*J. Enzyme Inhibition*, 1997, Vol. 12, pp. 155–160
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Printed in India

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by HINARI on 12/17/11
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## *Short Communication*

# NOVEL SELECTIVE THIOL INHIBITORS OF NEUTRAL ENDOPEPTIDASE CONTAINING HETEROCYCLES AT $P'_2$ POSITION

GABRIELE NORCINI*, GABRIELE MORAZZONI, FELICE POCCHIARI, FRANCESCO SANTANGELO and CLAUDIO SEMERARO

*R&D, Zambon Group Spa, Via Lillo del Duca 10, 20091 Bresso (Milan), Italy*

*(Received 3 December 1996)*

*Keywords:* 3.4.24.11 Neutral Endopeptidase; NEP; Thiol inhibitors; Structure–Activity Relationship

## INTRODUCTION

The Atrial Natriuretic Factor (ANF) is a 28 amino acid peptide produced by the heart in response to atrial distension. By interacting with a biological receptor generating cGMP[1] it causes rapid natriuresis and diuresis, decreases blood pressure and counteracts the effects of activation of the Renin-Angiotensin System.[2] Despite the significant efficacy and extreme potency of ANF, its clinical usefulness is limited by a short half life and a poor oral bioavailability.[3]

One strategy to circumvent these limitations is to prevent the degradation of endogenous ANF catalyzed by Neutral Endopeptidase EC 3.4.24.11 (NEP).[4,5]

* Corresponding author.



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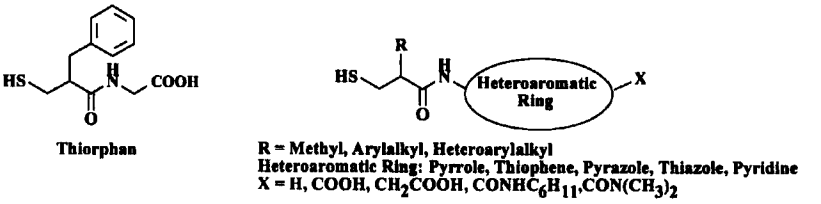


FIGURE 1 Structure of thiorphan and general structure of compounds studied.

The inhibition of NEP[6] increases the circulating ANF that has demonstrated therapeutically beneficial haemodynamic and renal effects in pathological states. In particular, Candoxatrilat[7] is in advanced clinical development for the treatment of congestive heart failure.

We have recently reported a series of phosphonoamide inhibitors of NEP, characterized by the presence of a heteroaromatic ring at the $P'_2$ position.[8] Continuing our line of research, we synthesized a series of thiol-inhibitors by the introduction of the same kind of modifications in the original structure of thiorphan,[9] one of the more recognized NEP inhibitor, even though thiorphan shows a significant inhibition also on the Angiotensin Converting Enzyme (ACE) (Figure 1). Thus, we obtained the potent NEP inhibitors **6g–s** with an enzyme selectivity higher than thiorphan, since our compounds are not active on ACE even at a micromolar concentration.

## CHEMISTRY

The synthetic pathway is shown in Scheme 1. Compounds **1a–f** were obtained by malonic synthesis starting from the appropriate organic halide, followed by hydrolysis of the esters with potassium hydroxide in a water-methanol mixture. The reaction of **1a–f** with dimethylamine and formaldehyde in water gave the Mannich bases **2a–f** in reasonable yield. Heating at 120°C produced the unsaturated acids **3a–f**. Thioacetic acid was mixed with **3a–f** without solvent and heated to 100°C for 3 h affording the desired beta-acetylmercaptopropionic acids **4a–f**.[10] The reaction of carboxylic acids with heterocyclic amines[11] in the presence of a condensing agent such as dicyclohexylcarbodiimide was unsuccessful. Only activation into the corresponding acyl chlorides, prepared by reaction with thionyl chloride in toluene, allowed condensation yielding N-heterocyclo amides **5g–p**.

