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HIGHLY POTENT AND SELECTIVE INHIBITORS OF ENDOTHELIN CONVERTING ENZYME

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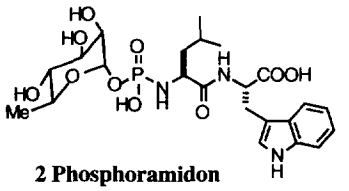
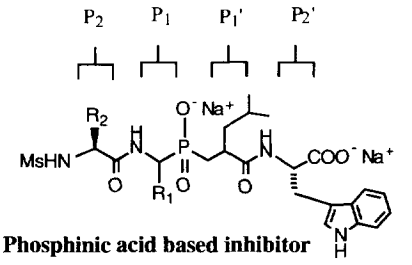
Abstract: Phosphinic acid derivatives, represented by structure **1**, have been synthesized and evaluated as endothelin converting enzyme (ECE) inhibitors. Several of these compounds (for example, **1b**, **1c**, and **1f**) were found to be potent inhibitors of ECE with a high degree of selectivity against neutral endopeptidase (NEP). Copyright © 1996 Elsevier Science Ltd

Endothelin (ET-1),[1,2] a potent vasoconstrictor and mitogenic agent, is a 21-amino acid peptidal hormone which has been implicated in a variety of pathophysiological conditions such as hypertension, congestive heart failure, renal failure, cerebral vasospasm, and atherosclerosis.[3-5] Endothelin is locally produced in various cell types under different physiological stimuli. Endothelin converting enzyme (ECE) cleaves the Trp_{21}-Val_{22} bond of big endothelin, the biologically inactive precursor to endothelin, at the last step of endothelin biosynthesis.



2 Phosphoramidon
ECE IC_{50} = 100 nM[15]
NEP IC_{50} = 4.2 nM[12]



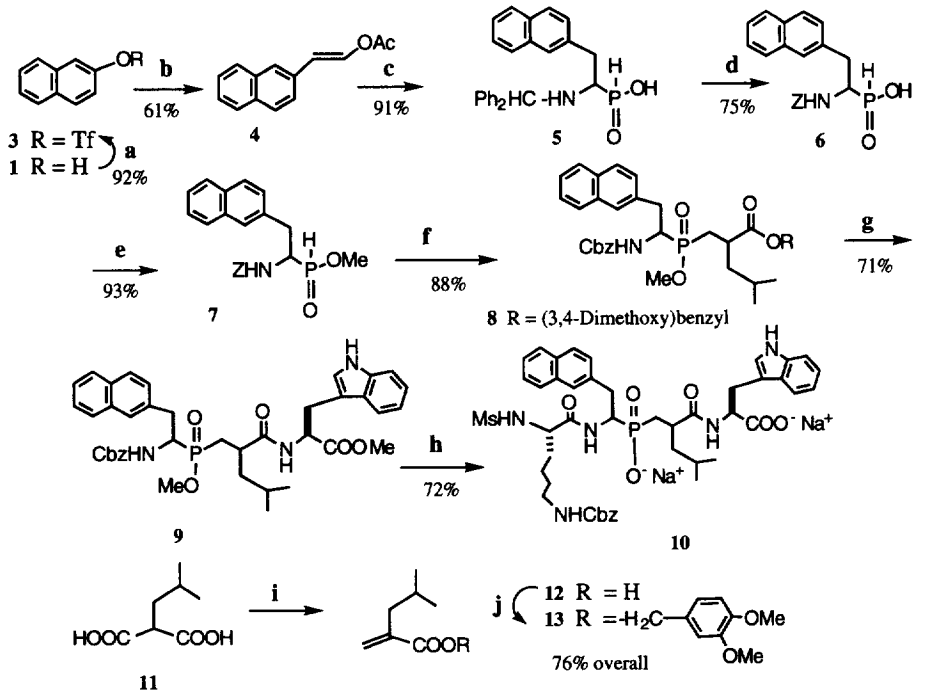
1 Phosphinic acid based inhibitor

Inhibition of endothelin as a therapeutic tool has attracted considerable attention in the management of cardiovascular, renal, and cerebrovascular diseases.[3,4] While there has been considerable progress in the discovery of endothelin receptor antagonists,[6] this progress has not been matched in the area of ECE inhibitors. Several of the known ECE inhibitors[7-12] have either low potency against ECE or poor selectivity against other metalloproteases like neutral endopeptidase (NEP). This report outlines the discovery of potent and highly selective phosphinic acid-derived inhibitors of ECE which bear structural similarity to phosphoramidon at the P_1' and P_2' sites.

