Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 15, pp. 1795-1800, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00236-3

DUAL METALLOPROTEASE INHIBITORS. II. EFFECT OF SUBSTITUTION AND STEREOCHEMISTRY ON BENZAZEPINONE BASED MERCAPTOACETYLS

Jeffrey A. Robl,* Ligaya M. Simpkins, Richard Sulsky, Ellen Sieber-McMaster, Jay Stevenson, Yolanda F. Kelly, Chong-Qing Sun, Raj N. Misra, Denis E. Ryono, Magdi M. Asaad, J. Eileen Bird, Nick C. Trippodo, and Donald S. Karanewsky

Bristol-Myers Squibb Pharmaceutical Research Institute
P.O. Box 4000, Princeton, N.J. 08543-4000

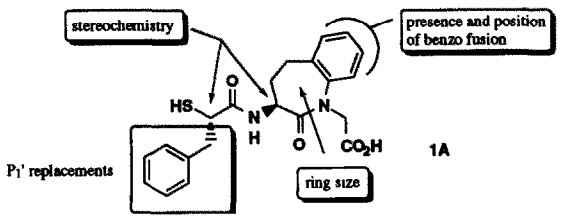
Abstract: A structure-activity study of dual-acting ACE/NEP inhibitor **1A** was initiated in order to ascertain what parameters effect *in vitro* activity versus ACE and NEP. Unlike NEP, ACE was found to be remarkably tolerant to a wide variety of permutations with respect to both the lactam nucleus and the pharmacophore side chain.

The development of dual-acting inhibitors of the zinc metalloproteases angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP) is currently an active field of study in cardiovascular research.[1] It has been shown that co-administration of selective ACE and NEP inhibitors in models of both hypertension and heart failure has a beneficial synergistic effect over the administration of selective ACE or NEP inhibitors alone.[2] Consequently dual-acting inhibitors may offer a new therapy for the treatment of high blood pressure, congestive heart failure, and chronic renal failure.

The major difficulty in this field centers about the development of a single compound which can effectively inhibit two distinct enzymatic binding sites. Towards this goal, our efforts have concentrated on the utilization of mercaptoacyl dipeptides as dual-acting inhibitors of ACE and NEP. This class of compounds has been proposed to bind to both metalloproteases via a "frame-shift" mechanism, with the mercaptoacyl pharmacophore acting as the zinc binding element in either a monodentate (NEP) or bidentate (ACE) fashion.[3] Subsequently we have shown that replacement of the dipeptide portion of these compounds with conformationally restricted analogs can lead to inhibitors with excellent *in vitro* potency against both enzymes.[4] One of the more potent inhibitors discovered was **1A** (Figure 1), a benzazepinone based mercaptoacetyl which displayed good potency against ACE and NEP both *in vitro* and *in vivo* (Table 1).

In this communication we expand on the structure-activity relationships developed previously in this class of compounds. With **1A** as our lead compound, a determination of the effect of stereochemistry, ring size, aromatic ring fusion and positioning, and P_1' replacement[5] was made with respect to inhibitory potency.

Figure 1



A variety of dipeptide surrogates **2** were examined to this end[6] (Figure 2). The effect of benzo-fusion and position was assessed through the use of amines **2C** and **2B** respectively as the dipeptide surrogates. Utilization of compound **2D**, obtained upon resolution of the corresponding racemic amine with D-tartaric acid, made possible a comparison of the importance of stereochemistry at the C-3 center. Azocine **2E** and alanine-N-phenylglycine derivative **2F** addressed the question of ring size and conformational restriction respectively. Reaction of the requisite amines **2A-F** with (S)-α-(acetylthio)-2-benzenepropanoic acid (**3**)[3,4] utilizing EDAC, or preferably BOP reagent,[7] gave the intermediate protected amide. Saponification of the protecting groups in the absence of oxygen led to the desired thiol acids **1A-F** in generally good overall yield.

