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SOLID PHASE SYNTHESIS OF PHOSPHINIC ACID ENDOTHELIN CONVERTING ENZYME INHIBITORS

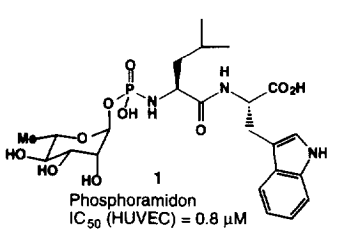
John Lloyd,* Joan B. Schmidt, John T. Hunt, Joel C. Barrish, Deborah K. Little, and Adrienne A. Tymiak.

The Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000

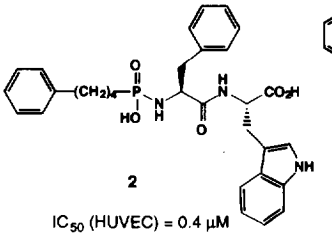
Abstract: We have synthesized a series of phosphinic acids by solid phase peptide synthesis where we have explored effect on inhibition of changes in the P_2' binding site. The most potent compounds show inhibition of ECE similar to phosphoramidon.

Endothelins (ET) are a group of vasoconstrictive, bicyclic polypeptides[1] that may be responsible for numerous pathophysiological conditions.[2] They exert their actions through extracellular receptors of which multiple subtypes have been characterized.[3] Endothelins are produced from the cleavage of the C-terminal portion of the prohormone to yield the mature, active 21 amino acid ETs.[1] The most important endopeptidase responsible for this conversion has been characterized as a zinc metalloproteinase.[4] Inhibition of this endothelin converting enzyme (ECE) would block the effects of ETs regardless of receptor subtype.

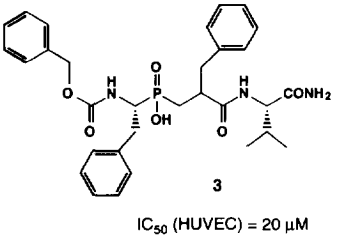
Figure



1
Phosphoramidon
IC_{50} (HUVEC) = 0.8 μM



2
IC_{50} (HUVEC) = 0.4 μM



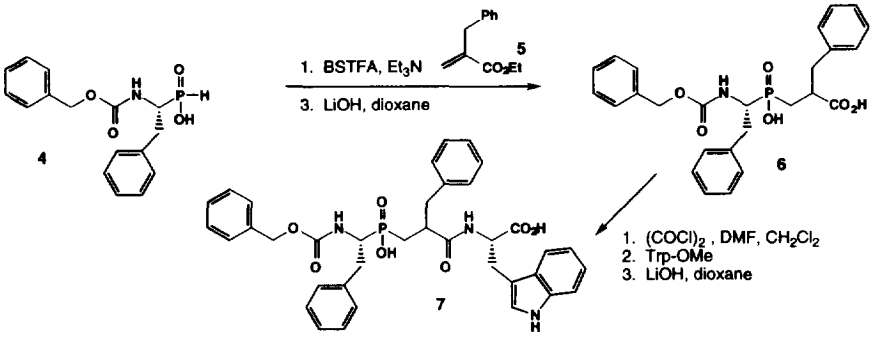
3
IC_{50} (HUVEC) = 20 μM

At the time we began our investigation of ECE inhibitors, phosphoramidon (**1**, Figure) was the most potent inhibitor known. We hoped to discover compounds with greater inhibitory potency and increased chemical and metabolic stability as well as selectivity for ECE over other closely related metalloproteinases such as neutral endopeptidase (NEP, EC 3.4.24.11) Screening of our library of metalloproteinase inhibitors led to the discovery of the phosphonamide **2**, that showed potency similar to phosphoramidon. Unfortunately, it contained the chemically labile phosphonamide functionality. Using this compound as a template, computer based similarity searching of the BMS library led us to the phosphinic acid **3** that was a mixture of diastereomers (1:1) at the carbon β to phosphorus. Although this compound was significantly less active than phosphoramidon, this structural prototype had the desired stability for a pharmaceutical agent, therefore, we investigated modification of the C-terminal (P_2') residue.

