Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 18, pp. 2193-2198, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00311-4

DUAL METALLOPROTEASE INHIBITORS. IV. UTILIZATION OF THIAZEPINES AND THIAZINES AS CONSTRAINED PEPTIDOMIMETIC SURROGATES IN MERCAPTOACYL DIPEPTIDES

Jagabandhu Das,* Jeffrey A. Robl, Joyce A. Reid, Chong-Qing Sun, Raj N. Misra, Baerbel R. Brown, Denis E. Ryono, Magdi M. Asaad, J. Eileen Bird, Nick C. Trippodo, Edward W. Petrillo, and Donald S. Karanewsky§

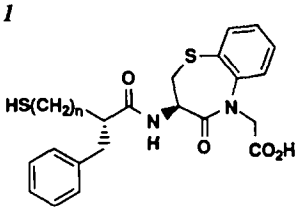
Bristol-Myers Squibb Pharmaceutical Research Institute
P.O. Box 4000, Princeton, N.J. 08543-4000

Abstract: A structure-activity study of the dual acting ACE/NEP inhibitors related to **1a** and **1b** was undertaken to determine the parameters critical for activity versus ACE and NEP *in vitro*.

Angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP) are zinc metallo-enzymes, intimately involved in regulation of the renin-angiotensin-aldosterone system and atrial natriuretic peptide (ANP), respectively. Selective ACE inhibitors prevent angiotensin II (AII) induced hypertension,[1] while NEP inhibitors have been shown to cause vasodilation, natriuresis and suppression of renin and aldosterone secretion by preventing ANP degradation.[2] Due to the functionally opposed hormonal actions of AII and ANP, simultaneous inhibition[3] of ACE and NEP is expected to have a synergistic effect in lowering vascular resistance and inhibiting activation of renin-angiotensin-aldosterone system. Not surprisingly, development of a single agent which inhibits both enzymes has attracted considerable attention in recent years.[4]

In a recent communication,[5] we have disclosed dual-acting ACE/NEP inhibitors that incorporate dipeptidomimetic surrogates in mercaptoacyl containing dipeptides. From these studies, benzothiazepinone derivatives **1a** and **1b** (Figure 1) were identified to be potent inhibitors of both ACE and NEP *in vitro*.[5] In this communication we describe structure-activity relationship studies on compounds related to **1** incorporating non benzo-fused thiazepines and thiazines as dipeptidomimetic surrogates. SAR studies were undertaken to study the effects of ring size, aromatic ring fusion, substitution and position of the sulfur heteroatom on potency both *in vitro* and *in vivo*.

Figure 1



1a (n = 0) ACE I_{50} = 13 nM NEP I_{50} = 64 nM

1b (n = 1) ACE I_{50} = 17 nM NEP I_{50} = 1.0 nM

A variety of monocyclic peptide mimetics were prepared and their structures are outlined in Figure 2. The effect of the benzofusion was studied by preparing compounds containing monocyclic ring systems **4a-d**. An analog containing ring **4e** assessed the effect of geminal disubstitution in the thiazepinone ring while analogs incorporating rings **4f** and **4g** enabled us to study the effect of the position of the sulfur atom in the thiazepine ring as well as substitution on the ring system itself. Analogs with the 6-membered thiazine rings **4h-j** addressed the importance of ring size and stereochemistry for inhibitory potencies within this class of compounds.

Thiol acids **2** and **3** were synthesized in generally excellent yield by condensation of the respective amines **4a-i** with either (S)-2-(acetylthio)-3-benzenepropanoic acid[6] (**5**) or (S)-3-(acetylthio)-2-benzylpropionic acid[7] (**6**) in presence of EDAC or preferably BOP reagent, followed by aqueous base hydrolysis under anaerobic conditions. Compound **3j** was prepared via epimerization of **3i** by prolonged exposure of **3i** to aqueous base (RT, 6 h).

