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4-SUBSTITUTED PROLINE DERIVATIVES THAT INHIBIT ANGIOTENSIN CONVERTING ENZYME AND NEUTRAL ENDOPEPTIDASE 24.11.

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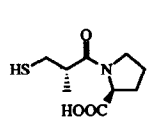
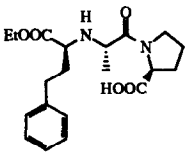
Abstract: Analogs of captopril and enalapril with a thiorphan-like substituent at the 4-position of the proline ring were synthesized and found to be dual inhibitors of angiotensin converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP).

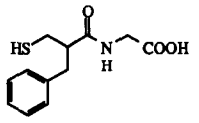
Angiotensin converting enzyme (ACE) inhibitors are one of the most widely used classes of antihypertensive agents today. Captopril and enalapril, the two most prescribed ACE inhibitors, are effective in treating nearly half of all the hypertensive patients. When coadministered with a diuretic, their efficacy level goes up significantly.[1] Diuretics have many undesired side effects such as aldosterone secretion, elevation of plasma renin levels, and nonspecific ion excretion leading to hypokaliemia. Inhibitors of neutral endopeptidase (NEP), on the other hand, have been found to produce diuresis with selective natriuresis in animal models and in humans.[2] It has been hypothesized that the combined action of inhibitors of ACE and NEP would produce a potent antihypertensive effect with fewer side effects than by a combination of an ACE inhibitor and a diuretic.[3] Therefore, we decided to synthesize and evaluate compounds that inhibit both ACE and NEP.



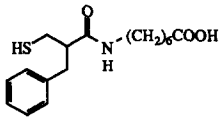
captopril (IC_{50}=23 nM)



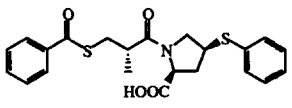
enalapril
enalaprilat: IC_{50}=1.2 nM



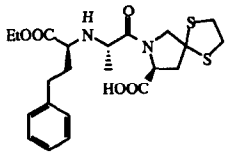
thiorphan IC_{50}=6 nM



SQ 29072 K_i=26 nM



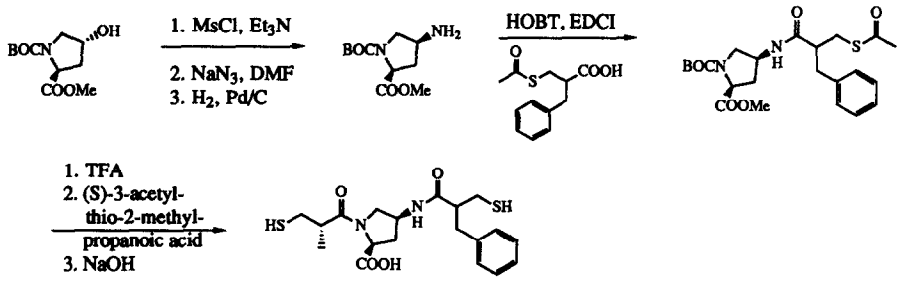
zofenopril



spirapril

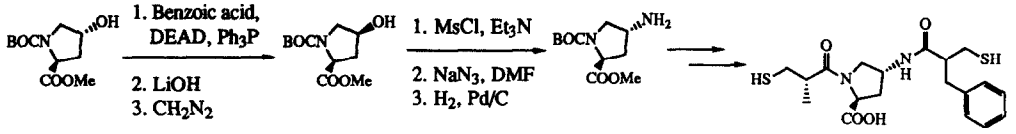
ACE and NEP are zinc metalloproteases that are inhibited by captopril and thiorphan respectively. We decided to design a novel series of compounds by joining these two structures with a variety of linkers. The structure activity relationship (SAR) for each compound was therefore examined. It has been found that placing a substituent at the 4-position of the proline ring of captopril and enalapril, as exemplified by zofenopril and spirapril, leads to potent and long acting ACE inhibitors.[4] We decided to link a thiorphan-like structure at this position. It has been reported that the carboxylic acid of thiorphan could be extended out, as exemplified by SQ 29072, with little loss in activity.[5] Keeping these considerations in mind, we designed the following initial targets in which the zinc chelating group, the P_1' residue and the amide linkage necessary for the NEP inhibitory activity are attached to the 4-position of the proline ring of captopril and enalapril. The carboxylic acid group in these targets serves as the C-terminal acid required for both ACE and NEP inhibitory activities.