Phosphoramidon (**2**) is a weak inhibitor of ECE. It shows several fold higher potency towards NEP.[12] Also the chemical instability of the phosphoramidate group makes it of limited therapeutic interest. Phosphinic acids are chemically stable and can be readily synthesized. A representative example is shown in the Scheme.

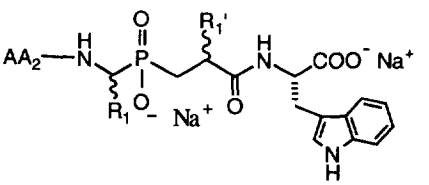
The intermediate **6** was synthesized from the vinyl acetate precursor **4** according to the literature procedure.[13] Conjugate addition of methyl phosphinate **7** to the α,β-unsaturated ester **13** gave the phosphinic acid derivative **8** as a mixture of diastereomers which was further elaborated into final targets[14] as described.

Scheme

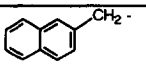
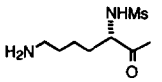
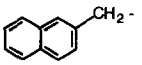
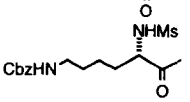
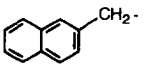
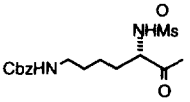
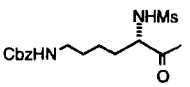
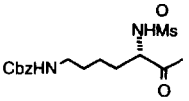
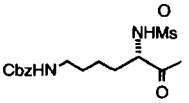
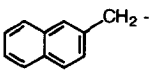
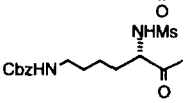
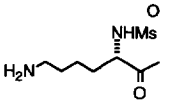
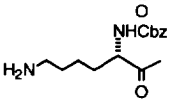
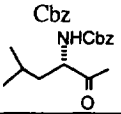


Reagents and conditions: a. Tf_2O/Py/RT b. Vinyl acetate/$Pd(OAc)_2$/Ph_3P/DMF/Et_3N c. H_3PO_2/Ph_2CHNH_2/dioxane/HCl d. (i) TFA/anisole/85–90 °C/3 h; (ii) NaOH (10%)/CbzCl e. TMS-CH_2N_2/dioxane-MeOH (7:1, v/v) f. NaH (cat)/THF; then **13** g. (i) TFA:anisole (5:2, v/v)/0 °C; (ii) H-Trp-OMe.HCl/DMF/HOBT/1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride/N-Methylmorpholine h. (i) H_2/Pd-C (10%)/EtOH; (ii) α-Ms(ε-Cbz)Lys-OH/DMF/HOBT/1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride/N-Methylmorpholine (iii) NaOH (aq)/THF i. (i) HCHO (aq, 40%)/Et_3N; (ii) 1N HCl j. (3,4-dimethoxybenzyl)alcohol/DCC/DMAP/CH_2Cl_2/0 °C/18 h.

The IC_{50} values were determined using ECE isolated from guinea pig lung membrane according to the reported protocol.[15] The data are presented in the table. A basic amino group at the P_1 position resulted in poor potency (entry **1e**). The corresponding N-Cbz derivatives (**1a**, **1l-m**) were found to have low micromolar activity. However, incorporation of a P_2 amino acid moiety greatly enhanced potency. Among the various amino acid groups examined, α-N-Cbz or N-methanesulfonyl bearing leucine and lysine residues were found to be optimal at the P_2 site. The lysine side chain could carry either a free or Cbz-protected amino group (compare **1b** and **1c**). The P_1 site could accommodate various hydrophobic residues. Among these are 2-naphthylmethyl, benzyl and isobutyl groups. The presence of a bulky hydrophobic substituent at P_1' site is essential for potency. The potency seems to decreases in the order *i*-Bu>*i*-Pr>Me as substituents at P_1' site (compare **1f**, **1g** and **1i**, **1o**). Additionally, it was found that for optimal potency the P2′ site had