Synthesis

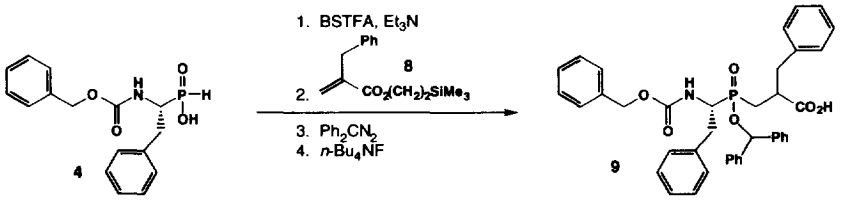
Our original solution phase synthesis of C-terminal modified analogs of the phosphinic acid **3** relied on amide formation by an acid chloride coupling reaction (Scheme 1). The phosphinic acid **4** was prepared and resolved by a known route.[5] Silylation and Michael reaction[6] with the ethyl acrylate **5** followed by base hydrolysis of the carboxylic acid ester provided the key intemediate **6**. Acid chloride formation and coupling with the amino ester followed by hydrolysis provided the C-terminal tryptophan compound **7** as a prototype.

Scheme 1



For the rapid synthesis of a large number of analogs we planned to use automated solid phase synthesis. This methodology would allow a combinatorial approach to simultaneous C- and N-terminal modifications in subsequent studies. Earlier screening in our labs indicated that inhibitors with a C-terminal acid functionality were generally more potent than C-terminal amides like the prototype **3**. Therefore, we designed our solid phase synthetic approach to use FMOC protection protocols and Wang-type amino acid resins which we planned to couple to a common phosphinic-carboxylic acid portion.[7] We also protected the phosphinic acid as the benzhydryl ester which could be removed under the resin cleavage conditions. The synthesis of the necessary intermediate was accomplished simlar to the above synthesis (Scheme 2). Michael reaction of the phosphinic acid **4** with the trimethylsilylethyl acrylate **8** was followed by formation of the benzhydryl phosphinic acid ester. Fluoride ion deprotection provided the critical intermediate **9**.

Scheme 2

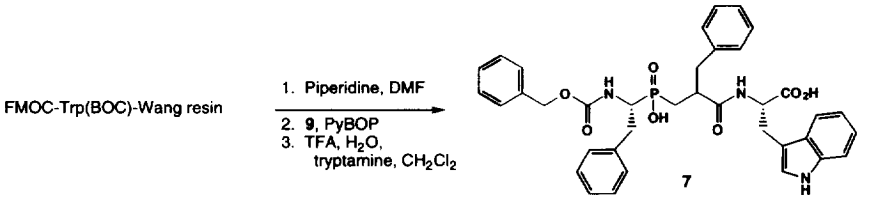


Single coupling of the carboxylic acid **9** with H_2N-Trp-Wang resin using PyBOP[8] (Scheme 3) was fairly efficient (Kaiser test). Subsequent resin cleavage and deprotection,[9] however, led to a low yield of the desired product and a variety of side products including one identified by mass spectrometry to have resulted from reaction of benzhydryl cation with the tryptophan nitrogen. The use of BOC protected tryptophan reduced the formation of this product and addition of tryptamine to the cleavage cocktail[10] provided greatly enhanced cation scavenging. Separation from the tryptamine and its trapping products was accomplished by base extraction and ether washing. The crude product was then purified by reversed phase column chromatography (CHP-20) which provided partial separation of the diastereomers that resulted from the uncontrolled stereochemistry of the Michael addition.

