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Discovery of endothelin antagonists

Masahiro Neya

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-3, 5-chome, Tokodai, Tsukuba, Ibaraki, 300-26, Japan

Abstract: Novel cyclic pentapeptides, WS7338A, B, C and D [cyclo-(AA1-AA2-D-Trp-D-Glu-Ala)] were isolated as Endothelin (ET) receptor antagonists from the culture broth of *Streptomyces* sp. No. 7338. Based on conformational analysis of these seeds, we defined the minimum structural requirements for binding affinity and prepared a series of acylated tripeptides with ETA receptor binding affinity. Through extensive chemical modification of the lead tripeptide, we have discovered a highly potent and selective ETA receptor antagonist FR139317. Further modification of this series of tripeptide ET antagonists has led to the discovery of a highly potent and selective ETB receptor antagonist FR164343. The discovery and structure-activity relationship of these ET antagonists and the pharmacological profile of FR139317 and FR164343 are described.

1. Introduction

Endothelin 1 (ET-1), which consists of 21 amino acid residues with two disulfide bonds, was first isolated from cultured porcine vascular endothelial cells in 1988 and has been found to be the most potent and long-lasting vasoconstrictor peptide (1). Subsequently, a family of mammalian ET isopeptides (ET-1, ET-2 and ET-3) has been identified (2). ET receptors have been divided into two different subtypes, ETA (ET-1 selective) and ETB (nonselective for the isopeptides) (3, 4). ETA receptors are distributed predominantly in vascular smooth muscle, heart and intestine, whereas ETB receptors are found in cerebral cortex, kidney and trachea. ETs have been implicated in the pathogenesis of several disease states such as hypertension, acute myocardial infarction, renal failure and asthma (5). Therefore the discovery of receptor subtype selective antagonists will help to clarify the pathophysiological roles of ETs and should also give potentially useful therapeutic agents.

In the course of our screening program we discovered novel four ET receptor antagonists, WS7338A **1**, B **2**, C **3** and D **4** in the culture broths of *Streptomyces* sp. No. 7338 (6). The structure and antagonistic activities of these compounds in the ET receptor binding assay are shown in Table 1. Activity at the ETA receptor was measured by inhibition of ^{125}I-labeled ET-1 binding to porcine aorta membranes. Activity at the ETB receptor was measured by inhibition of ^{125}I-labeled ET-1 binding to porcine kidney membranes. These results indicated that WS7338B **2** is the most potent and selective ETA receptor antagonist among these WS 7338 compounds (7).

TABLE 1. Structure and Binding Affinity of WS7338 Compounds for ET Receptors

Compd.	Structure	IC50(μM) ETA (Porcine Aorta)	IC50(μM) ETB (Porcine Kidney)
WS7338A **1**	cyclo-(D-Val-Leu-D-Trp-D-Glu-Ala)	0.93	N.T.
WS7338B **2**	cyclo-(D-allolle-Leu-D-Trp-D-Glu-Ala)	0.27	50.0
WS7338C **3**	cyclo-(D-Val-Val-D-Trp-D-Glu-Ala)	43.0	N.T.
WS7338D **4**	cyclo-(D-Leu-Val-D-Trp-D-Glu-Ala)	2.00	N.T.

Conformation analysis on the basis of NMR data revealed that **2** was stabilized by two intramolecular hydrogen bonds as judged by the presence of two amide protons showing small temperature coefficients ($\Delta\delta/\Delta T$[ppb/deg]: D-alloIle (1.7), D-Glu (1.9)) (8, 9). These results suggested that the hydrogen bonds Glu NH to alloIle C=O and alloIle NH to Glu C=O form a type II β-turn in the D-alloIle-Leu-D-Trp-D-Glu region and an inverse γ-turn in the D-Glu-Ala-D-alloIle region (Figure 1). Using this information regarding peptide backbone conformation as the starting point, we designed linear peptide derivatives.