Table

Entry	AA_2	R_1	R_1'	ECE IC_{50} nM	NEP IC_{50} nM
1a	Cbz		$-CH_2CH(CH_3)_2$	3300	
1b			$-CH_2CH(CH_3)_2$	65	18% @ 300
1c			$-CH_2CH(CH_3)_2$	25	13% @ 300
1d		$-CH_2(C_6H_5)$	$-CH_2CH(CH_3)_2$	500	
1e	H	$-CH_2(C_6H_5)$	$-CH_2CH(CH_3)_2$	31% @ μM	
1f		$-CH_2CH(CH_3)_2$	$-CH_2CH(CH_3)_2$	40	320
1g		$-CH_2CH(CH_3)_2$	$-CH_3$	57% @10 μM	370
1h			$-CH(CH_3)_2$	800	
1i		$-CH_2(C_6H_5)$	$-CH(CH_3)_2$	390	19% @ 300
1j		$-CH_2(C_6H_5)$	$-CH(CH_3)_2$	500	800
1k		$-CH_2(C_6H_5)$	$-CH(CH_3)_2$	250	0 %@ 1000
1l	Cbz	$-CH_2(C_6H_5)$	$-CH(CH_3)_2$	1300	
1m	Cbz	$-CH(CH_3)_2$	$-CH(CH_3)_2$	5000	
1n	Cbz	$-CH_2(C_6H_5)$	$-CH(CH_3)_2$	6000	
1o		$-CH_2(C_6H_5)$	$-CH_2CH(CH_3)_2$	160	9% @ 300

to carry a tryptophan residue bearing a free carboxylic group at the C-terminus. Carboxamides and esters of carboxylic acids were inactive (data not presented). It is noteworthy that compounds **1b**, **1c**, **1f**, and **1o**, which meet the above structural paradigm, are potent ECE inhibitors.

Several of the phosphinic acid derivatives presented in the table were tested against neutral endopeptidase 24.11 (NEP) according to the reported procedure.[16] In general, these compounds were weak inhibitors of NEP. The ECE selectivity of these compounds is apparent from the data presented for compounds **1b**, **1c**, and **1f** which inhibited ECE at 65, 25, and 40 nM, respectively and were essentially inactive towards NEP.

In conclusion, by modification of the structure of phosphoramidon, we have generated several phosphinic acid derivatives which are potent and selective inhibitors of ECE.

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References and Notes

1. Yanagisawa, M.; Kurihara, H.; Kimura, S.; Tomobe, Y.; Kobayashi, M.; Mitsuit, Y.; Yazaki, Y.; Goto, K.; Masaki, T. *Nature* **1988**, *322*, 411.
2. Two additional isoforms of endothelin (endothelin-2 and endothelin-3) have been identified. Inoue, A.; Yanagisawa, M.; Kimura, S.; Kasuya, Y.; Miyauchi, T.; Goto, K.; Masaki, T. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2863.
3. Epstein, F. H. *N. Engl. J. Med.* **1995**, *333*, 356 and references cited therein.
4. Doherty, A. M. *J. Med. Chem.* **1992**, *35*, 1493.
5. Rubanyi, G. M.; Polokoff, M. A. *Pharmacol. Rev.* **1994**, *46*, 325.
6. Battistini, B.; Botting, R.; Warner, T. *Trends Pharmacol. Sci.* **1995**, *16*, 217.
7. Chan, L.; Chittanada, A.; Shapiro, J. I.; Shanley, P. F.; Schrier, R.W. *Am. J. Physiol.* **1994**, *266*, F135.
8. Bihovsky, R.; Levinson, B. L.; Loewi, R. C.; Erhardt, P. W.; Polokoff, M. A. *J. Med. Chem.* **1995**, *38*, 2119.
9. Tsurumi, Y.; Fujie, K.; Nishikawa, M.; Kiyoto, S.; Okuhara, M. *J. Antibiotics* **1995**, *48*, 169.
10. Bertensaw, S. R.; Talley, J. J.; Rogers, R. S.; Carter, J. S.; Moore, W. M.; Branson, L. M.; Koboldt, C. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1953.
11. Bertenshaw, S. R.; Rogers, R. S.; Stern, M. K.; Norman, B. H.; Moore, W. M.; Jerome, G. M.; Branson, L. M.; McDonald, J. F.; McMahon, E. G.; Palomo, M. A. *J. Med. Chem.* **1993**, *36*, 173.
12. Fukami, T.; Hayama, T.; Amano, Y.; Nakamura, Y.; Arai, Y.; Matsuyama, K.; Yano, M.; Ishikawa, K. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1257.
13. Baylis, E. K.; Campbell, C. D.; Dingwall, J. G. *J. Chem. Soc. Perkin I*, **1984**, 2845.
14. The compounds presented in the table are a mixture of all four possible diastereomers corresponding to P_1 and P_1' centers, in approximately equal ratio, as judged by NMR data.
15. For details of IC_{50} determinations, see: Fawzi, A. B.; Cleven, R. M.; Wright, D. L. *Anal. Biochem.* **1994**, 222, 342.
16. Syberts, E. J.; Chiu, P. J. S.; Watkins, R. W.; Vemulapalli, S. J. *Hypertens.* **1990**, *8* (suppl 7), S161.

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