Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 6, pp. 619-624, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00091-1

OPTIMIZATION OF RETRO-THIORPHAN FOR INHIBITION OF ENDOTHELIN CONVERTING ENZYME

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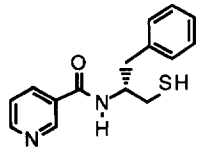
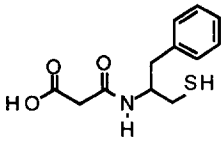
Abstract: The structural requirements of retro-thiorphan, *N*-[1*R*,*S*-benzyl-2-mercaptoethyl]malonamic acid **2**, analogs for the inhibition of endothelin converting enzyme (ECE) were investigated. Although based on a single amino acid residue, *N*-[1*R*-(*1H*-indol-3-ylmethyl)-2-mercaptoethyl]-2-phenylacetamide **28** was found to be two times more potent than the widely utilized reference inhibitor phosphoramidon.

Endothelin-1 (ET-1) is a potent, peptidic vasoconstrictor originally isolated from conditioned medium of cultured porcine aortic endothelial cells.[1] The final step of biosynthesis of this peptide requires post-translational cleavage of a precursor peptide big ET-1 at the Trp^{21}-Val^{22} amide bond by endothelin converting enzyme (ECE), a membrane-bound zinc metalloprotease.[2] Phosphoramidon, a potent inhibitor of neutral endopeptidase 24.11 (NEP),[3] has been shown to suppress the secretion of ET-1 from cultured endothelial cells[4,5] and block the pressor response induced by big ET-1 in vivo.[6] Interestingly, thiorphan, a potent thiol inhibitor of NEP,[3,7] is not effective in similar experiments.[4,6] However, through selective random screening of our compound library of zinc metalloprotease inhibitors the non-peptidic β-thiol 3-pyridyl derivative **1**, an analog of retro-thiorphan, *N*-[1*R*,*S*-benzyl-2-mercaptoethyl]malonamic acid **2**,[8] was found to exhibit moderate ECE inhibition and was active in both cell culture and tissue contraction assays.



Compound **1**

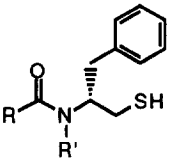


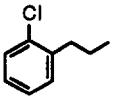
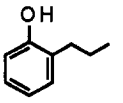
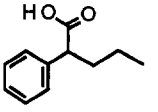
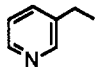
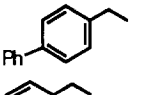
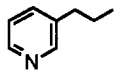
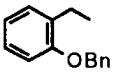
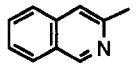
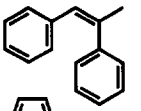
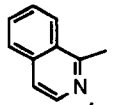
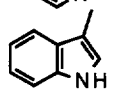
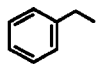
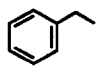
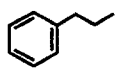
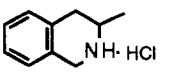
Retro-thiorphan **2**

In this study, ECE was partially purified from porcine primary aortic endothelial cells using DE52 anion exchange column chromatography, and the enzyme activity was determined by radioimmunoassays using antibodies which specifically recognize the C-terminal tryptophan of ET-1.[9] Under these conditions, retro-thiorphan **2** at 20 μM inhibited ECE activity by 28%. The 3-pyridyl derivative of retro-thiorphan, *N*-[1*R*-benzyl-2-mercaptoethyl]nicotinamide **1**, improved ECE inhibitory activity to 66% when tested at the same concentration (IC_{50} = 7.8 ± 2.3 μM; mean ± SEM, n = 3). Therefore, the P_2' position of the β-thiol retroamide **1** was subsequently modified (Table 1). Representative examples in this class of compounds indicate that due to steric limitations those with wide rigid lipophilic substituents at the P_2' position, such as compound **18**, are poorer ECE inhibitors than **1** and a pyrrolidine functionality, compound **21**, can totally abolish the ECE inhibition.

Systematic modifications of the P_1' position of the β-thiol retroamide series of compounds were also carried out, and as exemplified in Table 2, a variety of analogs derived from different amino acids were

Table 1. Effects of P_2' Modifications of **1** on ECE Inhibition



R (P_2')	R'	Cpd	% Inhibn @ 20 μM	R (P_2')	R'	Cpd	% Inhibn @ 20 μM
	H	**1**	66		H	**13**	57
	H	**3**	52		H	**14**	58
	H	**4**	48		H	**15**	48
	H	**5**	58		H	**16**	74
	H	**6**	66		H	**17**	63
	H	**7**	76		H	**18**	23
	H	**8**	36		H	**19**	54
	H	**9**	67		H	**20**	67
	H	**10**	58		H	**21**	0
	Me	**11**	24				
	H	**12**	49		H	**22**	33

The IC_{50} value for compound **1** is 7.8 ± 2.3 μM (n = 3).