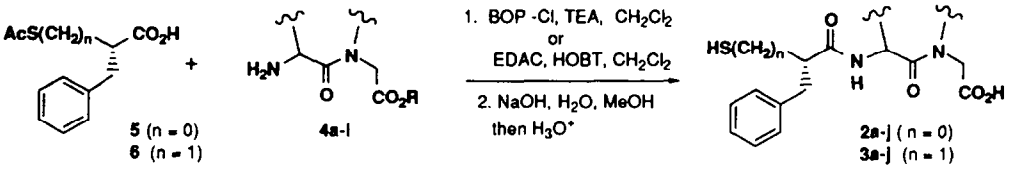
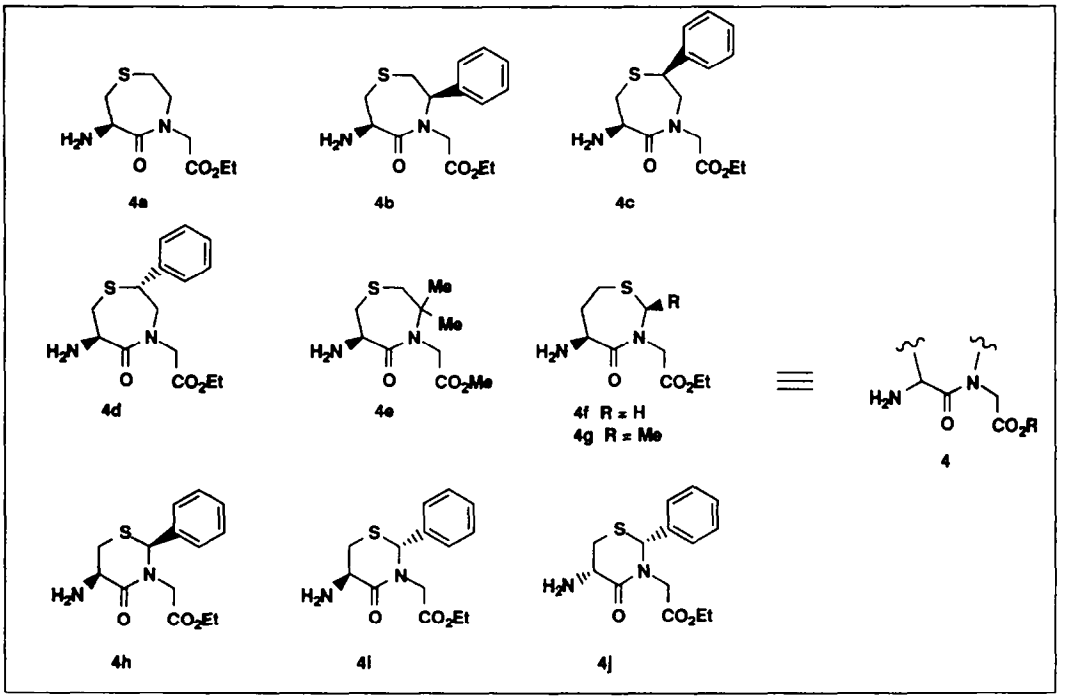


