71−2.05 (m, 6H), 3.47 (m, 1H), 3.53 (d, J = 9.4 Hz, 1H), 3.74 (s, 3H), 3.76 (d, J = 17 Hz, 1H), 4.66 (d, J = 17 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 176.75, 170.36, 61.74, 54.37, 52.70, 52.06, 37.23, 33.38, 31.04, 21.66, 8.70, 4.58.

(3*S*)-*trans*-3-Aminohexahydro-7-(2-methylpropyl)-2-oxo-1*H*-azepine-1-acetic Acid, Methyl Ester (5l). A solution of amine **5k** (405 mg, 1.59 mmol) in HOAc (10 mL) and MeOH (3 mL) was charged with PtO_2 on carbon (30% by weight, 122 mg) and subsequently hydrogenated (45 psi) on a Parr apparatus at 50 °C. An additional 60 and 30 mg of PtO_2 on carbon were added after 60 and 104 h, respectively. After a total of 5 days, the solution was passed through a polycarbonate filter and the filtrate was concentrated to give an oil. The oil was dissolved in CH_2Cl_2 and subsequently washed with saturated $NaHCO_3$. Drying of the solution (Na_2SO_4) followed by removal of the solvent gave crude **5l** (120 mg, 29%) as an oil: TLC R_f 0.30 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.90 (d, J = 6.0 Hz, 3H), 0.94 (d, J = 6.4 Hz, 3H), 1.25 (m, 1H), 1.56−2.06 (m, 8H), 3.4 (m, 1H), 3.68 (d, J = 17.1 Hz, 1H), 3.73 (s, 3H), 4.12 (d, J = 13 Hz, 1H), 4.62 (d, J = 17.1 Hz, 1H), 5.26−5.30 (s, 2H).

General Procedure for the Coupling of Aminoazepinones 5 with (*S*)-2-(Acetylthio)benzenepropionic Acid. [α*S*-(α*R,2*R**,3*R**)]-3-[[2-(Acetylthio)-1-oxo-3-phenylpropyl]amino]hexahydro-α-(1-methylethyl)-2-oxo-1*H*-azepine-1-acetic Acid, Methyl Ester (6d).** A solution of (*S*)-2-(acetylthio)benzenepropionic acid[9] (463 mg, 2.06 mmol) and free amine **5d** (416 mg, 1.72 mmol) in CH_2Cl_2 (20 mL) was cooled in an ice bath and treated with TEA (260 μL, 189 mg, 1.86 mmol) followed by (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent; 770 mg, 1.74 mmol). The mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h. The solvent was removed by rotary evaporation, and the residue was dissolved in EtOAc and washed successively with 0.5 N HCl, H_2O, 50% saturated $NaHCO_3$, and brine. After drying (Na_2SO_4), the organic layer was filtered and concentrated *in vacuo*. Flash chromatography (1:3 EtOAc:hexanes as eluant) afforded diastereomerically pure **6d** (743 mg, 96%) as an oil: TLC R_f 0.62 (1: EtOAc: hexanes); ^{1}H NMR ($CDCl_3$) δ 0.78 (d, J = 6.8 Hz, 3H), 0.96 (d, J = 6.0 Hz, 3H), 1.11−1.44 (m, 2H), 1.70−2.23 (m, 6H), 2.32 (s, 3H), 3.00 (dd, J = 7.7, 1.41 Hz, 1H), 3.29 (m, 2H), 3.56 (d, J = 14.9 Hz, 1H), 3.70 (s, 3H), 4.30 (pseudo-t, 1H), 4.52 (dd, J = 6.2, 10.9 Hz, 1H), 4.91 (d, J = 10.7 Hz, 1H), 7.15−7.31 (m, 5H), 7.43 (d, J = 5.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 194.55, 173.03, 171.25, 169.07, 137.60, 129.20, 128.39, 126.82, 61.56, 52.73, 51.90, 48.16, 43.49, 36.82, 31.22, 30.39, 27.41, 27.37, 19.47, 18.64.