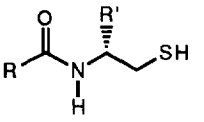
prepared. The D-tryptophan derivative **28** showed significant improvement in ECE inhibitory activity with an IC50 of 1.7 ± 0.1 μM (mean ± SEM, n = 3). In contrast, analogs with large aromatic substituents such as 4-(1-naphthyl)phenyl (compound **34**)[10] or with a small aliphatic group (compounds **23** and **24**) in the P_1' position showed markedly diminished inhibitory activity. Although the 3-pyridylalanine derivative **31** gave a comparable inhibition as that of compound **28** when measured at 20 μM, a full dose-response curve analysis

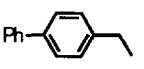
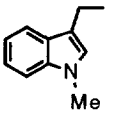
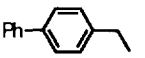
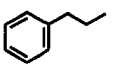
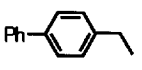
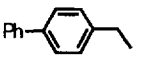
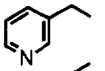
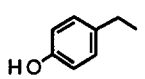
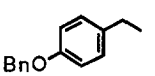
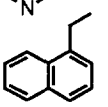
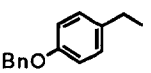
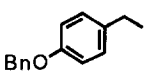
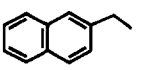
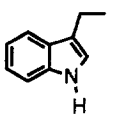
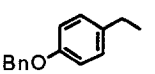
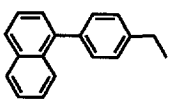
indicated that compound **31** was about threefold weaker (IC_{50} = 5.2 ± 0.4 μM, n = 3) due to a rapid decrease in the inhibitory activity upon dilution. In all cases examined, the natural amino acid based β-thiol retroamide was found to be significantly less potent than the unnatural enantiomer, for example the enantiomer of compound **35** (Table 3) inhibited ECE activity by only 19% when tested at 20 μM.

The structure activity relationships for D-tryptophan based β-thiol retroamide **28** in ECE inhibition were investigated further (Table 3). For example, both basic and acidic groups, compounds **37** and **38**, respectively, as well as conformationally constrained ring systems, compounds **36** and **42**, were incorporated into the molecule. However, following the trend for the phenylalanine series of compounds, none of these variations improved the ECE inhibition. Furthermore, the tether between the tryptophan *N*-terminus and the sulfhydryl group in compound **28** appears to be optimal for coordination of the zinc ion at the active site of ECE, that is significant decrease in the inhibitory activity was observed even with compound **40**[11] having only a single methylene group added to the tether.

The importance of hydrogen bonding in ECE inhibition by this class of compounds was also examined. Compound **29**[12] (Table 2) demonstrates that the enhanced potency of the tryptophan analog **28** is not due to

Table 2. Effects of P_1' Modifications on ECE Inhibition



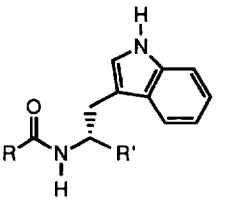
R (P_2')	R'(P_1')	Cpd	% Inhibn @ 20 μM	R (P_2')	R'(P_1')	Cpd	% Inhibn @ 20 μM
		**23**	30			**29**	68
		**24**	21			**30**	16
		**25**	39			**31**	91
		**26**	56			**32**	51
		**27**	15			**33**	10
		**28**	84			**34**	0

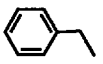
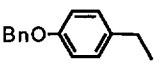
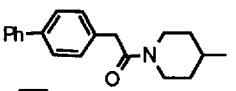
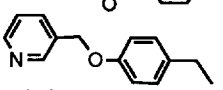
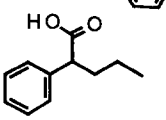
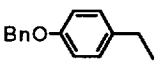
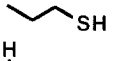
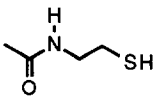
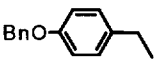
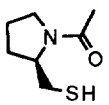
The IC_{50} values for compounds **28** and **31** are 1.7 ± 0.1 μM and 5.2 ± 0.4 μM, respectively (n = 3).

the indole NH-group interacting as a hydrogen bond donor since **29** still retains the activity. However, *N*-methylation of the amide moiety reduces the inhibitory potency compared to the parent compound as illustrated with **10** and **11** (Table 1).