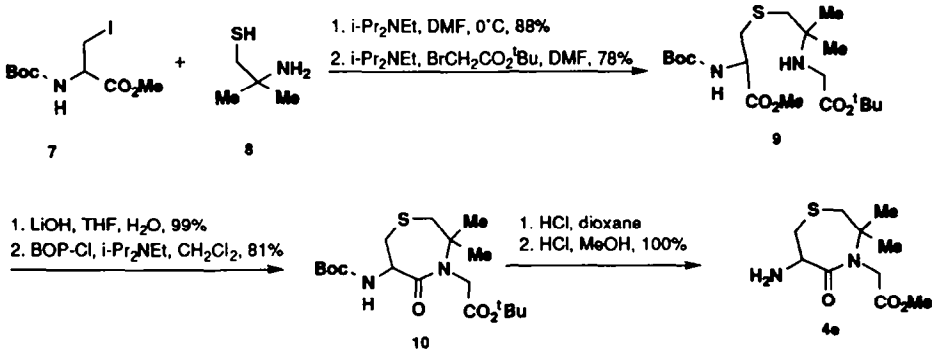
Figure 2



Amino-thiazepines **4a-d**[8] and amino-thiazines **4h-i**[9] were prepared according to the procedures described in the literature. Preparation of 3,3-dimethylthiazepinone **4e** is outlined in Scheme I. S-Alkylation of aminothiol **8**[10] with iodide **7**[11] in presence of Hunig's base in DMF at 0°C, followed by alkylation of the intermediate amine with t-butyl bromoacetate in DMF gave **9** in 69% overall yield. The stereochemical purity of **9** was not determined at this stage.[12] Hydrolysis of the methyl ester with aqueous LiOH, followed by intramolecular coupling of the intermediate amino acid with BOP-Cl afforded thiazepinone **10** in 81% yield

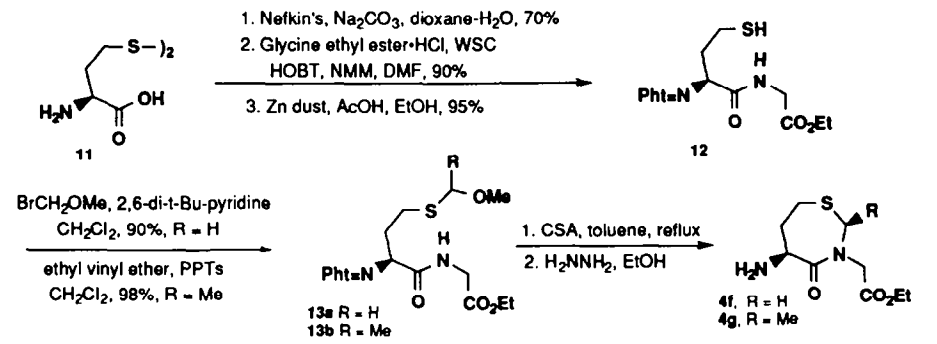
from **9**. Removal of BOC-protecting group and concommitant hydrolysis of the t-butyl ester with HCl in dioxane followed by subsequent treatment with methanolic HCl afforded amino ester **4e** as a hydrochloride salt quantitatively.

Scheme I



The synthesis of thiazepinones **4f** and **4g** started from a common precursor, L-cystine **11**, and is depicted in Scheme II. Protection of the amine functionality in **11** with a phthalimide group, coupling the resulting acid with glycine ethyl ester under standard peptide coupling conditions, and subsequent reductive cleavage of the disulfide with zinc dust in ethanolic acetic acid afforded thiol **12** in 60% overall yield. To prepare **4f**, thiol **12** was alkylated with bromomethyl methyl ether in dichloromethane in presence of 2,6-di-t-butylpyridine to form **13a**[9] (R = H) in 90% yield. Slow addition of **13a** via syringe pump to a refluxing solution of camphorsulfonic acid in toluene effected cyclization to the thiazepine ring. Subsequent removal of pthalimido protecting group by reaction with ethanolic hydrazine formed **4f** in 45% yield (2 steps). To synthesize **4g**, thiol **12** was treated with ethyl vinyl ether in dichloromethane in the presence of pyridinium p-toluenesulfonate to give **13b** which was further converted to **4g** in 10-14% overall yield under conditions similar to that described for **4f**. The absolute and relative stereochemistry of **4g** was established by NOE studies and by single crystal X-ray crystallographic analysis of its N-pthalimido derivative.

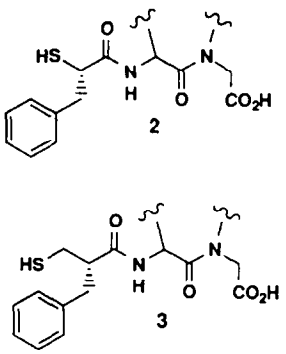
Scheme II



Compounds **2** and **3** were assayed for their ability to inhibit ACE and NEP *in vitro*.[13] 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 among compounds with respect to their ability to inhibit ACE *in vivo*.[14] ED_{50} values were determined from plots of percent maximal inhibition versus dose after intravenous (i. v.) administration. The data for compounds **1-3** are listed in Table 1.