General Procedure for the Saponification of 6 to Inhibitors 2 or 3. [α*S*-(α*R,3*R**)]-Hexahydro-3-[(2-mercapto-1-oxo-3-phenylpropyl)amino]-α-(1-methylethyl)-2-oxo-1*H*-azepine-1-acetic Acid (2d).** A solution of **6d** (658 mg, 1.47 mmol) in CH_3OH (10 mL) was purged with argon for 30 min and then cooled to 0 °C and treated with 1 N NaOH (5.9 mL, previously purged with argon for 30 min). Bubbling of argon through the solution was maintained throughout the addition and length of the reaction. After stirring at 0 °C for 3 h and at room temperature for 3 h, the mixture was acidified with 5% $KHSO_4$ and extracted with EtOAc. The EtOAc extract was washed with H_2O and brine and then dried (Na_2SO_4), filtered, and concentrated to give a solid. Trituration of the solid with 1:3 EtOAc:Et_2O (30 mL) gave pure **2d** (451 mg, 78%): TLC R_f 0.72 (5:95 HOAc:EtOAc); mp 217−219 °C; $[\alpha]_D$ = −90.4° (*c* 0.5, CH_3OH); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 0.79 (d, J = 6.8 Hz, 3H), 1.01 (d, J = 6.4 Hz, 3H), 1.45 (m, 2H), 1.71−2.26 (m, 5H), 3.08 (dd, J = 7.3, 13.7 Hz, 1H), 3.24 (dd, J = 6.8, 13.7 Hz, 1H), 3.37 (m, 1H), 3.61 (m, 2H), 4.56 (dd, J = 6.4, 9.4 Hz, 1H), 4.80 (d, J = 10.7 Hz, 1H), 7.15−7.40 (m, 5H), 7.80 (d, J = 6.0 Hz, 1H); ^{13}C NMR ($CDCl_3$/CD_3OD) δ 173.60, 172.63, 171.83, 137.79, 129.47, 128.61, 127.12, 62.41, 52.98, 44.60, 44.12, 41.71, 31.34, 27.57, 27.40, 27.22, 19.67, 18.78. Anal. Calcd for $C_{20}H_{28}N_2O_4S$: C, 61.20; H, 7.19; N, 7.14; S, 8.17. Found: C, 61.26; H, 7.27; N, 7.14; S, 8.03.

(3*S*)-*trans*-Hexahydro-3-phthalimido-2-oxo-7-propyl-1*H*-azepine-1-acetic Acid, Methyl Ester (7g). A solution of **7i** (767 mg, 2.07 mmol) in methanol (10 mL) and EtOAc (10 mL) was hydrogenated (balloon) over palladium on carbon (10%, 62 mg) at room temperature for 1 h. The solution was filtered through Celite, and the solvent was removed by rotary evaporation to give pure **7g** (771 mg, 100%) as a white foam: TLC R_f 0.29 (1:1 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 0.99 (t, J = 7.2 Hz), 1.38 (m, 2H), 1.81−2.17 (m, 8H), 2.74 (m, 1H), 3.43 (m, 1H), 3.71 (s, 3H), 3.73 (d, J = 17.2 Hz, 1H), 4.66 (d, J = 17.2 Hz, 1H), 5.06 (dd, J = 1.9, 12.6 Hz, 1H), 7.70 (m, 2H), 7.82 (m, 2H); IR ($CHCl_3$ film) 1751, 1715, 1649, 1387, 1208, 719 cm^{-1}.

(3*S*)-*trans*-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-hexahydro-7-(2-hydroxyethyl)-2-oxo-1*H*-azepine-1-acetic Acid, Methyl Ester (7j). An ice-chilled solution of aldehyde **16** (309 mg, 0.90 mmol) in MeOH (5 mL) was treated portionwise with $NaBH_4$ (68 mg, 1.80 mmol). After 1.5 h, the reaction was quenched with H_2O; the mixture was warmed to room temperature and then partitioned between EtOAc and 1 N HCl. The aqueous layer was extracted twice with additional EtOAc, and the pooled organic extracts were washed with saturated $NaHCO_3$ and brine and then dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (2:1 EtOAc:hexanes followed by EtOAc as eluant) provided azepinone **7j** (292 mg, 94%) as a clear oil: TLC R_f 0.30 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.84−2.08 (m, 8H), 3.74 (m, 6H), 3.94 (d, J = 17.1 Hz, 1H), 4.32 (m, 1H), 4.51 (d, J = 17.1 Hz, 1H), 5.91 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 173.21, 170.28, 155.10, 79.85, 59.12, 57.59, 53.96, 52.67, 52.12, 34.21, 31.93, 30.87, 28.39, 21.68.

