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# NON-PEPTIDIC INHIBITORS OF NEUTRAL ENDOPEPTIDASE 24.11
## 1. DISCOVERY AND OPTIMIZATION OF POTENCY

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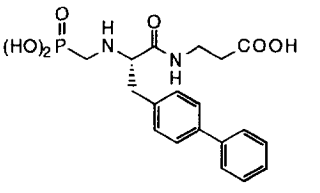
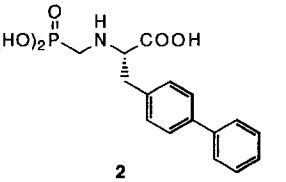
***Abstract:*** Although based on a single α-amino acid residue, *N*-phosphonomethyl-(S)-(4-phenyl)phenylalanine (**2**) was discovered to produce strong inhibition of the zinc metalloprotease, neutral endopeptidase (NEP 24.11). Structural optimization of this new lead culminated with the design of the phosphonic acid tetrazole **17** (CGS 26303), a non-peptidic and extremely potent NEP inhibitor.

Neutral endopeptidase (NEP 24.11) is a membrane-bound zinc metalloprotease involved in the degradation of various physiologically important peptides, including the enkephalins and atrial natriuretic peptide (ANP), a vasorelaxing and diuretic cardiac hormone. Potentiation of endogenous ANP levels through NEP inhibition is currently under clinical evaluation as an alternative therapy for hypertension and congestive heart failure.[1] Originally modeled on the enkephalin substrates,[2] all potent NEP inhibitors contain a modified di- (or tri-) peptide backbone, embracing a critical secondary amide, and linked to a zinc-chelating element (e.g. thiol, carboxylic acid, hydroxamic acid or phosphorus-containing acid).[3] A similar strategy was applied to the design of the phosphonomethyl dipeptide **1** (CGS 24592), recently disclosed as a potent ($IC_{50}$ = 1.9 nM) and long-acting NEP inhibitor.[4] During extensive pharmacological studies with **1**, we noticed its very slow hydrolysis in bicarbonate solution (25-30% conversion after 26 days at 37°C in 0.25 M $NaHCO_3$) to the truncated derivative **2**, a transformation probably facilitated by the participation of the neighboring phosphonic acid group. Although, in the absence of a typical dipeptidic framework, **2** was anticipated to be inactive as an NEP inhibitor, *in vitro* testing revealed its unexpectedly potent NEP inhibitory activity ($IC_{50}$ = 15 nM).[5]




**1 (CGS 24592)**

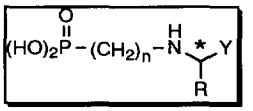


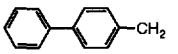
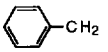
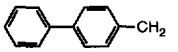
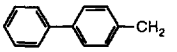
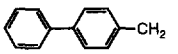
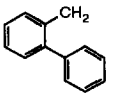
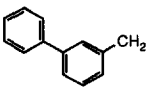
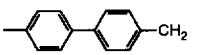
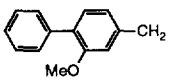
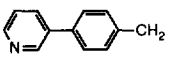
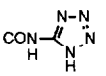
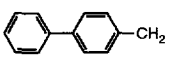
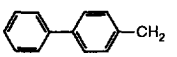
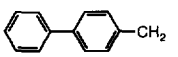
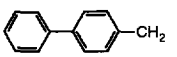
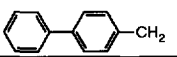

**2**

The structure of **2** represents a significant departure from those of the known potent NEP inhibitors,[3] thereby offering a unique opportunity for the design of novel non-peptidic inhibitors. In this Letter, we report a SAR investigation that culminated with the discovery of a structurally unique and highly potent inhibitor of NEP.

In the initial phase of our study, we focused on simple derivatizations of the C-terminal carboxylic acid functionality and on modifications of the (S)-4-biphenylmethyl substituent of **2** (Table 1).[6]