In the mercaptoacetyl series (n = 0), a comparison of unsubstituted thiazepine analog **2a** and its benzo-fused counterpart **1a** showed that deletion of the phenyl ring caused a decrease in both ACE and NEP inhibitory activities *in vitro* by 5-fold and 4-fold respectively. Introduction of a β-phenyl substituent *alpha* to the lactam nitrogen, giving **2b**, increased activity versus ACE by two-fold relative to **2a** but activity versus NEP was reduced significantly. The corresponding phenyl substituted regioisomer **2c** experienced a decrease in both ACE and NEP inhibitory potency as compared with **2a**. The diastereomer of **2c**, compound **2d**, retained most of the ACE and NEP activities of the benzo-fused analog **1a**, indicating the importance of stereochemistry at this center for optimal inhibitory activity. Although slightly less active versus ACE *in vitro*, compound **2d** compares favorably *in vivo* with compound **1a** in the AI pressor assay. Compound **2e**, possessing a geminal dimethyl substituted thiazepine is a potent inhibitor of ACE. Unfortunately incorporation of the methyl groups led to a 6-fold drop in NEP activity versus the unsubstituted analog **2a**. A comparison of **2a** with **2f** indicates that the placement of the sulfur in the thiazepine ring system has a minimal effect on inhibitory potency against either enzyme. The β-methyl substituted analog of **2f**, compound **2g**, exhibited an increase in both ACE and NEP activities (5-fold and 2-fold respectively).

Table 1



2

3

Cmpd	NEP (I_{50}, nM)	ACE (I_{50}, nM)	[a] ACE ED_{50} (μmol/kg, iv)
1a	64	13	0.14
1b	1.0	17	3.96
2a	244	67	4.4
2b	2020	29	0.49
2c	2043	290	[b] NT
2d	99	40	<0.15
2e	1559	13.8	NT
2f	535	61	NT
2g	251	11.7	NT
2h	380	73	NT
2i	1352	25	2 75
2j	3820	20	0 21
3a	2.4	100	NT
3b	40	22	NT
3d	0 9	33	>1.5
3h	2.7	115	NT
3i	2.5	167	NT
3j	126	123	NT
SQ 28603[15]	9.4	32,000	NT

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

Replacement of the seven-membered thiazepine nucleus by the related the six-membered thiazine system (compare **2b** and **2h** respectively) resulted in a modest loss in activity versus ACE (2-fold) but an enhancement in activity versus NEP (5-fold). Surprisingly, the corresponding diastereomers of **2h**, phenyl

isomer **2i** and amide isomer **2j** were 3-fold more potent as ACE inhibitors but were 4 to 10-fold less active versus NEP as compared to **2h**.

Replacement of the mercaptoacetyl pharmacophore with the homologated mercaptopropanoyl group in general greatly enhanced NEP inhibitory activity without significantly affecting ACE inhibitory activity *in vitro* (compare **1b**, **3a**, **3b**, **3d**, **3h-j** with **1a**, **2a**, **2b**, **2d**, and **2h-j**, respectively). The most potent compound in this series, **3d** is roughly 100-fold more potent than **2d** as an NEP inhibitor *in vitro* and maintains a comparable level of ACE inhibitory activity *in vitro*. Unfortunately the mercaptopropanoyl analogs as a class failed to display reasonable potency *in vivo*. In the AI pressor assay, the potencies of both mercaptoacetyls **1a** and **2d** are at least an order of magnitude greater than those of the mercaptopropanoyls **1b** and **3d**, respectively. This same phenomenon has been observed in other series of mercaptoacyl containing inhibitors .[5]

In conclusion, we described the synthesis of several substituted and unsubstituted thiazepine and thiazine ring systems and their application to the generation of dual-acting ACE and NEP inhibitors. We demonstrated that in the mercaptoacetyl series (**2a-j**), replacement of the benzo-fused thiazepine nucleus with related sulfur containing monocyclic ring systems, in general, resulted in a dramatic attenuation of NEP inhibitory activity but only a modest loss in potency versus ACE. Compound **2d**, the only exception in this series, was similar to **1a** in its biological response versus ACE and NEP both *in vitro* and *in vivo*. In the mercaptopropanoyl series (**3a-j**), potent NEP inhibitory activity was retained to a significant extent by most of the monocyclic analogs although activity versus ACE, especially *in vivo*, was poor.