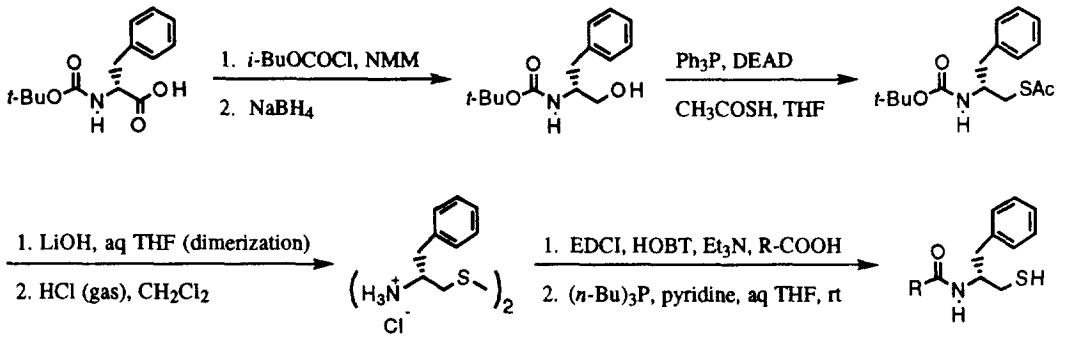
The β-thiol retroamide derivatives can be efficiently synthesized through the amino acid derived disulfide intermediate as illustrated with D-phenylalanine (Scheme 1). The coupling reactions between a variety of carboxylic acids and the disulfide component are nearly quantitative and the corresponding β-thiol retroamides are then easily obtained by reduction with tri-*n*-butylphosphine in aqueous solution of tetrahydrofuran in the presence of pyridine. Other reducing agents such as triphenylphosphine, sodium borohydride and 1,3-propanedithiol resulted in partial or complete desulfurization.

Table 3. Variations of the Tryptophan Analog **28**



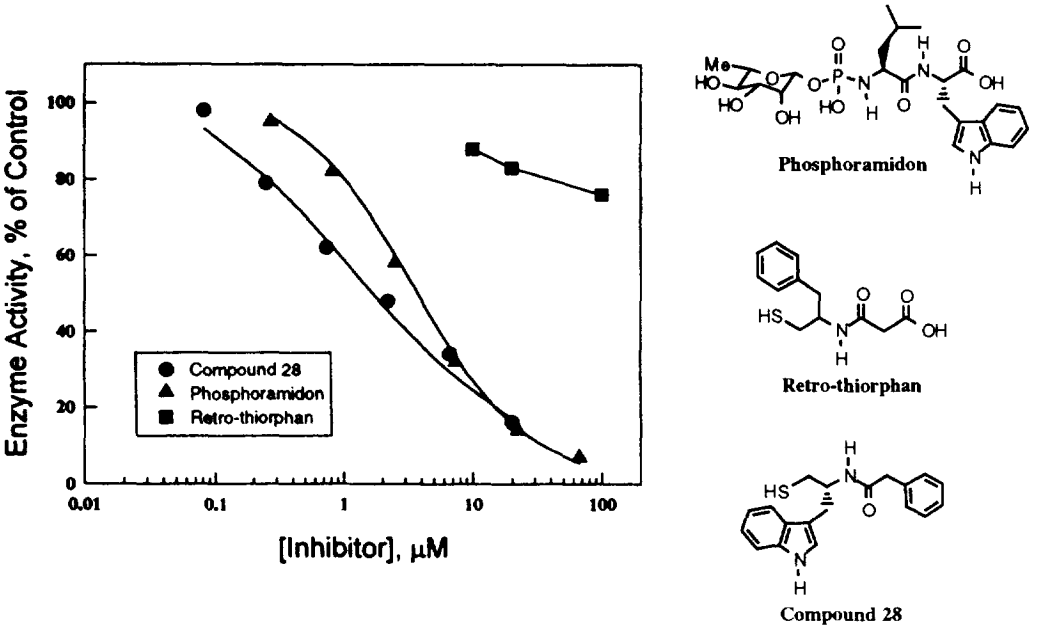
R	R'	Compound	IC_{50} (μM, n = 3)
		**28**	1.7 ± 0.1
		**35**	6.5 ± 1.3
		**36**	6.5 ± 1.2
		**37**	7.7 ± 2.9
		**38**	5.4 ± 0.67
		**39**	5.2 ± 1.1
		**40**	> 20
		**41**	> 20
		**42**	> 20

Scheme 1



The phosphorus-containing phosphoramidon has been widely utilized as a reference inhibitor of ECE. However, development of this compound as an orally active drug has not been pursued due to the presence of an acid labile phosphorus-nitrogen bond. Attempts have been made in the modification of phosphoramidon using a thiol-containing group to replace the phosphoramidate moiety, but the potencies of the resulting compounds have been disappointing ($IC_{50} > 10\ \mu M$).[13] In this study, we have optimized a chemically more stable retro-thiorphan to generate compound **28**. This compound is about twofold more potent than phosphoramidon in the inhibition of ECE (Figure 1). Clearly, a comparison of the potencies of **28** and phosphoramidon in vivo is necessary in the future. Furthermore, the differential structure activity relationships for retro-thiorphan analogs in the inhibition of ECE and other zinc metalloproteases need to be addressed.

Figure 1. Inhibition of ECE by Compound **28**, Phosphoramidon and Retro-thiorphan



Acknowledgments. We thank the Ciba Analytical Chemistry Staff for providing the analytical data on the compounds described in this study.

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(*Received in USA* 16 *November* 1995; *accepted* 5 *February* 1996)