*Table 1 . Effects of Structural Modifications of 2 on NEP Inhibition*

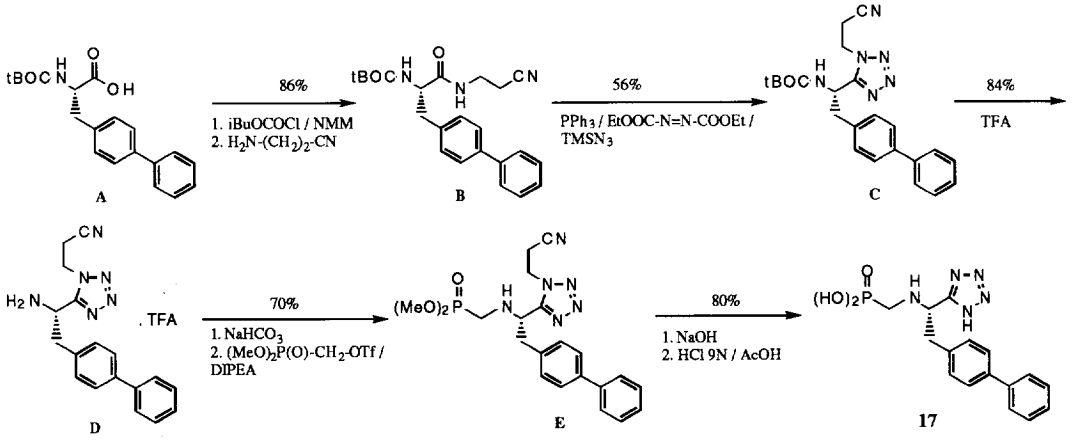


| Cpd | * | n | Y | R | $IC_{50}$ (μM) | Method of synthesis |
|---|---|---|---|---|---|---|
| **2** | (S) | 1 | COOH |  | 0.015 | ref.[6] |
| **3** | (S) | 1 | COOH |  | >1 | ref.[6] |
| **4** | (S) | 1 | $CONH_2$ |  | 1.3 | ref.[7] |
| **5** | (S) | 1 | CONHBn |  | 0.308 | ref.[8] |
| **6** | (R) | 1 | COOH |  | >1 | ref.[6] |
| **7** | (R,S) | 1 | COOH |  | >1 | ref.[9] |
| **8** | (R,S) | 1 | COOH |  | >1 | ref.[9] |
| **9** | (S) | 1 | COOH |  | 0.236 | ref.[9] |
| **10** | (S) | 1 | COOH |  | 0.014 | ref.[10] |
| **11** | (S) | 1 | COOH |  | >1 | ref.[11] |
| **12** | (S) | 1 |  |  | 0.0014 | ref.[12] |
| **13** | (S) | 2 | COOH |  | >10 | ref.[13] |
| **14** | (S) | 1 | $CH_2COOH$ |  | 0.015 | ref.[14] |
| **15** | (S) | 1 | $(CH_2)_2COOH$ |  | 0.643 | ref.[15] |
| **16** | (S) | 1 | CH=CHCOOH[a] |  | 0.032 | ref.[15] |

[a] E-isomer.

The critical importance of both the carboxylic acid group and the (S)-4-biphenylmethyl substituent in **2** is clearly demonstrated by the weak activity of the analogues **3-8**. The length of the 4-biphenylmethyl group appears to be optimum for NEP binding, as indicated by the diminished potency of compound **9**. Meta substitution of the proximal phenyl ring by a methoxy group (e.g. **10**) had no significant effect on NEP inhibition, but replacement of the distal phenyl ring in **2** with the basic 3-pyridyl group (e.g. **11**) produced a loss of inhibitory activity, a result consistent with the known lipophilic nature of the NEP $S_1'$ subsite. Excellent NEP inhibition was recovered with **12**, in which the acidic group had been introduced as a tetrazole. Comparing the relative inhibitory activity of **1** and **12**, it is interesting to note that a 5-amino tetrazole moiety can successfully substitute for a β-alanine residue at the C-terminus. However, since **12** still retained a secondary amide linkage, further optimization was pursued. Previous SAR studies with NEP inhibitors have indicated that some structural flexibility exists regarding the position of the zinc-chelator relative to the C-terminal carboxylic acid.[16] Therefore, homologations of the N- and C-termini were also investigated. The β-aminoethyl phosphonic acid **13** was inactive, but the elongated C-terminal carboxylic acids **14-16** retained some NEP inhibitory activity, despite their lack of an amide bond.[17] In particular, extension of the carboxyl end of **2** by one methylene (i.e. **14**) had no measurable impact on the inhibitory activity.

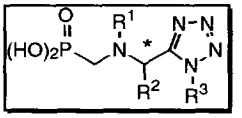
Considering that a C-terminal acidic group was required to achieve potent NEP inhibition with these compounds, we directed our subsequent efforts to finding surrogates for the carboxylic acid group in **2**. It was quickly discovered that a significant increase in potency could be achieved with a tetrazole ring (Table 2). A synthetic procedure was selected to provide a convenient access to the tetrazole analogues of *N*-tBOC-(L)-α-amino acids. Serving our purpose, the method of Duncia et al.[18] turned out to be mild and generally applicable, affording derivatives selectively protected on the tetrazole ring at $N^1$. After deprotection of the primary amine, the phosphonomethyl group was readily installed by alkylation, as described for the preparation of **2**.[6] Sodium hydroxide-induced β-elimination of the cyanoethyl group, followed by demethylation of the phosphonates completed the synthesis of the desired α-amino phosphonic acids. The synthesis of **17** is representative (Scheme).