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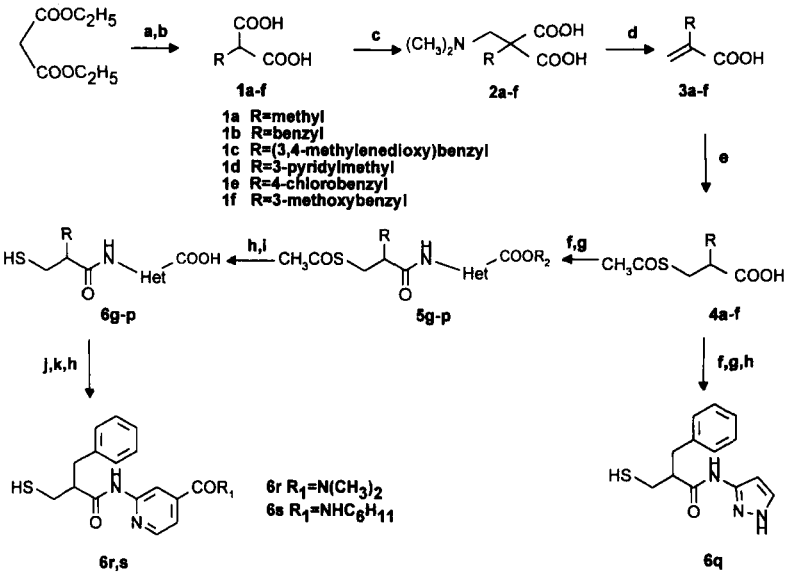


SCHEME 1 General Scheme of Synthesis. **a**: NaOMe/MeOH, RCl; **b**: KOH, MeOH/water; **c**: HCHO 33%, dimethylamine; **d**: heat (120°C); **e**: thioacetic acid; **f**: $SOCl_2$, toluene; **g**: $R_2OOC$-Het-$NH_2$, $R_2 = CH_3$ or $C_2H_5$, TEA, toluene; **h**: NaOH/MeOH, water; **i**: HCl, water; **j**: t-$C_4H_9COCl$, NaOH; **k**: DCC, *N*-hydroxysuccinimide then *N*,*N*-dimethylamine or cyclohexylamine.

Finally the simultaneous hydrolysis of the acetylmercapto group and the carboxylic ester gave **6g–p**. The unsubstituted pyrazole derivative **6q** was obtained in a similar way.

The carboxylic acid of **6i**, after protection of the thiol group, was activated as the N-hydroxysuccinimido ester and then condensed with dimethylamine or cyclohexylamine yielding the corresponding amides. The final hydrolysis led to **6r,s**.

## DISCUSSION

Compounds **6g–s** were tested for *in vitro* inhibition of NEP[12] and the results are summarized in Table I.

The introduction of a heterocyclic aminoacid maintained, and at the same time, improved the inhibitory effect on NEP in comparison with thiorphan. This effect did not seem to be related to stereoelectronic characteristics of the heterocycle, since pyridyl, pyrrolyl, thiophenyl and thioazolyl rings were all well accepted. As expected the presence of an aromatic

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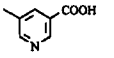
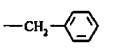
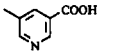
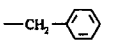
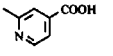
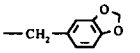
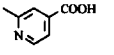
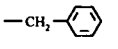
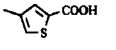
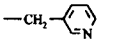
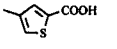
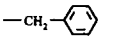
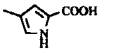
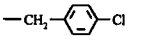
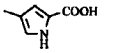
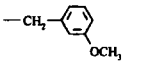
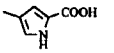
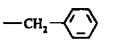
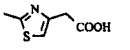
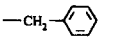
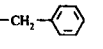
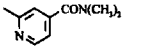
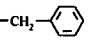
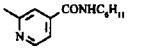
TABLE I Effect of structural modification on NEP inhibition


| *Compound* | *R* | Het—$COR_1$ | $IC_{50}$ (nM)[†] |
|---|---|---|---|
| **6g** |  |  | 60 |
| **6h** |  |  | 5 |
| **6i** |  |  | 3 |
| **6j** |  |  | 5.5 |
| **6k** |  |  | 2 |
| **6l** |  |  | 13 |
| **6m** |  |  | 2 |
| **6n** |  |  | 2 |
| **6o** |  |  | 5 |
| **6p** |  |  | 9 |
| **6q** |  |  | 23 |
| **6r** |  |  | 5 |
| **6s** |  |  | 250 |
| Thiorphan: | *N*-(3-phenyl-2-mercaptomethyl-1-oxopropyl)glycine | | 18 |
| Glycoprilat: | *N*-[3-(3,4-methylene dioxyphenyl)-2-(mercapto-methyl)-1-oxopropyl]glycine | | 19 |

[†] NEP was obtained from rat kidney cortex membrane. Assay were carried out as described in ref. 12. The $IC_{50}$ value of the compounds tested was determined using increasing concentration in triplicate. Each value represent the mean of two experiments.