Scheme I



1 MW = 410

Scheme II

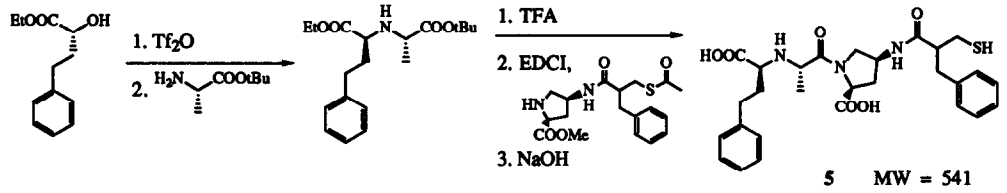


2

Compound **1** with a 4-cis-substituent on the proline ring was synthesized as shown in Scheme I. Functional group manipulations on the BOC-protected L-4(R)-hydroxyproline methyl ester gave the corresponding 4-cis-amino derivative. Coupling of this amine with the racemic 2-benzyl-3-acetylmercapto-propanoic acid[6] gave the proline derivative with a 4-cis-thiorphan-like substituent. Deprotection followed by coupling with the commercially available (S)-3-acetylthio-2-methyl-propanoic acid and hydrolysis afforded **1**. Compound **3** was prepared similarly using the corresponding biphenyl derivative. Compound **2** was prepared from the BOC-protected-4(S)-hydroxyproline methyl ester, which in turn was prepared by inversion of the 4(R) compound as shown in Scheme II. The enalapril-thiorphan compounds, **5** and **6** were synthesized utilizing the appropriately substituted proline derivative in the synthesis of enalapril (Scheme III). Compounds **4**, **7** and **8** were synthesized by reacting the appropriate 4-substituted proline derivative with the isocyanate of the

retrothiorphan fragment[7] which was prepared in situ by the Curtius rearrangement of 3-acetylthio-2-benzyl-propionic acid.

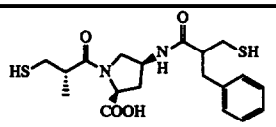
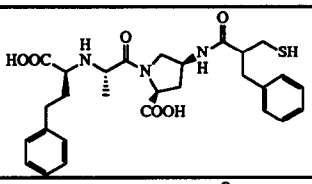
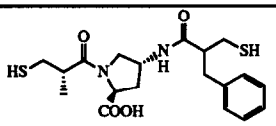
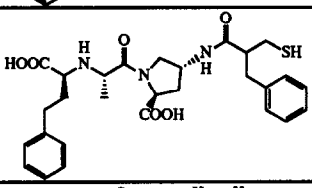
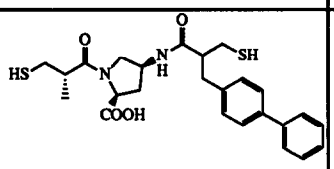
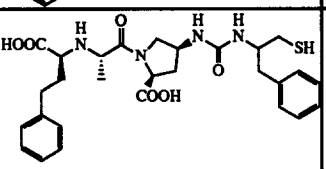
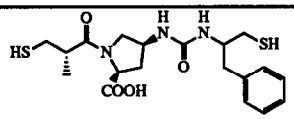
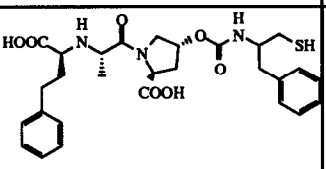
Scheme III



5 MW = 541

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 buffer, 37 °C for 30 min). The product histidyl-leucine was reacted with o-phthalaldehyde (2

Table I: In vitro activity of ACE/NEP inhibitors

Number	Structure	IC_{50} (nM) ACE	NEP	Number	Structure	IC_{50} (nM) ACE	NEP
1		87	14	5		42	149
2		18	849	6		253	> 1000
3		696	20	7		43	1240
4		147	118	8		506	> 1000

mg/ml in methanol) and measured spectrophotometrically at 360 nM as described by Cushman and Cheung.[8] Inhibition of NEP was determined by the extent of hydrolysis of the substrate glutaryl-Ala-Ala-Phe-2-naphthyl amide using a modified 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).[9,10] The results were expressed as IC_{50} values (Table I).