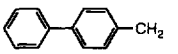
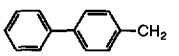
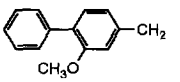
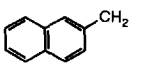
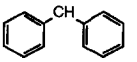
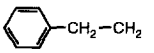
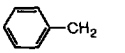
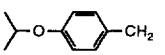
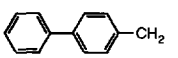
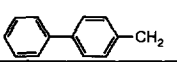



Scheme

Replacement of the carboxylic acid in **2** with a tetrazole afforded **17** (CGS 26303), a compound identified to be among the most potent NEP inhibitors reported ($IC_{50}$ = 0.93 ± 0.1 nM, n=9). Results indicated again that, in this series, the presence of an (S)-4-biphenylmethyl substituent was critical for optimum binding. Contrary to the SAR developed with thiol-containing inhibitors of NEP,[19] the α-amino phosphonic acids specifically require the (S) configuration for inhibitory activity (compare **17** vs. **18**).[4] As in the previous series, methoxy substitution of the proximal phenyl ring did not affect significantly the potency of the inhibitor (compare **19** and **17**). Interestingly, although the carboxylic acid **3** was inactive (Table 1), its tetrazole analogue **23** was a relatively potent NEP inhibitor, thereby indicating that the tetrazole ring represents more than a simple carboxylic acid surrogate, but also participates in additional productive binding interactions with the active site. Replacement of the biphenyl group with a 4-isopropoxyphenyl substituent, as in **24**, led to a compound about 15 times less potent than **17**, but slightly more active than the unsubstituted analogue **23**. Methylation of the amino group (e.g. **25**) resulted in a substantial loss of activity, as did the $N^1$-benzylation of the tetrazole moiety (e.g. **26**).

***Table 2. Inhibition of NEP by Tetrazole Analogs of 2***




| Cpd | * | $R^1$ | $R^2$ | $R^3$ | $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **17** | (S) | H |  | H | **0.00093** |
| **18** | (R) | H |  | H | >1 |
| **19** | (S) | H |  | H | 0.0015 |
| **20** | (S) | H |  | H | 0.364 |
| **21** | (R,S) | H |  | H | >1 |
| **22** | (S) | H |  | H | >10 |
| **23** | (S) | H |  | H | 0.026 |
| **24** | (S) | H |  | H | 0.015 |
| **25** | (S) | $CH_3$ |  | H | 0.530 |
| **26** | (S) | H |  | $CH_2Ph$ | >1 |

In a selectivity screening, **17** did not inhibit angiotensin-converting enzyme (ACE) or thermolysin (TLN), but showed modest activity ($IC_{50}$ = 1.1 μM) as an inhibitor of endothelin-converting enzyme (ECE), an endopeptidase bearing some homology with NEP.[20,21] *In vivo*, **17** displayed potent and sustained NEP inhibition, and even showed pharmacological effects consistent with a functional blockade of ECE.[22] Unfortunately, **17** was devoid of any significant oral activity in rats.

In conclusion, with the discovery of **2**, we have demonstrated for the first time that potent NEP inhibition could be achieved with a compound based on a *single α-amino acid residue* instead of the usual dipeptide motif. Optimization of the potency has led to the phosphonic acid tetrazole **17** (CGS 26303), which belongs to a new structural type of highly potent and non-peptidic NEP inhibitors with interesting pharmacological properties. Unfortunately, the poor oral bioavailability of **17** limits its potential as a therapeutic agent. This issue is addressed in the following communication.[23]

***Acknowledgments.*** We thank Dr. A. Jeng and Mrs. P. Savage for their valuable contribution, and the Ciba Analytical Chemistry Staff for providing the analytical data on the compounds described in this study.

**References and Notes**

(1) Ruskoaho, H. *Pharmacol. Rev.* **1992**, *44*, 479.

(2) Llorens, C.; Gacel, G.; Swerts, J.-P.; Perdrisot, R.; Fournié-Zaluski, M.-C.; Schwartz, J.-C.; Roques, B. P. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 1710.

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

(4) De Lombaert, S.; Erion, M. D.; Tan, J.; Blanchard, L.; El-Chehabi, L.; Ghai, R. D.; Sakane, Y.; Berry, C.; Trapani., A. J. *J. Med. Chem.* **1994**, *37*, 498.