(3*S*)-*trans*-7-(Cyclopropylmethyl)-3-(2,3-dihydro-1,3-dioxo-1*H*-isoindol-2-yl)hexahydro-2-oxo-1*H*-azepine-1-acetic Acid, Methyl Ester (7k). Following the procedure of Suda,[10] a solution of alkene **7i** (700 mg, 1.89 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added to a solution of diazomethane in Et_2O followed by $Pd(OAc)_2$ (7 mg, 0.03 mmol). The reaction mixture bubbled vigorously and gradually turned clear. After 10 min, an additional 25 mL of ethereal diazomethane was added and the mixture was stirred for an additional 50 min at 0 °C. The solution was filtered through Celite and concentrated by rotary evaporation. Flash chromatographic purification of the resulting oil (1:2 EtOAc:hexanes as eluant) provided azepinone **7k** (717 mg, 99%) as a white foam: TLC R_f 0.30 (1:1 hexanes:acetone); ^{1}H NMR ($CDCl_3$) δ 0.16 (m, 2H), 0.52 (m, 2H), 0.70 (m, 1H), 1.25−2.18 (m, 6H), 2.72 (m, 1H), 3.62 (m, 1H), 3.72 (s, 3H), 3.78 (d, J = 17.6 Hz, 1H), 4.74 (d, J = 17.6 Hz, 1H), 5.01 (d, J = 13.2 Hz, 1H), 7.68 (m, 2H), 7.83 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.65, 170.71, 160.85, 133.85, 131.91, 123.33, 61.79, 55.09, 52.67, 52.09, 36.51, 31.13, 28.77, 22.69, 8.78, 4.55, 4.46.

(3*S*)-*trans*-7-Cyclopentylhexahydro-2-oxo-3-phthalimido)-1*H*-azepine-1-acetic Acid, Methyl Ester (7m). A mixture of compound **14** (2:1 mixture, 700 mg, 1.89 mmol) and Pd on carbon (10% by wt, 150 mg) in MeOH (5 mL) was hydrogenated (balloon) at room temperature for 5 h. The reaction mixture was filtered through Celite and concentrated to give diastereomerically pure **7m** (594 mg, 97%) as a white foam: TLC R_f 0.30 (1:1 hexanes:acetone); ^{1}H NMR ($CDCl_3$) δ 1.21 (m, 1H), 1.25 (m, 1H), 1.57 (s, 3H), 1.68 (m, 3H), 1.70−2.19 (m, 5H), 2.73 (m, 2H), 3.14 (dt, J = 11.1 Hz, 1H), 3.55 (d, J = 17.1 Hz, 1H), 3.72 (s, 3H), 4.89 (d, J = 17.1 Hz, 1H), 5.15 (d, J = 12.6 Hz, 1H), 7.69 (m, 2H), 7.82 (m, 2H); IR (CH_2Cl_2 film) 2924, 2868, 1751, 1715, 1653, 1468, 1387 cm^{-1}.

[3*S*-(1R*,3α,7*β*)]-Hexahydro-α-ethyl-2-oxo-3-phthalimido-7-propyl-1*H*-azepine-1-acetic Acid, Methyl Ester (7o). A mixture of compound **12b** (580 mg, 1.46 mmol) and Pd on carbon (10%, 60 mg) in EtOAc (10 mL) was hydrogenated (balloon) at room temperature for 3.5 h. The reaction mixture was filtered through Celite and concentrated to give pure **7o** (558 mg, 95%) as an oil: TLC R_f 0.52 (1:9 EtOAc:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 7.2 Hz, 3H), 1.01 (t, J = 7.0 Hz, 3H), 1.31 (m, 1H), 1.43 (m, 1H), 1.70 (m, 2H), 1.80−2.11 (m, 6H), 2.19 (m, 1H), 2.67 (m, 1H), 3.42 (m, 1H), 3.70 (s, 3H), 4.90 (dd, J = 3.8, 5.1 Hz, 1H), 5.07 (d, J = 8.8 Hz, 1H), 7.69 (m, 2H), 7.82 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 171.99, 170.64, 168.56, 134.08, 132.40, 123.54, 62.45, 57.41, 55.50, 52.13, 34.26, 29.55, 29.27, 22.72, 22.66, 20.78, 14.21, 11.45.

