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# α-MERCAPTOACYL DIPEPTIDES THAT INHIBIT ANGIOTENSIN CONVERTING ENZYME AND NEUTRAL ENDOPEPTIDASE 24.11.

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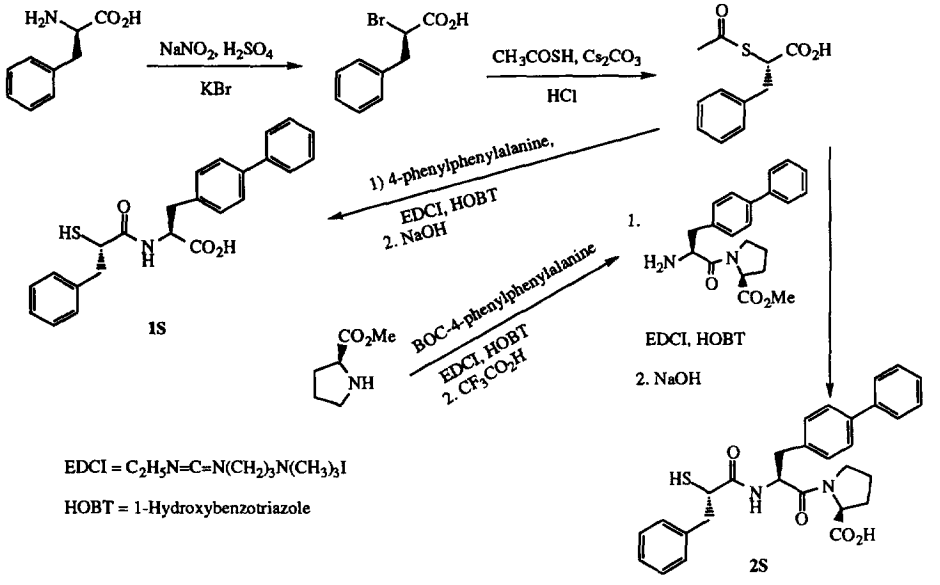
**Abstract:** α-Mercaptoacyl dipeptides were prepared and found to inhibit angiotensin converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP). Compounds with a proline as the C-terminal amino acid, and possessing the S-stereochemistry at the thiol bearing carbon were preferred for optimal ACE inhibition while, R-stereochemistry was preferred for optimal NEP inhibition. Compounds containing alternative amino acid residues at the C-terminal position displayed optimal dual ACE/NEP inhibition when the stereochemistry was 'S' at this carbon atom.

Medication for the treatment of hypertension today consists largely of angiotensin converting enzyme (ACE) inhibitors and calcium channel blockers prescribed alone or in combination with a diuretic.[2] Undesired side effects such as elevation of plasma renin levels, hypokalemia and hyperglycemia, upon treatment with a diuretic, make it desirable to have improved adjunct therapies available. It has been observed in animals and humans that, inhibitors of neutral endopeptidase 24.11(NEP) produce diuresis with selective natriuresis.[3] This observation has led to the idea of combined inhibition of ACE and NEP for the treatment of hypertension and congestive heart failure (CHF).[4] We disclose herein, our initial results in the search for compounds that inhibit both ACE and NEP.[5]

In search of new templates for the dual inhibition of ACE and NEP both of which are zinc metallo-proteases, we decided to initiate our study using the α-thiols, **1R** and **1S**, as lead compounds. An additional amino acid at the C-terminus was envisioned to increase hydrophilic and hydrophobic interactions between the inhibitor and the two enzymes leading to improved dual activity. A terminal proline residue is known to be ideal for optimal ACE inhibition.[6] Therefore, we synthesized **2-6** as some of our initial α-mercaptoacyl dipeptide dual inhibitor targets. Further structure activity relationship (SAR) studies were done using other amino acids at the C-terminal and central positions. A recent publication[5d] describing the SAR of β-mercaptoacyl dipeptide series of ACE/NEP inhibitors mentions the dual activity of two epimers of a close analog of **2S** without describing the SAR. Herein, we describe the SAR of α-mercaptoacyl series of dual ACE/NEP inhibitors.