(5) $IC_{50}$ data were determined spectrophotometrically in duplicate, as described in ref.4, using the substrate glutaryl-Ala-Ala-Phe-2-naphthylamide (Orlowski, M.; Wilk, S. *Biochemistry* **1981**, *20*, 4924) and kidney cortex membranes as the source of NEP (Maeda, T.; Balakrishman, K.; Mehdi, S. Q. *Biochem. Biophys. Acta* **1983**, *731*, 115). The inhibitory constants of thiorphan and phosphoramidon were determined as reference standards ($IC_{50}$ = 5 and 40 nM, respectively).

(6) The truncated α-amino phosphonic acid **2**, and its analogues shown in Table 1, were prepared by phosphonomethylation of the desired amino ester with $(MeO)_2P(O)CH_2OTf$ (Phillion, D. P.; Andrew, S. S. *Tetrahedron Lett.* **1986**, *27*, 1477 and ref.4), followed by sequential deprotections of the carboxylic (NaOH) and the phosphonic acids (TMSBr or HBr / AcOH).

(7) (S)-Dimethylphosphonomethyl-4(phenyl)phenylalanine (ref.4) was esterified with diazomethane and treated with ammonia in methanol to provide, after deprotection of the phosphonic acid (HBr / AcOH), amide **4**.

(8) Coupling of (S)-*N*-(dimethylphosphonomethyl)-4(phenyl)phenylalanine with *N*-benzylamine (EDC / HOBt) afforded the precursor to **5**.

(9) The biarylalanine methyl esters used in the preparation of **7**, **8** and **9** were synthesized by Suzuki biaryl coupling of the corresponding o-, m- or p- *N*-tBOC tyrosine triflate methyl ester with phenyl or tolyl boronic acid (Shieh, W.-C.; Carlson, J. A. *J. Org. Chem.* **1992**, *57*, 379).

(10) Suzuki biaryl coupling between vanillin triflate and phenyl boronic acid gave 4-phenyl-3-methoxy benzaldehyde. Reduction ($NaBH_4$), followed by bromination (NBS / $Ph_3P$) afforded 4-phenyl-3-methoxy benzyl bromide. Enantioselective phase transfer-catalyzed alkylation of the activated glycine derivative, $Ph_2C$=N-$CH_2$-COOtBu, under O'Donnell's conditions (O'Donnell, M. J.; Bennett, W. D.; Wu, S. *J. Am. Chem. Soc.* **1989**, *111*, 2353) led to the desired optically pure (S)-(4-phenyl-3-methoxy)phenylalanine used in the preparation of **10**.

(11) Palladium-catalyzed coupling between diethyl(3-pyridyl)borane and (S)-*N*-tBOC tyrosine triflate methyl ester produced the desired (S)-4-(3-pyridyl)phenylalanine precursor to **11** (Ishikura, M.; Kamada, M.; Terashima, M. *Synthesis* **1984**, 936).

(12) (S)-*N*-(dimethylphosphonomethyl)-4(phenyl)phenylalanine methyl ester (ref.4) was first *N*-protected (CbzCl / $NaHCO_3$) to prevent diketopiperazine formation. The carboxylic acid was liberated (NaOH), activated as a mixed anhydride (iBuOCOCl / NMM), and coupled with 5-aminotetrazole. Hydrogenolysis of the Cbz group gave the desired phosphonate precursor of **12**.

(13) Reductive amination ($NaBH_3CN$) of diethylphosphonoacetaldehyde (Varlet, J. M.; Collignon, N.; Savignac, P. *Synth. Commun.* **1978**, *8*, 335) with (S)-(4-phenyl)phenylalanine methyl ester was used to prepare the β-amino phosphonate moiety in **13**.

(14) (S)-*N*-tBOC-(4-phenyl)phenylalanine was activated as a mixed anhydride (iBuOCOCl / NMM) and treated with diazomethane. Wolff rearrangement of the diazoketone (PhCOOAg / MeOH / TEA; Gordon, E. M.; Godfrey, J. D.; Delaney, N. G.; Asaad, M. M.; Von Langen, D.; Cushman, D. W. *J. Med. Chem.* **1988**, 2199) produced the chiral β-amino ester used for the synthesis of **14**.

(15) (S)-*N*-tBOC-(4-phenyl)phenylalanine was converted in 2 steps to the corresponding aldehyde (1. iBuOCOCl then $NaBH_4$; 2. $(COCl)_2$ / DMSO / TEA). Wittig olefination ($Ph_3P$=CH-COOEt) gave the (E)-α,β-unsaturated γ-amino ester precursor to **16**. Hydrogenation ($H_2$ / Pd-C) of the double bond gave the saturated amino ester used in the synthesis of **15**.

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