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DESIGN AND SYNTHESIS OF PHOSPHINIC ACIDS THAT TRIPLY INHIBIT ENDOTHELIN CONVERTING ENZYME, ANGIOTENSIN CONVERTING ENZYME AND NEUTRAL ENDOPEPTIDASE 24.11

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Abstract. We have synthesized a series of phosphinic acids as inhibitors of a metalloprotease endothelin converting enzyme (ECE). Potent ECE inhibitors **4g** and **4o** were identified. These compounds are members of a novel class of ECE inhibitors that are also potent inhibitors of angiotensin converting enzyme and neutral endopeptidase. Copyright © 1996 Elsevier Science Ltd

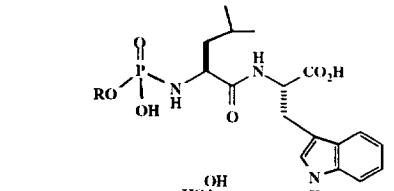
In 1988 the isolation of endothelin-1 (ET-1), a potent vasoconstrictive 21 amino acid peptide hormone from cultured endothelial cells, was described.[1] ET-1 is produced by proteolysis at Trp^{21}-Val^{22} of a less active 38 amino acid precursor big endothelin-1 (BET-1) by an endothelin converting enzyme (ECE).[1] We initiated a program to develop inhibitors of ECE as potential therapeutic agents since ET-1 has been implicated in the pathophysiology of hypertension, atherosclerosis, renal failure, and cerebral vasospasm.[2]

The identity of the enzyme responsible for conversion of BET-1 to ET in vivo has not been established with certainty. Neutral metalloprotease, aspartyl protease, and cysteine protease activities for the processing of BET-1 to ET-1 have been reported.[3] However, the metalloprotease inhibitor phosphoramidon **1** abolishes the pressor response induced by the infusion of BET-1 in rats[4] and inhibits the production of ET-1 by cultured endothelial cells,[5] suggesting that the physiologically relevant ECE activity resides with a membrane-bound neutral metalloprotease.[6] Phosphoramidon **1** also inhibits hypoxia-induced release of ET-1 in isolated perfused guinea pig lungs.[7]

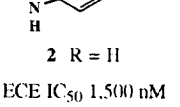
Herein we report studies toward the design and synthesis of phosphinic acids as inhibitors of a phosphoramidon-sensitive membrane-bound zinc metalloprotease ECE from partially purified guinea pig lungs.[8] These efforts resulted not only in the synthesis of potent ECE inhibitors, but also in the discovery of novel phosphinic acids that triply inhibit ECE, angiotensin converting enzyme (ACE), and neutral endopeptidase 24.11 (NEP).[9]

Our initial studies showed that phosphoramidon inhibits ECE with an IC_{50} of 500 nM. The desrhamnosyl derivative **2**[10] is also an ECE inhibitor demonstrating that the rhamnose residue of phosphoramidon is not necessary for inhibition. Furthermore, we have found that the Pfizer glutaramide **3**,[11] a dual ACE-NEP inhibitor, is an ECE inhibitor that is slightly less potent than phosphoramidon.

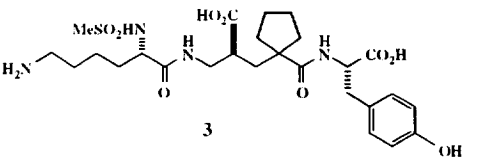
Our strategy for developing more potent ECE inhibitors was to build in appropriate residues at the P1 and P2 positions of a phosphoramidon-related structure. The fact that Trp^{21} occurs at the N-terminal side of the BET-1 cleavage site suggested incorporation of a hydrophobic P1 side chain to mimic the Trp^{21} (3-indolyl)-



1 R = H_3C ECE IC_{50} 500 nM



2 R = H ECE IC_{50} 1,500 nM



3 ECE IC_{50} 1,500 nM

methyl group of BET-1. For our initial investigation we chose a phenylmethyl substituent for this purpose. We were also influenced by the significant ECE inhibitory potency of the glutaramide **3**[11] which suggested that a cyclopentyl moiety could replace the P1' isobutyl group of phosphoramidon. This would provide a synthetic advantage in that it precludes generation of stereoisomers at this position.