This procedure was then automated using a multiple peptide synthesizer (Advanced ChemTech 350) to produce analogs with all the natural amino acids (except cysteine) and several unnatural and D-amino acids in the C-terminal position of the prototype inhibitor (Table). In some cases the diastereomers were partially separated and the diastereomer ratio (A:B) was determined by HPLC. The combined yield from **4** of both

diastereomers ranged from 30-87% (ave. 53%). Purity by HPLC ranged from 90-99% (ave. 95%). The stereochemistry of the isomers at the carbon β to phosphorus was not assigned.

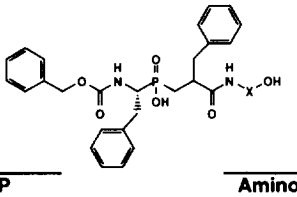
Scheme 3



Biological Results

The inhibitors were assayed for inhibition of ECE in human umbilical vein endothelial cells (HUVEC)[11]. They were also assayed for inhibition of NEP[12] which is also present in HUVEC and other endothelial cells and capable of degrading ETs. We felt that selectivity for ECE over NEP would be important for defining the role of ECE and determining its potential as a pharmaceutical target.

Table



Amino Acid	A:B	ECE $IC_{50}(\mu M)$	NEP $IC_{50}(\mu M)$
Trp	4:1 1:18	1.1 5.8	1.4 10
Val	>50:1 <1:50	1.7 80	3.0 >100
D-Val	13:1 1:4.5	>100 60	>100 >60
β-Ala	1.8:1	50	2.3
Asp	2.3:1	30	0.8
Arg	2.3:1	4.3	0.8
D-Arg	2.9:1	>100	>60
Nle	2.6:1	12	0.3
Thr	2.1:1	5	10
D-Thr	1:1.2 6:1	>100 >100	50 >100
Glu	2.7:1	25	3.0
D-Glu	1.6:1	>100	>100
Phe	1.8:1	10	0.8
D-Phe	1.9:1	>100	>60
Lys	3.5:1 1:1	30 60	>100 >100
Leu	14:1 1:1	20 23	4 7
Tyr	20:1 1:1	7 12	1 2.3
His	1.7:1	20	10
Asn	2:1	20	10
Gln	>50:1 <1:50	6 >100	15 >100
Ala	1.8:1	8	0.3
Gly	*	80	0.9
Ile	>50:1	4	1.2

* isomers not separated by HPLC

The most active compound has C-terminal tryptophan (1.1 μM) and is equipotent to phosphoramidon in our assay system. Other active compounds have C-terminal residues of valine (1.7 μM), arginine (4.3 μM), threonine (5 μM), glutamine (6 μM), tyrosine (7μM), and isoleucine (4 μM). Unfortunately, this collection of inhibitors does not reveal any preference for specific functionality at the C-terminal amino acid. There may be a preference for α-amino acids since β-alanine is >5-fold less potent than alanine. Although asparagine is the P_2' resdiue in the natural substrate, the asparagine compound was not among the best inhibitors. The activity of several compounds, including our most potent compound, with tryptophan as the P_2' residue is interesting since Trp is the P_1 residue in the natural substrate. There are also some apparent inconsistencies such as the arginine compound is significantly more potent than the lysine compound; tryptophan is preferred over the relatively conservative replacement of phenylalanine; valine and isoleucine are preferred over leucine. Despite this lack of consistent SAR, these results suggest that there are some very specific interactions of the enzyme with the putative P_2' position. These compounds display the expected stereochemical preference of L-amino acids in the P_2' position and in cases where the A and B diastereomers were separated there is a preference for one of the two isomers. Unfortunately, the preferred isomer for ECE is also preferred for NEP. Of those compounds with activity less than 10 μM, Trp, Val, Thr, Gln, and Ile have NEP activity greater than 1μM. Any future investigations will concentrate on these C-terminal amino acids in combination with modifications at the other inhibitor subsites.

In conclusion, we have discovered several phosphinic acid containing inhibitors that have greater chemical stability, equivalent ECE potency, and 1000-fold less activity against NEP than phosphoramidon.

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