[*S*-(*R,*R**)]-α-[[2-(2,3-Dihydro-1,3-dioxo-1*H*-isoindol-2-yl)-1,6-dioxohexyl]amino]butanoic Acid, Ethyl Ester (10).** A solution of L-2-aminobutyric acid, ethyl ester hydrochloride (**9**; 9.70 g, 48.0 mmol) and 4-methylmorpholine (NMM; 6.44 mL, 5.92 g, 58.5 mmol) in DMF (100 mL) was treated with

acid **8**[8a] (9.48 g, 34.1 mmol) and 1-hydroxybenzotriazole hydrate (HOBT·xH$_2$O; 4.74 g, 35.1 mmol). The mixture was cooled in an ice bath and subsequently treated with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDAC; 7.84 g, 40.9 mmol). After 1 h at 0 °C and 2 h at room temperature, the mixture was partitioned between H_2O and EtOAc. The EtOAc extract was washed successively with H_2O, 0.5 N HCl, H_2O, 50% saturated aqueous $NaHCO_3$, and brine and then dried (Na_2SO_4), filtered, and concentrated to give the intermediate dipeptide alcohol (12.50 g, 94%) as a white foam which was used directly in the next reaction: TLC R_f 0.10 (1:1 EtOAc:hexane); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.4 Hz, 3H), 1.23 (t, J = 7.0 Hz, 3H), 1.40 (m, 2H), 1.55−1.80 (m, 3H), 1.91 (m, 1H), 2.24 (m, 1H), 2.46 (m, 1H), 3.62 (m, 2H), 4.18 (m, 2H), 4.56 (m, 1H), 4.87 (dd, J = 5.5, 10.8 Hz, 1H), 6.82 (d, J = 6.4 Hz, 1H), 7.76 (m, 2H), 7.82 (m, 2H); IR (CH_2Cl_2 film) 1717, 1670, 1539, 1385, 721 cm^{-1}; HRMS (FAB) calcd for $C_{20}H_{27}N_2O_6$ (M + H)$^+$ 391.1869, found 391.1860.

A −78 °C solution of oxalyl chloride (4.70 mL, 6.84 g, 54.9 mmol) in CH_2Cl_2 (114 mL) was treated dropwise with a solution of dry DMSO (5.75 mL, 6.33 g, 81.0 mmol) in CH_2Cl_2 (6 mL). After 10 min, a solution of the above alcohol (12.0 g, 30.7 mmol) in CH_2Cl_2 (50 mL) was added dropwise to the above mixture. Fifteen minutes after the addition, TEA (25 mL) was added and the mixture was stirred at −78 °C for 10 min and then warmed to 0 °C. The mixture was partitioned between EtOAc/Et_2O and H_2O. The organic layer was washed successively with 1 N HCl and brine and then dried (Na_2SO_4), filtered and concentrated. Flash chromatograph (1:1 EtOAc:hexanes as eluant) afforded aldehyde **10** (9.00 g, 75% from **8**) as a white solid: $[\alpha]_D$ + 17.0° (c 0.3, CH_2Cl_2); mp 125−127 °C; TLC R_f 0.34 (1: EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 7.4 Hz, 3H), 1.25 (t, J = 7.2 Hz, 3H), 1.57−1.77 (m, 3H), 1.90 (m, 1H), 2.24 (m, 1H), 2.32 (m, 1H), 2.52 (m, 2H), 4.15 (m, 2H), 4.54 (m, 1H), 4.83 (dd, J = 5.6, 10.8 Hz, 1H), 6.73 (d, J = 6.8 Hz, 1H), 7.75 (m, 2H), 7.87 (m, 2H), 9.76 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 201.43, 172.03, 168.17, 168.00, 134.45, 131.51, 123.71, 61.45, 54.22, 53.53, 42.93, 28.22, 25.42, 18.77, 14.08, 9.33; IR (KBr) 3289, 1734, 1659, 1549, 1385, 723 cm^{-1}; HRMS (FAB) calcd for $C_{20}H_{25}N_2O_6$ (M + H)$^+$ 389.1713, found 389.1705. Anal. Calcd for $C_{20}H_{24}N_2O_6$: C, 61.85; H, 6.23; N, 7.21. Found: C, 61.70; H, 6.13; N, 7.16.