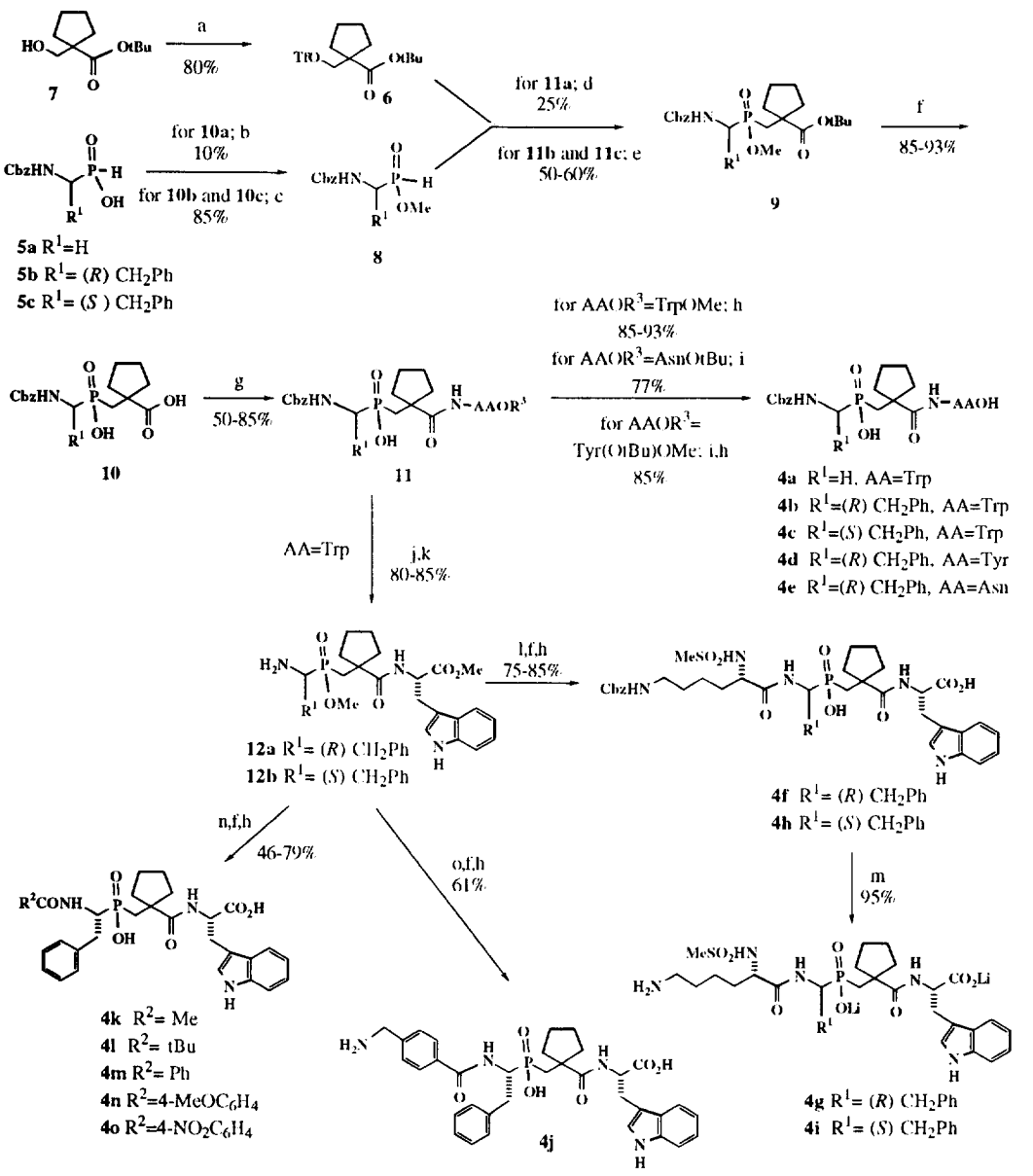
A series of phosphinic acids **4a-o** that embodies these features was synthesized as outlined in the Scheme. This synthetic route allowed us to introduce a P1 phenylmethyl group of defined stereochemistry dependent on the absolute stereochemistry of the starting phosphinic acids **5b** and **5c**.

The N-Cbz protected α-amino phosphinic acids **5a-c** were prepared by known methods[12,13] and the stereochemistry of **5b** and **5c** was established by correlation to the corresponding known phosphonic acids.[13,14] Triflate **6**, prepared from the alcohol **7**,[15] was reacted with the anion derived from the protected phosphinate ester **8** to afford the desired P-alkylated product **9**. Cleavage of the t-butyl ester of **9** with HCl/1,4-dioxane also resulted in loss of the phosphinate methyl ester, presumably via neighboring group participation of the liberated carboxylic acid,[16] to give the diacid **10**[17] in good yield. This key synthon was elaborated at the C-terminal by coupling to the appropriate amino acid ester under 1,1'-carbonyldiimidazole/DMF activation to give **11**. Deprotection of **11** afforded the diacids **4a-e**.

