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DUAL METALLOPROTEASE INHIBITORS. III. UTILIZATION OF BICYCLIC AND MONOCYCLIC DIAZEPINONE BASED MERCAPTOACETYLS

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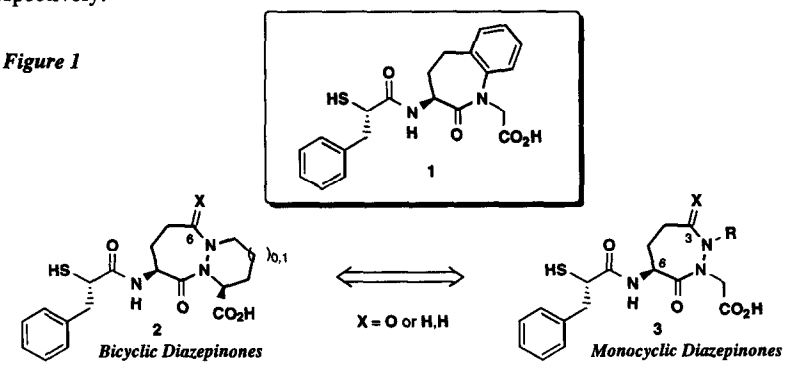
Abstract: A series of bicyclic and monocyclic diazepinones were incorporated as conformationally restricted dipeptide surrogates in mercaptoacetyl dipeptide dual-acting ACE/NEP inhibitors. A comparison was made between these two classes of compounds as well as with the previously disclosed ACE/NEP inhibitor **1**. Compound **2a** was found to exhibit high potency versus both enzymes *in vitro* as well as *in vivo*.

Angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP) are zinc metalloproteases responsible for angiotensin II (AII) formation and atrial natriuretic peptide (ANP) degradation, respectively. AII serves to raise blood pressure by both vasoconstriction and release of aldosterone. In contrast ANP, a peptidic cardiac hormone secreted by the heart in response to atrial distention, promotes the generation of cGMP via guanylate cyclase activation, which in turn causes vasodilatation, natriuresis, diuresis, and possibly inhibition of aldosterone formation. Because of the functionally opposed hormonal actions of AII and ANP, co-inhibition of ACE and NEP has the potential to act synergistically in lowering vascular resistance and inhibiting activation of the renin-angiotensin-aldosterone system. Indeed, co-administration of selective ACE and NEP inhibitors in models of both hypertension and congestive heart failure have shown a greater beneficial effect over that of single-acting ACE or NEP inhibitors given alone.[1] As a result, much effort has been expended recently towards the development of a single agent which would act as an inhibitor to both enzymes.[2]

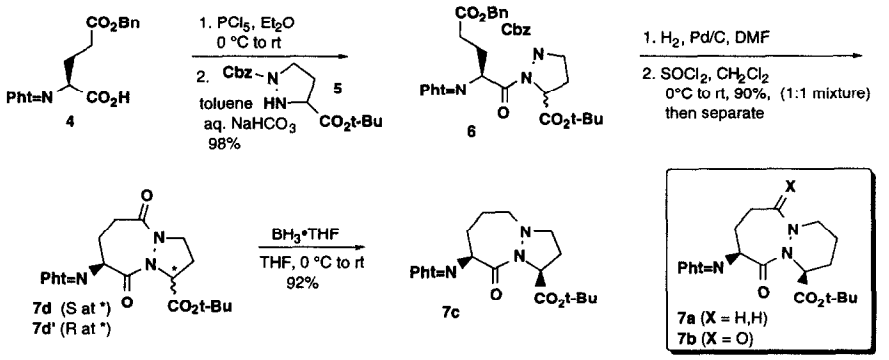
In a previous series of communications,[3] we have disclosed the generation of dual-acting ACE/NEP inhibitors based on the incorporation of dipeptidomimetic surrogates of Ala-Pro in mercaptoacyl dipeptides. From these initial studies, benzazepinone based mercaptoacetyl **1** was identified as a potent inhibitor of ACE and NEP both *in vitro* and *in vivo*. In this manuscript we extend the SAR of conformationally restricted mercaptoacetyls to include the introduction of bicyclic and monocyclic diazepinones leading to inhibitors of type **2** and **3** respectively.[4]