Figure 2

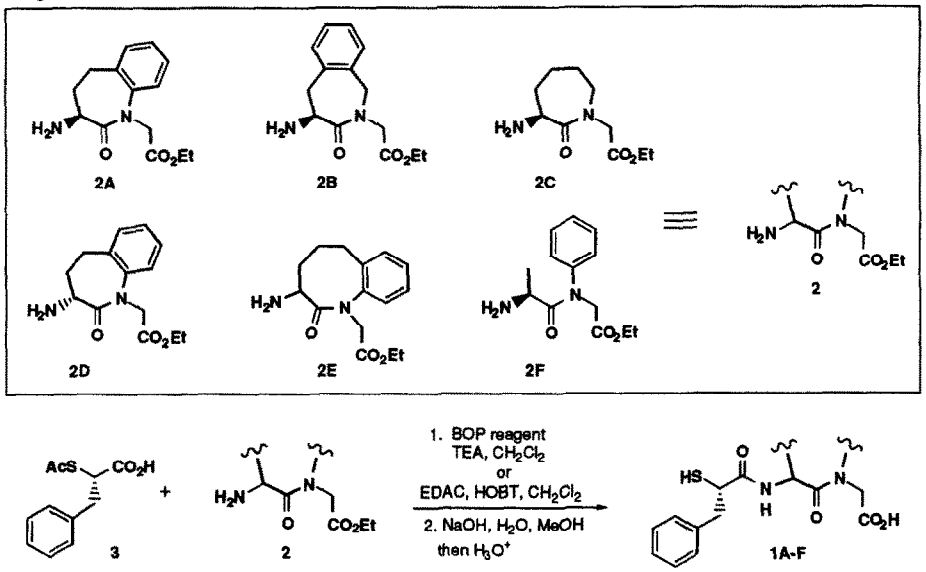


Table 1 lists *in vitro* data for compounds **1A-F** versus NEP and ACE.[8] Compounds which displayed good activity against both enzymes were evaluated in the angiotensin I (AI) induced pressor response assay in the normotensive rat,[8] thus allowing a comparison of performance among compounds with respect 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.

Not surprisingly, a comparison of **1A** and its diastereomer **1D** indicates that the proteinogenic (S) configuration at C-3 is critical for good inhibitory potency against both enzymes. Placement of the fused benzene ring in **1A** at the C-5,6 position, giving **1B**, resulted in a 30-fold diminution in potency versus NEP but only a small loss in activity with respect to ACE. Non-benzo fused azepinone **1C** was found to be twice as potent as **1B** versus NEP *in vitro*, but was still significantly less active than **1A**. As expected based on previous ACE structure-activity data,[9] azocinone based inhibitor **1E** was equipotent to azepinone **1A** (accounting for diastereomeric mixture) with respect to ACE. In sharp contrast though, this simple modification resulted in a dramatic loss in intrinsic potency versus NEP. Finally, the acyclic analog of **1A**, imino acid derivative **1F**, was clearly less potent than **1A** with respect to NEP, although equipotent versus ACE. This result once again

demonstrates that the utilization of conformationally restricted surrogates in mercaptoacetyl dipeptides usually results in an increase in inhibitory potency against NEP. These data taken together underscore a fundamental difficulty in designing potent mercaptoacetyl containing ACE/NEP inhibitors: while ACE is remarkably flexible with respect to a variety of structural modifications, NEP is not. Because of their reasonable intrinsic potencies, compounds **1C** and **1F** were tested for their ability to inhibit ACE *in vivo*. Both compounds were significantly less potent than the parent **1A**.

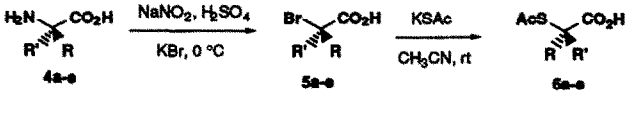
Table 1

Cmpd	NEP (I_{50}, nM)	ACE (I_{50}, nM)	[a] ACE ED_{50} (μmol/kg, iv)
1A	6	12	0.02
1B	175	33	[b] NT
1C	82	22	0.83
1D	560	306	NT
1E [c]	1124	25	NT
1F	137	14	>0.15 (42%)[d]

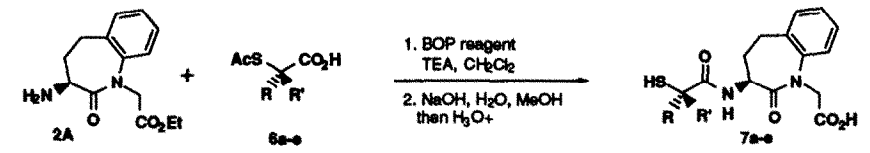
[a]dose required to effect 50% inhibition of the AI induced pressor response; [b]Not tested; [c]1:1 mixture of diastereomers at C-3; [d]maximal % inhibition at 0.15 μmol/kg

Having demonstrated that the benzazepinone nucleus is a suitably potent dipeptide surrogate in the mercaptoacetyl class of inhibitors, we turned our attention towards modification of the P_1' residue in an attempt to ascertain the effect on inhibitory potency *in vitro*. Scheme 1 outlines the synthesis of substituted thioglycolic acids **6a-e** in homochiral form and their conversion to inhibitors **7a-e**. The synthesis of acids **6a-e** follows literature precedent[10] in which D- or L-amino acids **4** are first converted to their respective bromo acids **5**. Treatment of **5** with potassium thiolacetic acid gives **6a-e** which may be conveniently isolated and purified as their dicyclohexylamine salts. BOP mediated coupling of **2A** with **6a-e** followed by deprotection thus afforded **7a-e** usually in good yields.