[3*S*-(3α,6β,9aα)]-Tetrahydro-3-ethyl-2,5-dioxo-6-phthalimidooxazolo[3,2-*a*]azepine-2,5(3*H*,6*H*)-dione (11). A solution of aldehyde **10** (8.70 g, 22.4 mmol) and TFA (66 mL) in $CHCl_3$ (700 mL) was refluxed under argon for 6 days. The solvent was removed by rotary evaporation, and the residue was azeotroped twice with CH_2Cl_2. Flash chromatography (5:95 acetone:CH_2Cl_2 as eluant) afforded bicyclic lactam **11** (5.61 g, 73%) as a white solid in 94% diastereomeric purity as determined by NMR. Diastereomerically pure material was obtained by recrystallization from EtOAc/CH_2Cl_2: $[\alpha]_D$ +103.4° (c 0.59, CH_2Cl_2); mp 185−190 °C dec; TLC R_f 0.51 (5:95 acetone:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, J = 7.5 Hz, 3H), 1.67−2.48 (m, 8H), 2.78 (m, 1H), 4.55 (dd, J = 2.1, 6.0 Hz, 1H), 4.77 (dd, J = 1.7, 12.4 Hz, 1H), 5.81 (d, J = 9.8 Hz, 1H), 7.73 (m, 2H), 7.86 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.79, 167.96, 166.25, 134.23, 123.61, 90.43, 57.74, 55.15, 35.73, 28.62, 24.59, 22.32, 7.91; IR (KBr) 1800, 1719, 1659, 1391, 1364, 1211, 1057, 721 cm^{-1}.

[3*S*-(1*R,3α,7β)]-Hexahydro-α-ethyl-2-oxo-3-phthalimido-7-(2-propenyl)-1*H*-azepine-1-acetic Acid, Methyl Ester (12b).** Neat $TiCl_4$ (520 μL, 897 mg, 4.73 mmol) was added to a mixture of **11** (515 mg, 1.50 mmol) and allyltrimethylsilane (2.0 mL, 12.2 mmol) in CH_2Cl_2 (25 mL) at 0 °C. After 28 h, the reaction was quenched with H_2O and the solution extracted with EtOAc. The EtOAc extract was washed with brine, dried (Na_2SO_4), filtered, and stripped. Flash chromatography (EtOAc followed by 1:99 HOAc:EtOAc as eluant) of the residue provided the impure allyl-substituted azepinone **12a** (R = H; 319 mg) as an oil: TLC R_f 0.66 (2:98 HOAc:EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.97 (t, J = 7.6 Hz, 3H), 1.75−2.12 (m, 7H), 2.54−2.76 (m, 2H), 2.89 (m, 1H), 3.49 (m, 1H), 4.75 (m, 1H), 5.05 (d, J = 10.5 Hz, 1H), 5.14 (d, J = 10.0 Hz, 1H), 5.23 (d, J = 16.8 Hz, 1H), 5.73 (m, 1H), 7.71 (m, 2H), 7.84 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 176.89, 170.98, 168.94, 134.61, 134.57, 133.62, 124.09, 119.04, 58.06, 57.43, 55.80, 37.47, 29.86, 29.56, 23.02, 15.62.