The amino diester **12a**, obtained by treatment of **11** (R^1 = (*R*)CH_2Ph, AA = Trp) with diazomethane followed by hydrogenolysis, was used as a common precursor for exploring SAR development at the N-terminus. Coupling of **12a** to N^{ε}-Cbz-N^{α}-MsLysOH then sequential acid cleavage of the phosphinate ester and base hydrolysis of the carboxylate ester furnished the tetrapeptide analog **4f**. Hydrogenolysis of **4f** gave the amino diacid **4g** with R absolute stereochemistry at the P1 stereocenter bearing the phenylmethyl substituent. The diastereomers of **4f** and **4g** of S stereochemistry at P1, **4h** and **4i**, were prepared in similar fashion from **12b**. Compounds **4j-o** were prepared by coupling **12a** to the appropriate acid chloride followed by deprotection.

Evaluation of the phosphinic acids **4a-o** for ECE inhibitory activity in vitro[8a] demonstrated a clear preference for the R (natural) stereochemistry at P1 (Table 1). The presence of the P1 R-phenylmethyl side chain improved potency by about two-fold as shown by comparison of **4a** and **4b**. Introduction of the P2 N^{α}-MsLysOH residue, **4f** and **4g**, afforded a further increase in potency. On the other hand, replacement of the P2' tryptophan residue with asparagine, **4e**, which corresponds to the P2' residue of BET-1, resulted in a substantial

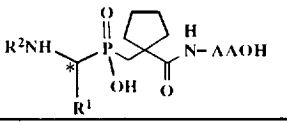
Scheme

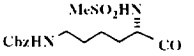
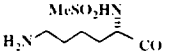
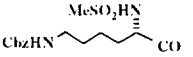
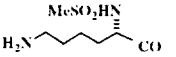


Reagents: (a) 1.5 equiv. Tf_2O, 7 eq. pyridine, CH_2Cl_2, -78°-0°C; (b) DEC, DMAP, MeOH; (c) CH_2N_2, EtOAc, MeOH; (d) NaH, THF, 6, 0°C-rt; (e) LDA, THF, -78°C then 6 -78°C to rt; (f) HCl, 1,4-dioxane; (g) L-AAOMe.HCl, 1,1'-carbonyldiimidazole, NMM, DMF; (h) aq. LiOH, MeOH; (i) TFA, CH_2Cl_2; (j) CH_2N_2, EtOAc; (k) H_2, 10% Pd/C, MeOH; (l) DEC, NMM, HOBT, N^{ε}Cbz-N^{ω}Ms-L-Lys, CH_2Cl_2; (m) H_2, 10% Pd/C, MeOH, 2 equiv. LiOH, H_2O; (n) R^2COCl, py, CH_2Cl_2; (o) 4-(BocNHCH$_2$)C$_6$H$_4$COOH, DEC, HOBT, CH_2Cl_2.

loss in potency. Tyrosine at the P2' position, **4d**, proved to be equivalent to tryptophan. Replacement of the Cbz group of **4b** with various acyl groups, **4j-o**, revealed a modest sensitivity to the nature of the acyl group with regard to ECE potency, wherein aroyl groups are preferred over an acetyl or a pivaloyl group. Notably, we have since examined the utility of the potent 4-aminomethylbenzoyl derivative **4j** as an affinity ligand for purification of ECE by affinity chromatography.[18]

Table 1. ECE inhibitory activity of phosphinic acids **4a-o**

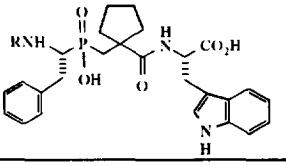


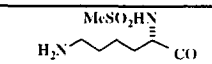
compound	R^1	P1 stereo-chem.*	R^2	AA	^{31}P nmr chemical shift[a] or formula[b]	ECE IC_{50} nM[c]
4a	H	-	Cbz	Trp	δ 32.17	420
4b	CH_2Ph	R	Cbz	Trp	$C_{34}H_{38}N_3O_7P.H_2O$	190
4c	CH_2Ph	S	Cbz	Trp	δ 33.64	>10,000
4d	CH_2Ph	R	Cbz	Tyr	$C_{32}H_{34}N_2O_6PLi_3.3H_2O$[d]	120
4e	CH_2Ph	R	Cbz	Asn	$C_{27}H_{34}N_3O_8P.1.2H_2O$	>1,000
4f	CH_2Ph	R		Trp	$C_{41}H_{52}N_5O_{10}PS.1.1H_2O$	60
4g	CH_2Ph	R		Trp	$C_{33}H_{44}N_5O_8PSLi_2.2.5H_2O$[e]	70 (55[h])
4h	CH_2Ph	S		Trp	δ 32.70	>10,000
4i	CH_2Ph	S		Trp	δ 32.67	>10,000
4j	CH_2Ph	R	4-$(NH_2CH_2)C_6H_4CO$	Trp	$C_{34}H_{37}N_4O_6PLi_2.2.5H_2O$[e,f]	90 (80[h])
4k	CH_2Ph	R	CH_3CO	Trp	δ 33.18	600
4l	CH_2Ph	R	tBuCO	Trp	$C_{31}H_{34}N_3O_6P.1.5H_2O$[g]	550
4m	CH_2Ph	R	C_6H_5CO	Trp	$C_{33}H_{36}N_3O_6P.H_2O$	250
4n	CH_2Ph	R	4-$MeOC_6H_4CO$	Trp	δ 33.44	160
4o	CH_2Ph	R	4-$NO_2C_6H_4CO$	Trp	$C_{33}H_{35}N_4O_8P.2H_2O$[g]	50