Scheme 1



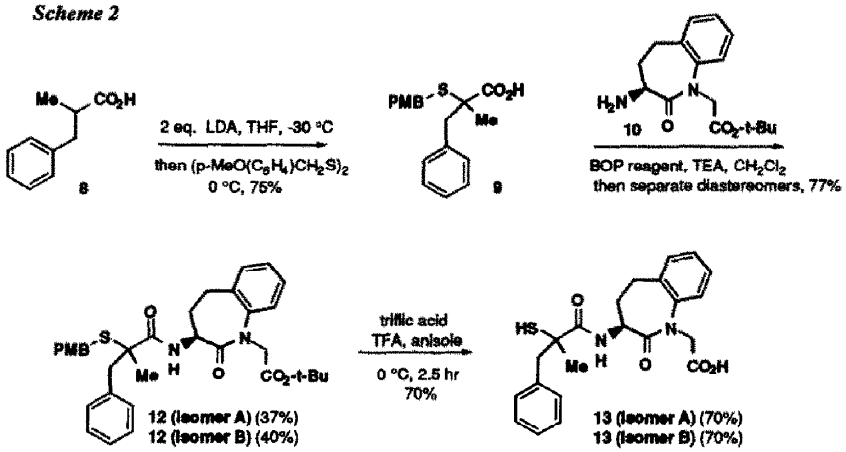
6	R	R'	4→6 (% overall)
a	H	$PhCH_2$	57
b	c-$C_6H_{11}CH_2$	H	45
c	Me	H	50
d	n-Bu	H	9
e	i-Bu	H	19



In the case of **6** where neither R or R' are hydrogen, the above methodology cannot be applied and therefore a different route was required. Scheme 2 depicts the chemistry utilized for the synthesis of **13**, an inhibitor possessing a tertiary thiol group as part of the mercaptoacetyl pharmacophore. α-Methyl

dihydrocinnamic acid was metallated with LDA and the resulting dianion was quenched with p-methoxybenzyldisulfide[11] to afford **9**. Coupling of racemic **9** to homochiral amine **10**[12] gave **12** as a chromatographically separable mixture of isomers. The p-methoxybenzyl group and the t-butyl ester were removed simultaneously with triflic acid in neat TFA. A definitive stereochemical assignment of the isomers was not made.

Scheme 2



In vitro data for $P_{1'}$ modified inhibitors are listed in Table 2. Compound **7a**, the benzyl substituted $P_{1'}$ diastereomer of **1A**, was poorly active versus ACE but retained good potency with respect to NEP. The observed activity of **7a** and **1D** (*vide supra*) indicate that ACE is especially sensitive to variations in stereochemistry about the mercaptoacetyl portion of the molecule. Both isomers of **13** displayed relatively poor activity *in vitro* against ACE and NEP. This may be attributed to either steric congestion about the thiol zincophile or the inability of the pharmacophore to adopt a reasonable conformation suitable for binding. The

Table 2

Cmpd	R	R'	NEP (I_{50}, nM)	ACE (I_{50}, nM)	[a]ACE ED_{50} (μmol/kg, iv)
1A	$PhCH_2$	H	6	12	0.02
7a	H	$PhCH_2$	14	284	[b]NT
7b	c-$C_6H_{11}CH_2$	H	4260	22	NT
7c	Me	H	1850	24	NT
7d	n-Bu	H	36	5.4	0.06
7e	i-Bu	H	7	6.3	0.23
13 (Iso A)	$PhCH_2$, Me		2000	63	NT
13 (Iso B)			865	183	NT

[a]dose required to effect 50% inhibition of the AI induced pressor response; [b]Not tested

most surprising observation in this series was seen in the case of **7b**, where a >700-fold loss of potency versus NEP was observed as compared to its aromatic analog **1A**. Coupled with the *in vitro* activity of **7c**, the data suggest that very large and very small aliphatic groups do not productively interact with the $S_{1'}$ subsite of NEP.

Better *in vitro* activity was observed with medium sized aliphatic residues such as those found in **7d** and **7e**. In fact, **7e** was found to be slightly more potent than **1A** *in vitro* versus ACE while retaining excellent potency versus NEP. Unfortunately, ACE *in vivo* testing proved **7e** to be 10-fold less potent than its benzyl analog **1A**. In contrast, **7d** not only exhibited similar potency to that of **1A** in this assay but also displayed better duration of action at a comparative dose of 0.5 μmol/kg, iv ($t_{1/2} \approx 40$ min for **7d**, $t_{1/2} \approx 20$ min for **1A**).