Compounds **1-5** were synthesized by coupling racemic 2-mercaptoacetyl-3-phenylpropionic acid with either (S)-4-phenylphenylalanine in the case of **1R** and **1S** or the appropriate dipeptide ($AA_1$-Pro-OMe) as shown in Scheme I. The resulting mixture of diastereomers were separated into the SS and RS diastereomers which were

**Scheme I**



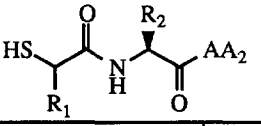

hydrolyzed with three equivalents of NaOH to give corresponding SS and RS α-thiols. No or very little racemization was seen at the carbon bearing the α-thiol. Racemic 2-mercaptoacetyl-3-phenylpropionic acid was prepared by diazotization of racemic phenylalanine followed by displacement with KBr. Treatment of the resulting α-bromo acid with thiolacetic acid and cesium carbonate afforded the desired α-thiol acid.[7,8] Compounds **6-9** were synthesized similarly by coupling (S)-2-mercaptoacetyl-3-methylbutyric acid with the appropriate dipeptide, 4-phenyl-Phe-$AA_2$, followed by hydrolysis. (S)-2-mercaptoacetyl-3-methylbutyric acid was prepared as per the literature procedure starting from D-valine.[7] The unnatural amino acids required for the synthesis of **1-9** were either commercially available or prepared as per the literature procedure.[9]

The compounds thus prepared were tested for their ability to inhibit ACE and NEP. Inhibition of ACE was determined by the extent of hydrolysis of hippuryl-L-histidyl-L-leucine (2 mM; 35-40 μg of partially purified ACE; pH=8.3, 37 °C for 30 min). The product histidyl-leucine was reacted with o-phthalaldehyde (2 mg/ml in methanol) and measured spectrophotometrically at 360 nM as described by Cushman and Cheung.[10] Inhibition of NEP was determined by the extent of hydrolysis of the substrate glutaryl-Ala-Ala-Phe-2-naphthyl amide using a modification of the procedure of Orlowski and Wilk (4.2 μg of protein from rat kidney cortex membranes, 500 μM substrate, pH=7.4, 25 °C for 25 min).[11,12] The results were expressed as $IC_{50}$ values (Table I).

The following SAR was observed upon examination of the data in Table I. Optimal ACE/NEP inhibitory activity is obtained with S-stereochemistry of the compounds at the carbon bearing the zinc-binding thiol group. Compounds with R-stereochemistry have decreased ACE inhibitory activity (compare **1S** vs **1R**, **2S** vs **2R** and **5S** vs **5R**). Changing the steric bulk of $R_1$ impairs the dual activity displayed by this series (compare **2S** vs **3S** and **2R** vs **3R** and **2S** vs. **6**). Substituting the biphenyl group at $R_2$ with α- or β-naphthyl groups led to

significant drop in the NEP inhibitory activity (compare **2**, **4** and **5**). It is interesting to note that the α-naphthyl group of **4S** and **4R** helps these molecules have highly desirable ACE inhibitory activity while making them inactive as NEP inhibitors. Examination of the activity of **1-6** leads to an important observation that while the S-diastereomer has better ACE inhibitory activity, the R-diastereomer has better NEP inhibitory activity in this series of compounds.

**Table I.** In vitro activity of α-mercapto peptides.[a]




| Number | $R_1$ | Stereo-chemistry | $R_2$ | $AA_2$ | $IC_{50}$ (nM) for inhibition of ACE | NEP |
|---|---|---|---|---|---|---|
| **1S** | benzyl | S | 4-phenylbenzyl- | OH | 80 | 654 |
| **1R** | " | R | " | " | 688 | 100 |
| **2S** | " | S | " | L-Pro | 57 | 117 |
| **2R** | " | R | " | " | 229 | 59 |
| **3S** | piperonyl | S | " | " | 183 | 385 |
| **3R** | " | R | " | " | 378 | 222 |
| **4S** | benzyl | S | 1-naphthylmethyl | " | 14 | >1000 |
| **4R** | " | R | " | " | 27 | >1000 |
| **5S** | " | S | 2-naphthylmethyl | " | 18 | 705 |
| **5R** | " | R | " | " | 304 | 583 |
| **6** | isopropyl | S | 4-phenylbenzyl | " | 319 | 500 |
| **7** | " | S | " | L-Val | 175 | 33 |
| **8** | " | S | " | L-Tyr | 62 | 28 |
| **9** | " | S | " | L-Ser | 100 | 43 |

[a] All compounds had satisfacory IR and $^1$H-NMR and the elemental analyses were found to be within ±0.4% of the calculated values.

Replacing the C-terminal proline residue with alternative amino acids gave compounds with greatly improved NEP inhibitory activity (compare **2S** vs **7**, **8** and **9**). These compounds also display more potent ACE inhibitory activity than **6**. It is evident that this series of molecules prefer some degree of rotational freedom in the C-terminal carboxylic acid portion. This is in sharp contrast with the potent dual ACE/NEP inhibitors[5e] with highly rigid frame work in the C-terminal portion.