Acknowledgement: We are grateful for the technical assistance provided by Maxine Fox, Mary Giancarli, Balkrishna Panchal, and Hong Sun Cheung. We also thank Ms. Yolanda Pan for NOE studies and Ms. Mary F. Malley for X-ray crystallographic studies.

References and Notes:

§Present address: Idun Pharmaceuticals, 3050 Science Park Rd., San Diego, CA, 92121

1. Chatterjee, K. *Heart Dis. Stroke* **1992**, *1*, 128.
2. (a) Schwartz, J. C.; Gros, C.; Lecomte, J. M.; Bralet, J. *Life Sci.* **1990**, *47*, 1279. (b) Brenner, B. M.; Ballerman, B. J.; Gunning, M. E.; Zeidel, M. L. *Physiol. Rev.* **1990**,*70*, 665. (c) Roques, B. P.; Noble, F.; Dauge, V.; Fournie-Zaluski, M.-C.; Beaumont, A. *Pharm. Rev.* **1993**, *45*, 87. (d) Wilkins, M. R.; Unwin, R. J.; Kenny, A. J. *Kidney Internat.* **1993**, *43*, 273. (e) Welches, W. R.; Brosnihan, K. B.; Ferrario, C. M. *Life Sci.* **1993**, *52*, 1461. (f) MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Bohacek, R. S.; Clarke, F. H.; Ghai, R. D.; Sakane, Y.; Berry, C. J.; Peppard, J. V.; Trapani, A. J. *J. Med. Chem.* **1993**, *36*, 3821.
3. (a) Seymour, A. A.; Swerdel, J. N.; Abboa-Offei, B. *J. Cardiovasc. Pharm.* **1991**, *17*, 456. (b) Krulan, C.; Ghai, R. D.; Lappe, R. W.; Webb, R. L. *FASEB J* **1993**, *7*, A247. (c) Pham, I.; Gonzalez, W.; El Amrani, A.-I. K.; Fournie-Zaluski, M.-C.; Philippe, M.; Laboulandine, I.; Roques, B. P.; Michel, J.-B. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 1339. (d) Roques, B. P.; Beaumont, A. TiPS. **1990**, *11*, 245.
4. Recent work includes: (a) Chackalamannil, S.; Wang, Y.; Haslanger, M. F. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1003. (b) Mimura, T.; Nakamura, Y.; Nishino, J.; Sawayama, T.; Komiya, T. Deguchi, T.; Kita, A.; Nakamura, H.; Matsumoto, J. *J. Med. Chem.* **1992**, *35*, 602. (c) Neustadt, B. R. *Curr. Opin. Invest. Drugs* **1993**, *2*, 1175. (d) Gomez-Monterrey, I.; Turcaud, S.; Lucas, E.; Bruetschy, L.; Roques, B. P.; Fournie-Zaluski, M. C. *J. Med. Chem.* **1993**, *36*, 87. (e) Fournie-Zaluski, M. C. *C. R. Soc. Biol.* **1992**, *186*,