In summary, a variety of modifications were made to the dual-acting ACE/NEP inhibitor **1A** with respect to both the benzazepinone nucleus and the mercaptoacetyl pharmacophore. Within this limited series of compounds it has been shown that: i) azepinones are preferred to azocinones as dipeptide surrogates with respect to NEP[13] ; ii) the $P_{2'}$ residue (azepinone ring) must possess the proteinogenic (S) configuration for optimal *in vitro* potency; and iii) benzo-fusion at the C-6,7 position of the azepinone ring is preferred to fusion at the C-5,6 position. Concerning the mercaptoacetyl pharmacophore, both benzyl and iso-butyl are preferred $P_{1'}$ residues for good NEP and ACE activity *in vitro*. In addition, ACE is highly sensitive to the stereochemistry at this position.

At the outset of this program, our goal was to discover a potent dual-acting ACE/NEP inhibitor within the class of mercaptoacyl conformationally restricted inhibitors. From the SAR developed here and in our previous communication,[4] compound **1A** was chosen as the most potent lead compound in the series we define as monocyclic benzo-fused azepinones.

Acknowledgment: Appreciation is expressed to Edward Petrillo for helpful suggestions in this program. We are also grateful for the technical assistance provided by Maxine Fox, Mary Giancarli, Balkrushna Panchal and Hong Sun Cheung.

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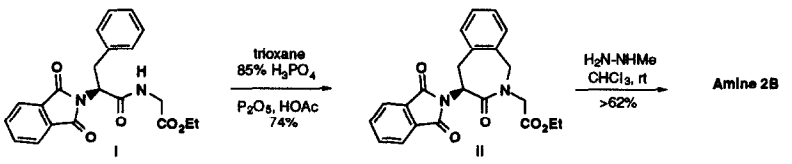
5. The benzyl substituent (termed the $P_{1'}$ residue) in **1A** is believed to bind in the $S_{1'}$ subsite in NEP and in the S_1 subsite in ACE according to the binding model proposed in reference 3.
6. Compounds **2A**, **2D**, and **2E** were prepared according to the procedures in (a) Watthey, J. W. H.; Stanton, J. L.; Desai, M. Babiarz, J. E.; Finn, B. M. *J. Med. Chem.* **1985**, *28*, 1511. (b) Parsons, W. H.; Davidson, J. L.; Taub, D.; Aster, S. D.; Thorsett, E. D.; Patchett, A. A.; Ulm, E. H.; Lamont, B. I. *Biochem. Biophys. Res. Comm.* **1983**, *117*, 108. Compound **2C** was prepared in an analogous fashion to that described in Sreenivasan, U.; Mishra, R. K.; Johnson, R. L. J. *Med. Chem.* **1993**, *36*, 256. Amine **2F** was prepared by activation of N-Cbz-alanine (isobutyrylchloroformate, NMM, DMF) followed by treatment with DMAP and N-phenylglycine ethyl ester (44% yield). The resulting protected dipeptide was subjected to catalytic hydrogenation (10% Pd/C in EtOH, 50 psi) to afford **2F** quantitatively. The synthesis of compound **2B** was obtained by a modification of the procedure described in Flynn, G. A. European Patent Application 0 249 223, 1987 and is outlined below. Dipeptide **i** (5.9 mmol, obtained by standard amino acid coupling condensation of N-phthaloyl-L-phenylglycine and glycine ethyl ester hydrochloride) and trioxane (8.3 mmol) were added to an 80 °C mixture of P_2O_5 (9.6 g), 85% H_3PO_4 (6 mL), and HOAc (36 mL). After 6.5 hours, additional trioxane (8.3 mmol) was added and heating was continued for 16 hours. Aqueous work-up gave **ii** in 74% yield. Removal of the phthalimido protecting group with N-methyl-hydrazine afforded the desired amine **2B**.



7. The use of BOP reagent resulted in the formation of less racemization product (<2%) as compared to ethyl-3-(3-dimethylamino)propylcarbodiimide•HCl (EDAC)/HOBT (5-15%).
8. The ACE and NEP *in vitro* assays and the AI pressor *in vivo* assay was run according to the procedures described in references 3 and 4.
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13. A similar structure-activity profile (poor NEP activity, good ACE activity) was seen in the comparison between other azepinones and azocinones. This data will be presented in the near future.

(*Received in USA* 5 *April* 1994; *accepted* 19 *May* 1994)