A solution of **12a** in MeOH (15 mL) and CH_2Cl_2 (5 mL) was treated with excess ethereal diazomethane in Et_2O for 10 min at 0 °C. The excess diazomethane was destroyed by the addition of HOAc, and the solvent was removed by rotary evaporation. Flash chromatography (5:95 EtOAc:CH_2Cl_2 as eluant) provided pure methyl ester **12b** (179 mg, 30% fron **11**) as an oil: TLC R_f 0.52 (1:9 EtOAc:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 7.4 Hz, 3H), 1.69−2.05 (m, 7H), 2.56 (m, 1H), 2.68 (m, 1H), 2.93 (m, 1H), 3.51 (m, 1H), 3.70 (s, 3H), 4.86 (m, 1H), 5.06 (d, J = 10.5 Hz, 1H), 5.13 (d, J = 10.0 Hz, 1H), 5.22 (d, J = 17.0 Hz, 1H), 5.75 (m, 1H), 7.70 (m, 2H), 7.82 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 171.58, 170.31, 168.21, 133.96, 133.82, 132.05, 123.27, 118.07, 62.38, 57.19, 55.22, 51.88, 36.41, 29.29, 28.86, 22.40, 22.33, 11.15; IR (KBr) 1738, 1715, 1649, 1387, 719 cm^{-1}; HRMS (FAB) calcd for $C_{22}H_{27}N_2O_5$ (M + H)$^+$ 399.1905, found 399.1920.

(3*S*)-*trans*-7-(2-Cyclopenten-1-yl)hexahydro-2-oxo-3-phthalimido-1*H*-azepine-1-Acetic Acid (14). A solution of bicyclic lactam **13**[8a] (800 mg, 2.55 mmol) and 3-(trimethylsilyl)cyclopentene (2.85 g, 20.4 mmol) in CH_2Cl_2 (60 mL) was treated dropwise with $SnCl_4$ (1.0 M in CH_2Cl_2, 5.1 mmol). After stirring at room temperature for 18 h, the reaction was quenched by the addition of H_2O and the mixture subsequently extracted three times with EtOAc. The combined organic extracts was washed with brine, dried (Na_2SO_4), filtered, and concentrated to give an oil. Flash chromatography (1:1 EtOAc:hexanes followed by 2:98 HOAc:EtOAc as eluant) provided the slightly impure carboxylic acid (788 mg, 81%) as a foam: TLC R_f 0.55 (5.95 HOAc:EtOAc).

The acid (768 mg, 2.01 mmol) was dissolved in MeOH (7 mL) and Et_2O (10 mL) and treated with an excess of ethereal diazomethane for 10 min. The excess CH_2N_2 was destroyed by the addition of HOAc, and the solvent was removed by rotary evaporation. Flash chromatography (1:2 EtOAc:hexanes as eluant) provided compound **14** (640 mg, 65% from **13**) as a white foam (2:1 mixture of diastereomers at the asterisk as determined by NMR): TLC R_f 0.60 (2% HOAc in EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.61 (m, 1H), 1.90−2.20 (m, 5H), 2.43 (m, 1H), 2.82 (m, 1H), 3.20 (m, 2H), 3.46 (d, J = 17.6 Hz, 1H), 3.59 (d, J = 17.6 Hz, 1H), 3.72 (s, 3H), 4.88 (dd, J = 17, 16.9 Hz, 1H), 5.15 (t, J = 10.6 Hz, 1H), 5.7 (m, 1H), 5.92 (m, 1H), 7.69 (m, 2H), 7.84 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.35, 133.88, 133.79, 133.21, 132.09, 130.91, 123.36, 65.94, 54.89, 53.62, 53.24, 52.15, 47.46, 31.76, 29.43, 28.51, 28.36, 27.59, 22.35; IR (CH_2Cl_2 film) 2922, 1777, 1751, 1715, 1653, 1468, 1385 cm^{-1}.

(3*S*)-*trans*-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-hexahydro-2-oxo-7-(2-propenyl)-1*H*-azepine-1-acetic Acid, Methyl Ester (15). A solution of azepinone **7i** (609 mg, 2.45 mmol) in methanol (5 mL) at room temperature was treated with hydrazine monohydrate (133 μL, 137 mg, 2.74 mmol). After stirring at room temperature for 64 h, the reaction mixture was filtered to remove the precipitated byproduct, and the solid was washed well with methanol. The filtrate was concentrated, and the residue was triturated with CH_2Cl_2 to precipitate out additional byproduct. Filtration followed by removal of the solvent provided the crude amine as a pale yellow oil. The oil was redissolved in CH_2Cl_2 (10 mL) and treated sequentially with TEA (510 μL, 370 mg, 3.65 mmol) and di-*tert*-butyl dicarbonate (Boc_2O; 642 mg, 2.94 mmol). After 16 h at room temperature, the mixture was charged with additional TEA (170 μL) and Boc_2O (160 mg), and the reaction was continued for an additional 16 h. The mixture was diluted with CH_2Cl_2, subsequently washed with H_2O, and then dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography (1:6 EtOAc:hexane followed by 1:4 EtOAc:hexanes as eluant) gave compound **15** (651 mg, 78%) as a clear oil: TLC R_f 0.70 (1:9 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.85−2.08 (m, 6H), 2.60 (m, 2H), 3.41 (m, 1H), 3.68 (d, J = 17 Hz, 1H), 3.74 (s, 3H), 4.37 (m, 1H), 4.60 (d, J = 17 Hz, 1H), 5.10 (d, J = 11 Hz, 1H), 5.18 (d, J = 1.8 Hz, 1H), 5.70 (m, 1H), 5.89 (d, J = 6.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 173.56, 170.01, 155.10, 79.52, 61.56, 53.88, 52.55, 52.09, 31.76, 30.23, 28.39, 21.16.