612. (f) Flynn, G. A.; Beight, D. W.; Mehdi, S.; Koehl, J. R.; Giroux, E. L.; French, J. F.; Hake, P. W.; Dage, R. C. *J. Med. Chem.* **1993**, *36*, 2420. (g) Burkholder, T. P.; Huber, E. W.; Flynn, G. A. *Bioorg. Med. Chem Lett.* **1993**, *3*, 231. (h) Stanton, J. L.; Sperbeck, D. M.; Trapani, A. J.; Cote, D.; Sakane, Y.; Berry, C. J.; Ghai, R. D. *J. Med. Chem.* **1993**, *36*, 3829. (i) Fournie-Zaluski, M. C.; Gonzalez, W.; Turcaud, S.; Pham, I.; Roques, B. P.; Michel, J. B. *Proc. Natl. Acad. Sci.* **1994**, *91*, 4072. (j) Fournie-Zaluski, M-C.; Coric, P.; Turcaud, P.; Rousselet, N.; Gonzalez, W.; Barbe, B.; Pham, I.; Jullian, N.; Michel, J-B.; Roques, B. P. *J. Med. Chem.* **1994**, *37*, 1070.

5. Robl, J. A.; Simpkins, L. M.; Stevenson, J.; Sun, C. Q.; Murugesan, N.; Barrish, J. C.; Asaad, M. M.; Bird, J. E.; Schaeffer, T. R.; Trippodo, N. R.; Petrillo, E. W.; Karanewsky, D. S. *Bioorg. Med. Chem. Lett.* in press.
6. Strijtveen, B. ; Kellogg, R. M. *J. Org. Chem.* **1986**, *51*, 3664.
7. Giros, B.; Gros, C.; Schwartz, J. C.; Danvy, D.; Plaquevent, J. C.; Duhamel, L.; Duhamel, P.; Vlaiculescu, A.; Constentin, J.; Lecomte, J. M. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 666.
8. Yanagisawa, H.; Ishihara, S.; Ando, A.; Kanazaki, T.; Miyamoto, S.; Koike, H.; Iijima, Y.; Oizumi, K.; Matsushita, Y.; Hata, T. *J. Med. Chem.* **1987**, *30*, 1984.
9. Karanewsky, D. S. *US Patent* **1984**, 4, 460, 579.
10. Aminothiol **8** was prepared following the procedure described in : (a) Dewey, C. S.; Bafford, R. A. *J. Org. Chem.* **1965**, *30*, 491. (b) Elz, S.; Draeger, M.; Schunack, W. *Z. Naturforsch., B. : Chem. Sci.* **1987**, *42*, 348.
11. Iodide **7** was prepared from N-BOC-L-serine in three steps: (i) diazomethane treatment in ether/dichloromethane to form methyl ester, 94%, (ii) mesylation with $MeSO_2Cl$ and Et_3N in dichloromethane at -20°C to form a mesylate and (iii) subsequent treatment with NaI in acetone at RT, 60% overall yield. The stereochemical purity of **7** was not determined.
12. Partially racemic **4e** was coupled with (S)-α-(acetylthio)-2-benzenepropanoic acid **5** to obtain a diastereomeric mixture of products which were separated by silica gel chromatography. The absolute and relative stereochemistry of the pure diastereomer was based on comparison of its ^1H NMR spectrum with those of the corresponding azepine derivatives of known absolute and relative stereochemistry.
13. ACE inhibitory activity *in vitro* was determined using rat lung ACE and Hippuryl-His-Leu as substrate and NEP inhibitory activity *in vitro* was determined using a fluorometric assay with purified rat kidney NEP and Dansyl-Gly-Phe-Arg as substrate. For a description of these assays, see: Delaney, N. G.; Barrish, J. C.; Neubeck, R.; Natarajan, S. I.; Rovnyak, G. R.; Huber, G.; Murugesan, N.; Girotra, R.; Sieber-McMaster, E.; Robl, J. A.; Assad, M.; Cheung, H. S.; Bird, E.; Waldron, T.; Petrillo, E. W. *Bioorg. Med. Chem. Lett.* in press.
14. For a description of the AI pressor *in vivo* assay, see reference 5.
15. Margulies, K. B.; Cavero, P. G.; Seymour, A. A.; Delaney, N. G.; Burnett, Jr., J. C. *Kidney International* **1990**, *38*, 67.

(*Received in USA* 12 *July* 1994; *accepted* 15 *August* 1994)