Our attempts to postulate a mode of binding of these compounds to ACE and NEP has led to a "frame shift" hypothesis similar to the one published in a recent publication.[5d] From the known SAR of ACE inhibitors[6], it is reasonable to propose that the benzyl, biphenylmethyl and the prolyl groups of **2S** bind to the $S_1$, $S_1'$ and $S_{2'}$ subsites of ACE. The same pattern of binding of **2S** to NEP would not fulfill the requirement that there be a N-H bond between the $P_1'$ and $P_{2'}$ residues.[13] Therefore, **2S** must bind to NEP with benzyl, biphenylmethyl and

prolyl groups in the respective $S_1'$, $S_2'$ and $S_3'$ subsites. This postulate assumes that the α-thiol group of **2S** binds to the zinc atom in the active site of ACE and NEP.

Compounds **2S** (10 mg/kg intra arterial, n=7), **5S** (10 mg/kg intra arterial, n=4), **7** (10 mg/kg i.v., n=3) and **8** (10 mg/kg i.v., n=4) inhibited angiotensin-I (AI) induced pressor response in normotensive rats,[14] indicating that they are efficacious *in vivo* as ACE inhibitors. However, their duration of action, as measured by the time during which 50% or more of the AI pressor response was inhibited, was 50, 25, 70 and 60 min. respectively. Further structural modifications have led to compounds with highly desirable *in vivo* dual ACE/NEP activity profile. This work will be reported in future publications.

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**References and Notes:**

(1) Current address: Abbott Laboratories, D4PM, AP10/3, 100 Abbott Park Road, Abbott Park, IL 60064-3500.

(2) Waeber, B.; Nussberger, J.; Brunner, H. *Hypertension*; Laragh, J. H.; Brenner, B. M., Eds.; Raven Press: New York, 1990; Vol 2, pp. 2209-2232.

(3) Roques, B. P.; Noble, F.; Dauge, V.; Fournie-Zaluski, M. C.; Beaumont, A. *Pharmacol. Rev.* **1993**, *45*, 87.

(4) Roques, B. P.; Beaumont, A. *Trends Pharmacol. Sci.* **1990**, *11*, 245.

(5) For some of the more recent publications see the following and the references cited therein. (a) Bhagwat, S. S.; Fink. C. A.; Gude, C.; Chan, K.; Qiao, Y.; Sakane, Y.; Berry, C.; Ghai, R. D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2673. (b) Neustadt, B. R.; Smith, E. M.; Nechuta, T. L.; Bronnenkant, A. A.; Haslanger, M. F.; Watkins, R. W.; Foster. C. J.; Sybertz, E. J. *J. Med. Chem.* **1994**, *37*, 2462. (c) Das, J.; Robl, J. A.; Reid, J. A.; Sun, C. -Q.; Misra, R. N.; Brown, B. R.; Ryono, D. E.; Asaad, M. M.; Bird, J. E.; Trippodo, N. C.; Petrillo, E. W.; Karanewsky, D. S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2193. (d) Delaney, N. G.; Barrish, J. C.; Neubeck, R.; Natarajan, S.; Cohen, M.; Rovnyak, G. C.; Huber, G.; Murugesan, N.; Giritra, R.; Sieber-McMaster, E.; Robl, J. A.; Asaad, M. M.; Cheung, H. S.; Bird, J. E.; Waldron, T.; Petrillo, E. W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1783. (e) French, J. F.; Flynn, G. A.; Giroux, E. L.; Mehdi, S.; Anderson, B.; Beach, D. C.; Koehl, J. R.; Dage, R. C.; *J. Pharmacol. Exp. Ther.* **1994**, *268*, 180.

(6) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*, 483.

(7) Strijtveen, B.; Kellog, R. M. *J. Org. Chem.* **1986**, *51*, 3664.

(8) Assignment of R- and S-stereochemistry was checked by preparing optically pure R- and S-2-mercaptoacetyl-3-phenylpropionic acid from D- and L-phenylalanine and coupling it to the dipeptide and then comparing the spectral and physical properties for **1R** and **1S**. Spectral and biological data were used to confirm the stereochemistry of **2-5**.

(9) Shieh, W. -C.; Carlson, J. A. *J. Org. Chem.* **1992**, *57*, 379.

(10) Cushman, D. W.; Cheung, H. W. *Biochem. Pharmacol.* **1981**, *20*, 1637.

(11) Orlowski, M.; Wilk, S. *Biochemistry* **1981**, *20*, 4942.

(12) Sonnenberg, J. L.; Sakane, Y.; Jeng, A. Y.; Koehn, J. A.; Ansell, J. A.; Wennogle, L. P.; Ghai, R. D. *Peptides* **1988**, *9*, 173.

(13) Fournie-Zaluski, M. -C.; Lucas, E.; Waksman, G.; Roques, B. P. *Eur. J. Biochem.* **1984**, *139*, 267.

(14) AI pressor test was done as per the procedure described in: 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.

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