Figure 1



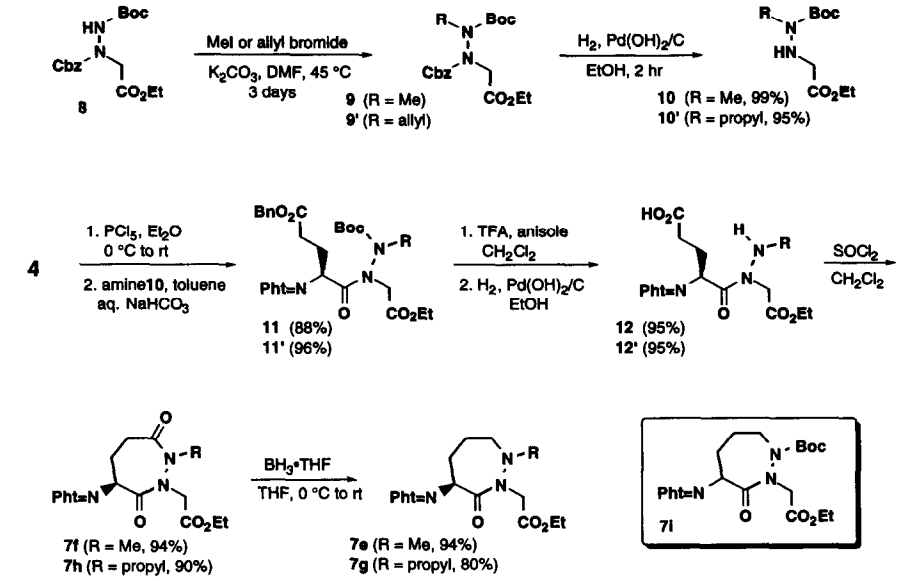
The core bicyclic nucleus represented in **2** was developed by Roche as an alanyl-proline replacement for their selective ACE inhibitor cilazapril.[5] The requisite phthalimido protected 7,6-fused bicyclic diazepinones **7a** and **7b** were generated following the Roche procedure. The corresponding 7,5-fused analogs, though not disclosed in the literature, were synthesized in an analogous fashion and is described in Scheme 1. Coupling of the acid chloride of (L)-N-phthalimido-γ-benzylglutamic acid (**4**) with racemic t-butyl 1-benzyloxycarbonylpyrrazolidine-3-carboxylate (**5**)[6] under biphasic conditions afforded **6** as a 1:1 mixture of diastereomers. Simultaneous removal of the benzyl ester and Cbz groups generated the intermediate amino acid which cyclized upon treatment with thionyl chloride to give **7d** and **7d'**. Chromatographic separation of the isomers was effected at this stage. A definitive stereochemical assignment of the isomers was made based on single crystal x-ray analysis of **7d'**. Selective reduction of the 6-oxo group in **7d** cleanly provided the corresponding dihydro analog **7c** in high yield. The high chemoselectivity of the reaction has been attributed to steric shielding about the C-3 amide carbonyl by the proximal phthalimido group.[5b]

Scheme 1



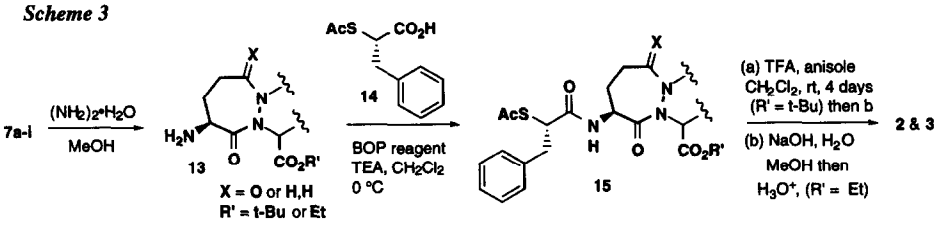
The utilization of monocyclic diazepinones for the generation of selective ACE inhibitors has been previously described by USV Pharma.[7] The synthesis of intermediate **7i** (precursor to dual inhibitor **3i**) followed their procedure. Unfortunately in our hands this route afforded monocyclic diazepinones in racemic form.[8] In addition, the corresponding 3-oxo analogs (diazepinediones) could not be synthesized by this method. We therefore sought to develop a new method to this class of compounds which would not only provide both the 3-oxo and 3-dihydro analogs in homochiral form, but would allow for the incorporation of various alkyl substituents at the N-2 nitrogen. We were gratified to discover that the chemistry utilized to generate the bicyclic diazepinones could also in part be applied to the synthesis of their related monocyclic analogs (Scheme 2). Thus, alkylation of the protected N-azaglycine derivative **8**[6] with methyl iodide or allyl bromide afforded **9** or **9'** respectively. Hydrogenation over Pearlman's catalyst removed the Cbz group in addition to effecting reduction of the allyl group of **9'** to propyl. Reaction of the weakly nucleophilic amines **10** and **10'** with the acid chloride of **4** provided the corresponding dipeptide derivatives **11** and **11'** in good yield. Sequential removal of the N-Boc group and the benzyl ester gave the amino acids which cleanly underwent intramolecular cyclization to give **7f** and **7h** respectively. The monocyclic diazepinediones were subsequently reduced to their dihydro analogs in a manner similar to that previously described.