(3*S*)-*trans*-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-hexahydro-2-oxo-7-(2-oxoethyl)-1*H*-azepine-1-acetic Acid, Methyl Ester (16). A solution of osmium tetraoxide (2.5% by wt in *t*-BuOH, 400 μL, 0.98 mmol) was added to a solution of compound **15** (554 mg, 1.63 mL) and $NaIO_4$ (731 mg, 3.42 mmol) in a 1:1 mixture of H_2O and dioxane (6 mL). After stirring at room temperature for 16 h, the mixture was filtered and concentrated in vacuo. The residue was directly flash chromatographed (1:4 EtOAc:hexanes as eluant) to give aldehyde **16** (309 mg, 55%) as a clear oil: TLC R_f 0.10 (1:3 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.51−2.08 (m, 6H), 3.04 (dd, J = 2.9, 6.4 Hz, 1H), 3.28 (dd, J = 2.9, 6.4 Hz, 1H), 3.34 (m, 1H), 3.73 (s, 3H), 4.11−4.26 (m, 1H), 4.29 (d, J = 2.94 Hz, 2H), 5.91 (s, 1H), 9.77 (s, 1H)); ^{13}C NMR ($CDCl_3$) δ 198.70, 173.80, 170.04, 91.15, 79.95, 69.99, 65.68, 62.55, 54.40, 54.08, 52.67, 52.15, 46.85, 31.88, 31.44, 28.39, 22.08.

Purification of Rat Kidney Neutral Endopeptidase. Neutral endopeptidase was solubilized and purified from rat kidney membrane fractions using, with slight modification, the procedure described by Almenoff and Orlowski.[15] The enzyme was solubilized from rat kidney membrane fractions with a 1% sodium deoxycholate solution in 50 mM Tris-HCl buffer (pH 7.6) and released from the membrane fragments by incubation at 37 °C for 90 min with papain (40 μg/mg of protein) and 5 mM dithiothreitol. Papain was completely separated from kidney metallopeptidases by gel filtration using Sephacryl S-200. Aminopeptidase activity, which constituted a major portion of kidney metallopeptidase activity, was effectively removed by hydrophobic chromatography using phenyl sepharose. Fractions containing endopeptidase activity were pooled, concentrated by ultrafiltration, dialyzed against 50 mM Tris-HCl buffer (pH 7.6), and stored at 4 °C.

***In Vitro* Inhibition of Purified Rat Kidney Neutral Endopeptidase.** This assay is based on the cleavage of the fluorescent dansylated tripeptide dansyl-Gly-Phe-Arg to form the strongly fluorescent dansyl-Gly which is extracted from the acidified mixture by ethyl acetate. Incubation mixtures employed for the assay contained (in mM) the substrate dansyl-Gly-Phe-Arg, 0.5; Tris-HCl buffer (pH 7.5), 50; enzyme preparation in 0.05% Triton X-100; and varied concentrations of inhibitor solution or water to a final volume of 0.25 mL. Inhibitor solutions were preincubated for 10 min with the enzyme peparation and buffer before the reaction was initiated by the addition of the substrate. Incubation was carried out at 37 °C for 30 min, and the reaction was stopped by the addition of 0.25 mL of 1 N HCl and by extraction of the dansyl-Gly product into 1.5 mL of ethyl acetate. Relative fluorescent intensity (RFI) of the ethyl acetate extract was measured using a Perkin-Elmer LS-5B luminescence spectrometer at the excitation and emission wavelengths of 342 and 510 nm, respectively, corrected for the RFI of a zero-time control and compared with assays lacking inhibitor.