[a] ^{31}P NMR spectra were recorded on the dilithium salts in D_2O relative to trimethyl phosphate at 0.0 ppm. [b] Satisfactory microanalyses were obtained for C, H, N, P (±0.4%) unless otherwise indicated. [c] IC_{50} determined as described in ref. 8a. [d] Trilithium salt. [e] Dilithium salt. [f] Calcd C, 59.39; Found C, 58.08. [g] C, H, N analysis. [h] IC_{50} versus purified enzyme (ref. 8b).

The potent ECE inhibitors **4g** and **4o** were screened against ACE[19] and NEP[20] and were also found to strongly inhibit these enzymes (Table 2). Compounds **4g** and **4o** are thus unique in that they are the first examples of protease inhibitors that effectively inhibit ECE, ACE, and NEP

Table 2. ECE, ACE, and NEP inhibitory activity of phosphinic acids **4g** and **4o**



		IC_{50} nM		
compound	R	ECE	ACE	NEP
4g		70	2.5	90
4o	4-$NO_2C_6H_4CO$	50	1.5	55

In summary, we have developed a series of potent phosphinic acid inhibitors of a metalloprotease ECE by incorporating structural features of phophoramidon **1** and the Pfizer dual ACE-NEP inhibitor **3**. The stereochemistry of the P1 side chain and the nature of the acyl group on the P1 nitrogen were shown to be important determinants of inhibitor potency. The enhanced potency of **4f** and **4g** relative to **4b** demonstrated that a P2 residue is well tolerated. Compounds **4g** and **4o** were found to not only inhibit ECE, but also ACE and NEP with IC_{50} values <100 nM for all three enzymes.

Given the potential role of ET in vascular disease, inhibition of ECE with concomitant inhibition of ACE and/or NEP may constitute an effective new therapeutic strategy for the treatment of hypertension and other cardiovascular disorders.[21] Consequently, novel protease inhibitors such as **4g** and **4o** may prove to be important pharmacological tools and could ultimately lead to therapeutic agents that act by inhibition of all three enzymes. Although determination of the physiological consequences of ECE inhibition with compounds such as **4g** and **4o** will be challenging given their lack of selectivity, this should not preclude interest in these as potential therapeutic agents.[22]

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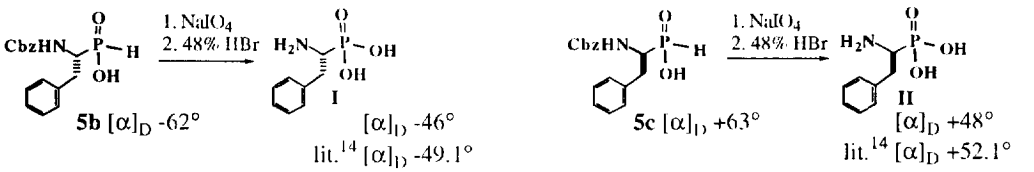

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17. Physical data for **10** (R^1 = (*R*)CH_2Ph): mp 144-145°C; $[\alpha]_D$ -52.0 ° (c 0.61 DMF); FAB MS *m/z* 446 $(M+H)^+$; ^{1}H NMR (DMSO-d6) δ 7.63 (1H, d, *J* 9.7 Hz), 7.29-7.15 (10 H, m), 4.94 (2H, m), 3.82 (1H, m), 3.08 (1H, d, *J* 13.7 Hz), 2.70 (1H, ddd, *J* 13.7, 5.7 Hz), 2.09-2.02 (4H, m), 1.69-1.60 (6H, m); ^{13}C NMR (DMSO-d6) δ 177.61, 156.03, 138.67, 137.37, 128.98, 128.26, 128.14, 127.51, 126.94, 126.14, 53.28 (d, $J_{C\text{-}P}$ 103 Hz), 64.98, 49.8 (d, $J_{C\text{-}P}$ 4.7 Hz), 36.99 (d $J_{C\text{-}P}$ 7.9 Hz), 34.17 (d $J_{C\text{-}P}$ 87.9 Hz), 32.71, 24.36; ^{31}P NMR (DMSO-d6) δ 45.03. Anal. calcd for $C_{23}H_{28}NO_6P$: C, 62.02; H, 6.34; N, 3.14; P, 6.95. Found: C, 62.24; H, 6.09; N, 3.25; P, 6.97.
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22. In vivo activity of **4g** will be reported separately.

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