Scheme 2



Scheme 3 outlines the methodology utilized to attach the mercaptoacetyl pharmacophore to the diazepine(di)one nucleus **7**. Treatment of **7** with hydrazine monohydrate gave amine **13**. Racemization free coupling of **13** with (S)-α-(acetylthio)-2-benzenepropanoic acid (**14**)[9] was best effected *via* activation of **14** with BOP reagent. In the case where R' is t-butyl, conversion of the ester **15** to the free carboxylic acid was first accomplished by treatment with TFA and anisole. Subsequent base hydrolysis removed the acetate group, thus generating the free thiol acid **2**. In the case where R' is ethyl, conversion of **15** to the thiol acid was performed simultaneously by treatment with base followed by acidification. All compounds were tested as their corresponding free acids.

Scheme 3

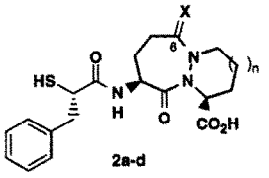


Compounds **2** and **3** were first assayed for their ability to inhibit ACE and NEP *in vitro*.[10] Inhibitors which showed good activity versus both enzymes were evaluated in the angiotensin I (AI) induced pressor response assay in the normotensive rat, allowing a comparison of performance among compounds with respect to their ability to inhibit ACE *in vivo*. ED_{50} values were determined from plots of percent maximal inhibition

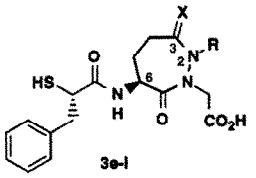
versus dose after intravenous (iv) administration. The data for compounds 1-3 are listed in Table 1. In addition, compounds which displayed acceptable activity in the AI pressor assay and possessed reasonable potency versus NEP *in vitro* were tested in the 1K-DOCA salt hypertensive rat assay.[10] In this low-renin model of hypertension, selective NEP inhibitors have been shown to lower mean arterial pressure (MAP) whereas ACE inhibitors are usually ineffective.

7,6-Fused bicyclic diazepinone **2a** proved to be a highly potent dual-acting inhibitor, possessing low nanomolar activity against both ACE and NEP. In addition, compound **2a** displayed exceptional activity in the AI pressor assay upon iv administration in the rat. The related 7,5-fused analog, **2c**, was 7-fold less active versus NEP but essentially equipotent with respect to ACE *in vitro*. Inspite of this, **2c** was significantly less active in the AI pressor rat assay. In the case of both the 7,6- and 7,5-fused bicyclic diazepinones, introduction of the C-6 oxo substituent led to a 5 to 6-fold diminution in activity versus NEP without significantly effecting ACE inhibitory potency *in vitro*. In the AI pressor assay though, diazepinedione **2b** was much less active than its dihydro analog **2a**.