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ring instead of a methyl group at the $P_1'$ position increased the potency of our inhibitors as demonstrated by comparison between **6h** and **6g**. In addition, the introduction of an electron-withdrawing atom such as chlorine (compare **6m** *vs* **6n**) or an electron-donating group such as methoxyl (compare **6m** *vs* **6o**) did not affect the inhibitory potency. The substitution of the phenyl ring of **6k** with the more polar pyridyl ring of **6l** gave only a slight decrease in potency.

Finally we studied the effects of other modifications, such as the elimination of the carboxylic acid or its transformation into a substituted amide. In line with the endopeptidase nature of NEP, both modifications led to a modest decrease in inhibitory potency. The unsubstituted pyrazole derivative **6q** was equipotent to thiorphan and the N,N-dimethylamide **6r** maintained practically the same activity as the corresponding carboxylic acid al-though the more hindered amide **6s** showed a relevant loss of potency. On the other hand, previous SAR studies with NEP inhibitors have already demonstrated that carboxylic acid is not essential for this activity.[14]

Thiorphan was tested in our laboratory as an ACE inhibitor[13] and showed a significant potency ($IC_{50} = 99\,nM$) whereas none of our compounds elicited inhibitory effects on ACE up to 1000 nM concentration. We introduced methylenedioxyphenyl into compound **6j**, a moiety present in glycoprilat which in the previous literature[15] had been reported as a dual ACE and NEP inhibitor with balanced activity: $IC_{50}ACE = 22\,nM$ and $IC_{50}NEP = 19\,nM$ respectively (internal data). However, **6j** was an effective NEP inhibitor not active on ACE ($IC_{50}ACE > 1000\,nM$) confirming that the introduction of heteroaromatic rings at the $P_2'$ position strongly increases the selectivity.

In conclusion, a general Structure–Activity Relationship could be drawn:

- The carboxylic acid is not essential i.e. **6q**, **6r**.
- The $S_1'$ site of the enzyme accepts aromatic substitutes without strict requirements i.e. **6j**, **6l**, **6n**, **6o**.
- The $S_2'$ site of the enzyme accepts high steric acid electronic tolerance i.e. **6h**, **6i**, **6k**, **6m**, **6p**.

Finally, our results contribute to the general understanding of the structural requirement of an effective NEP inhibitor.

***Acknowledgment***

We thank P. Castelnovo, R. Fattori and M. Gregori for providing analytical data on the compounds described in this study.

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[10] (S)-Beta-(acetylmercapto) isobutyric acid was also purchased from Janssen.
[11] The heterocyclic aminoacids were prepared as described in: (a) methyl 5-amino-nicotinate: Hawkins, G.F. and Roe, A. (1949) *J. Org. Chem. Soc.*, **14**, 328. (b) methyl 2-amino-isonicotinate: Ferrari, G. and Marcon, A. (1958) *Farmaco Ed. Scient.*, **13**, 485. (c) ethyl 4-aminopyrrole-2-carboxylate: Hale, W.J. and Hogt, W.J. (1915) *J. Am. Chem. Soc.*, **37**, 2538. (d) ethyl 5-aminothiophene-2-carboxylate: Sy, M. and De Malleray, B. (1962) *Bull. Soc. Chem. Fr.*, 1276. (e) 3-aminopyrazole: Austin, M.W., Blackborow, J.R., Ridd, J.H. and Smith, B.W. (1965) *J. Chem. Soc.*, 1051. (f) 2-amino-4-thiazolylacetic acid was a commercial sample.
[12] The NEP assay was performed according to the method of Llorens, C. and Schwartz, J.C. (1981) *Eur. J. Pharmacol.*, **69**, 113. Briefly, membrane from rat kidney cortex (Maeda, T., Balakrishnan, K. and Medi, S.Q. (1983) *Biochim. Biophys. Acta*, **731**, 115.) was used as the NEP preparation and [$^3$H]-Leu-Enkephalin was employed as substrate.
[13] The ACE activity was evaluated in purified rabbit lung preparation by a spectrophotometric technique using Furanacryloyl-phenyl-alanyl-glycyl-glycine (FAPGG) as substrate according to Holmquist, B., Bunning, P. and Riordan, J.F. (1979) *Anal. Biochem.*, **95**, 540.
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