Compound **1** with the 4-cis-substitution on the proline ring was found to be a potent inhibitor of NEP (IC_{50}=14 nM) and moderately active inhibitor of ACE (IC_{50}=87 nM). Changing the stereochemistry at the 4-position of this compound (see compound **2**) led to sixty fold decrease in NEP inhibition (NEPI) and five fold increase in ACE inhibition (ACEI). Increasing the steric bulk of the P_1'-substituent of the thiorphan portion (compare **1** and **3**) led to retention of NEPI and to a significant drop in ACEI. Modifying the thiorphan portion to retro-thiorphan subunit and joining it to the 4-cis-amino group of the proline ring (compound **4**) resulted in decrease of both NEPI and ACEI. In the enalapril-thiorphan series, change in the stereochemistry at the 4-position of the proline ring had a more dramatic effect on the dual activity. Thus, compound **6** was much less active than **5** as a dual ACE/NEP inhibitor. The thiorphan to retro-thiorphan change in this case (compare **5** and **7, 8**) led to retention of ACEI but significant drop in NEPI.

Inversion of the stereochemistry at the 4-position leading to the trans- orientation, increased the potency of ACEI in the captopril-thiorphan series but decreased the potency in the enalapril-thiorphan series. On the other hand, this change in stereochemistry led to decrease in potency of NEPI in both series. If one makes an assumption that the captopril or enalapril substructure of these molecules binds to ACE and thiorphan substructure to NEP, it appears that the nature of binding at the active site of ACE (thiol in captopril versus carboxylic acid and P_1 substituent in enalapril) has a profound effect on the tolerability of stereochemistry at the 4-position.

In conclusion, novel compounds that inhibit ACE and NEP were synthesized by incorporating a thiorphan unit at the 4-position of the proline ring of captopril and enalapril. The modification in the substituent at the 4-position had significant effect on the ACEI and NEPI. The preferred orientation of the substituent, with respect to the overall dual activity, was cis- to the carboxylic acid of proline. Compound **1** represents a novel structure exhibiting the dual ACE and NEP 24.11 inhibitory activities.

References: † Current address: Abbott Laboratories, Neuroscience Research, D4PM, AP10/3, One Abbott Park Road, Abbott Park, IL 60064-3500. (1) Waeber, B.; Nussberger, J.; Brunner, H. *Hypertension*; Laragh, J. H.; Brenner, B. M., Eds.; Raven Press: New York, 1990; Vol 2, pp. 2209-2232. (2) Roques, B. P.; Noble, F.; Dauge, V.; Fournie-Zaluski, M. C.; Beaumont, A. *Pharmacol. Rev.* **1993**, 45, 87-146. (3) Roques, B. P.; Beaumont, A. *Trends Pharmacol. Sci.* **1990**, 11, 245-249. (4) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, 5, 483-531. (5) Seymour, A. A.; Norman, J. A.; Asaad, M. M.; Fennel, S. A.; Swerdel, J. N.; Little, D. K.; Dorso, C. R. *J. Cardiovasc. Pharmacol.* **1990**, 16, 163-172. (6) Ondetti, M. A.; Condon, M. E.; Reid, J.; Sabo, E. F.; Cheung, H. S.; Cushman, D. W. *Biochemistry* **1979**, 18, 1427-1430.(7) Roques, B. P.; Lucas-Soroca, E.; Chaillet, P.; Costentin, J.; Fournie-Zaluski, M.-C. *Proc. Natl. Acad. Sci.* **1983**, 80, 3178-3182. (8) Cushman, D. W.; Cheung, H. W. *Biochem. Pharmacol.* **1981**, 20, 1637-1648. (9) Orlowski, M.; Wilk, S. *Biochemistry* **1981**, 20, 4942-4950. (10) Sonnenberg, J. L.; Sakane, Y.; Jeng, A. Y.; Koehn, J. A.; Ansell, J. A.; Wennogle, L. P.; Ghai, R. D. *Peptides* **1988**, 9, 173-180.

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