Table 1



2a-d



3e-l

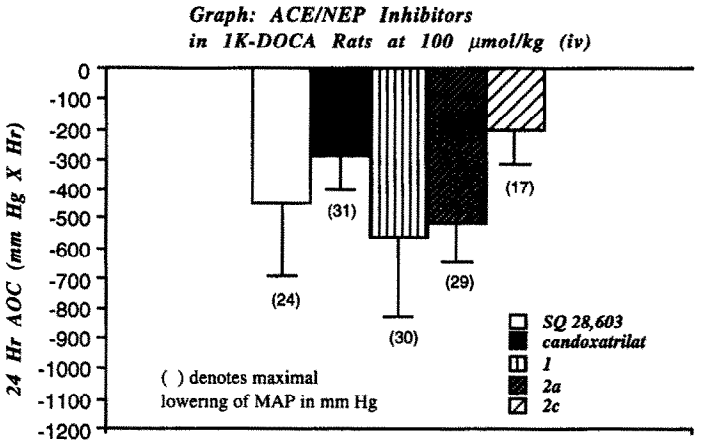
Compound	X	n	R	NEP (I_{50}, nM)	ACE (I_{50}, nM)	[a]ACE ED_{50} (μmol/kg, iv)	
1	---	---	---	6	12	0.02	
2a	H,H	1	---	3	8	0.01	*Bicyclic Diazepinones*
2b	O	1	---	16	7	0.23	
2c	H,H	0	---	22	12	0.12	
2d	O	0	---	140	20	[b]NT	
3e	H,H	---	methyl	484	16	NT	*Monocyclic Diazepinones*
3f	O	---	methyl	66	16	0.13	
3g	H,H	---	propyl	1490	6	NT	
3h	O	---	propyl	1922	10	NT	
3l[c]	H,H	---	H	486	60	NT	

[a]Dose required to effect 50% inhibition of the AI induced pressor response; [b]Not tested;
[c]1:1 mixture of diastereomers at C-6

In general, the monocyclic diazepine(di)ones were less potent dual-acting inhibitors as compared to their bicyclic counterparts. With the exception of **3f**, the monocyclic compounds displayed poor inhibitory potency against NEP although little variation in their activity with respect to ACE was observed. Smaller groups (R = H, methyl) were better tolerated than larger groups (R = propyl) at the N-2 position. The comparison between **2a** or **2b** and their corresponding seco analogs **3g** and **3h** respectively is especially interesting. The additional

conformational restriction present in the bicyclic inhibitors led to a dramatic enhancement in the ability of the compounds to inhibit NEP *in vitro*. We suspect that the monocyclic diazepinone 7-membered ring possesses a significantly different conformation than that present in the bicyclic ring system and that this conformational difference is influenced by the nature of the N-2 substituent. The *in vitro* data depicted here once again underscores the difficulty in designing dual-acting mercaptoacetyl ACE/NEP inhibitors: although ACE is relatively insensitive to a variety of structural modifications, NEP is not.

The initial lead compound in our program, benzazepinone **1**, is a potent inhibitor of both ACE and NEP *in vitro*. In addition this compound was shown to lower MAP in the 1K-DOCA salt rat hypertension assay, indicating inhibition of NEP *in vivo*.[3a] *In vivo* inhibition of NEP for **2a** was demonstrated in the 1K-DOCA salt rat hypertension assay. Compounds were given as bolus injections at 100 μmol/kg iv and mean arterial pressure (MAP) was measured versus time over a 24 hour period. The data in the Graph represents the compiled area-over-the-curve (AOC) during this time period. Under these conditions, compound **2a** was found to be at least as active as benzazepinone **1** and selective NEP inhibitor **SQ 28,603**.[11] Dual-acting inhibitor **2c** and selective NEP inhibitor candoxatrilat[12] were somewhat less effective under these conditions.



In conclusion, we have carried out structure-activity studies utilizing monocyclic and bicyclic diazepines as conformationally restricted surrogates in mercaptoacetyl containing dipeptide based ACE/NEP inhibitors. It has been demonstrated that, although both classes of inhibitors exhibit good potency versus ACE *in vitro*, the bicyclic based inhibitors were greatly superior with respect to intrinsic activity versus NEP. From these studies, a new lead compound **2a** was discovered. This compound possessed excellent *in vitro* potency against both enzymes and in addition demonstrated excellent potency in the AI pressor assay. Inhibitor **2a** also lowered mean arterial pressure in the 1K-DOCA salt rat, indicating inhibition of NEP *in vivo* as well. A

comparison of **2a** with other dual-acting ACE/NEP inhibitors will be presented in future disclosures. Studies focusing on the utilization of the mercaptoacetyl pharmacophore in conjunction with other conformationally restricted dipeptide surrogates are also forthcoming.

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