***In Vitro* Inhibition of Rabbit Lung Angiotensin-Converting Enzyme.** Compounds were evaluated for their ACE inhibitory potencies using the spectrophotometric assay of Cushman and Cheung.[16] In the assay, rabbit lung acetone powder extract was used as the source of ACE, and the rate of hippuric acid formation from the substrate hippuryl-L-histidyl-L-leucine (HHL) was monitored. Each 250 μL assay mixture contained the following at the indicated final concentration (in mM): phosphate buffer (pH 8.3), 100; NaCl, 300; HHL, 5; 100 μL of rabbit lung acetone powder extract (1:20 dilution); and 50 μL of varied concentrations of compound solution or vehicle. Following 30 min of incubation at 37 °C, the reaction was terminated by the addition of 250 μL of 1 N HCl followed by 1.5 mL of ethyl acetate. After mixing and centrifuging, a 1.0 mL aliquot of the ethyl acetate layer was removed and evaporated to dryness. Hippuric acid was reconstituted in 1 mL of water, and its concentration was determined spectrophotometrically at 228 nm.

***In Vivo* Biological Evaluation: AI Pressor Response Assay.** The procedure described is a modification of that reported by Rubin et al.[17] Sprague−Dawley male rats (220−240 g) were anesthetized and implanted with abdominal aortic and vena caval catheters. The rats were individually housed and placed on regular rat chow diet and water *ad libitum*. Approximately 2 weeks later, aortic blood pressure was monitored directly in the conscious rats. Four rats were used for each assay. Pressor responses to intravenous injections of AI (310 ng/kg) were obtained before (control) and at 2−30 min intervals up to 6 h after intravenous or oral administration of test compounds. Compounds were administered at various doses in 5% $NaHCO_3$ (intravenous) or 0.25% agar (oral). Inhibition (percent change from control) of the AI pressor response was determined at each interval. A dose−response curve was constructed using the time point after each dose showing maximum inhibition. The median effective dose (ED_{50}), which represents the dose required to effect 50% inhibition of the AI-induced pressor response, was determined by interpolation.

1-Kidney DOCA Salt Rat Assay. This low-renin model of hypertension was used to evaluate the ability of the compounds to lower MAP *in vivo*. The procedure described is a modification of that reported by Sybertz et al.[18] Male Sprague−Dawley rats (150 g) wre anesthetized with sodium pentobarbital (50 mg/kg ip), and the left kidney was ligated with nonabsorbable suture and removed. An indwelling catheter was implanted a short distance into the abdominal aorta terminating below the origin of the renal ateries. The abdominal muscle was then closed, a 100 mg pellet of deoxycorticosterone acetate (DOCA) was placed subcutaneously, and the abdominal skin was closed using stainless steel clips. The rats were individually housed and placed on 0.9% NaCl/0.2% KCl drinking water and regular rat chow diet. After a minimum of 2−3 weeks, a sustained hypertension of 165−175 mmHg was established. Each rat was placed in a harness which was secured to one end of a tightly wound spring. The other end of the spring was passed through the top of the cage containing the rat and attached to the lower end of a feed-through miniature swivel (King Chatham). The steel spring served as a protective cover for the catheter within it that connected the aterial cannula to the swivel connector. The upper portion of the swivel was provided with two connections: one to a Cobe transducer through a rotary fluid switch and the other to a pressurized (13 lb/in.^{2}) reservoir that provided a slow flow (0.03−0.06 mL/min) of saline into the aterial cannula to maintain patency.

The animals (N = 6−10) were dosed sid intravenously with the appropriate compound (100 μmol/kg) in 1 mL/kg 2.5% $NaHCO_3$ or with vehicle (1 mL/kg). Blood pressure was measured every 5 min over a 10 s period. Six such sets of data were averaged to give a mean value representing a 30 min sample. Measurements were continued over a 24 h period. The data were plotted as blood pressure (mmHg) versus time or, as in the case of Chart 1, are represented as the cumulative AOC over the 24 h period of study.

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