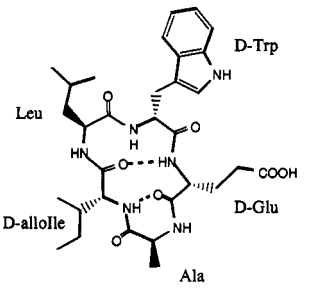


Fig. 1 Structure of WS7338B **2**

2. Design of Linear Tripeptide ET Receptor Antagonists

An independent investigation at Banyu Pharmaceutical Co. led to cyclic pentapeptide derivative, BQ-123 cyclo(D-Val-Leu-D-Trp-D-Asp-Pro) as a potent and selective ETA receptor antagonist through chemical modification of the same natural cyclic pentapeptide (10, 11). We attempted to define the minimum structural requirements on cyclic pentapeptides for ETA receptor binding, prior to an investigation of the structure-activity relationships of WS7338 compounds and their related cyclic peptide analogues. We first synthesized five different linear pentapeptides **5**, **6**, **7**, **8** and **9**, and examined the binding affinity (Figure 2). Among these five linear pentapeptide derivatives, **5**, **6** and **7** were found to retain almost equal potent binding affinity to the parent cyclic pentapeptide **2**. In contrast, **8** and **9** were completely inactive.

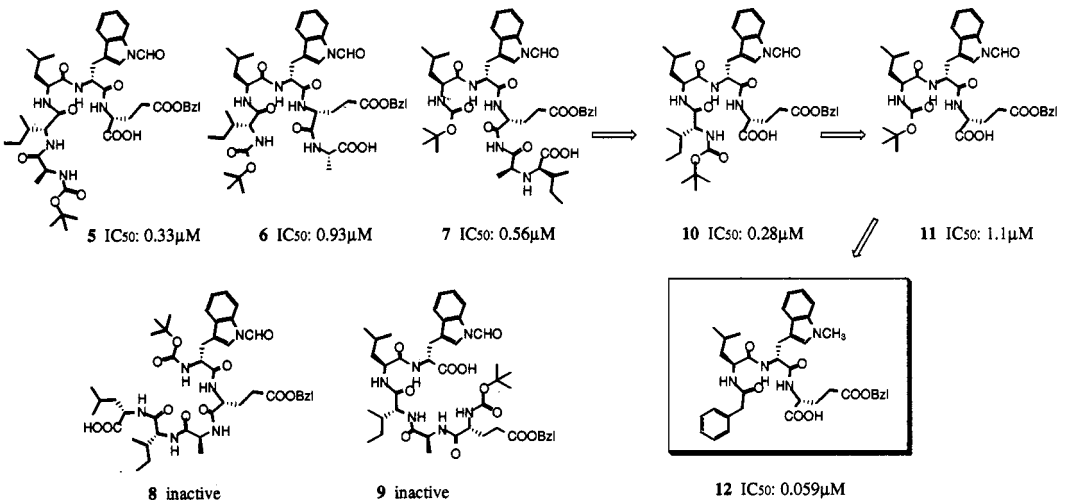


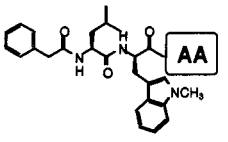
Figure 2. Determination of Minimum Structural Unit with ETA receptor Binding Affinity

On the basis of these results, we hypothesized that the essential binding region consists of only a few amino acid residues conserving a type II β-turn structure. In order to confirm this hypothesis we prepared a series of truncated peptide analogues and examined their binding affinities (Figure 2). The tetrapeptide derivative **10** lacking the N-terminal Ala residue in **5** was found to retain potency almost equal to the linear pentapeptide. The tripeptide derivative **11**, which was a 4-fold less potent than **10**, still exhibited fairly potent binding affinity. These results demonstrated that the acylated tripeptide R-CO-Leu-D-Trp(For)-D-Glu(OBzl)-OH was a minimum structural unit to retain ETA receptor binding affinity and was completely consistent with the type II β-turn region as expected in our hypothesis. Through chemical modification of the Boc group and 1-position substitution of the indole ring in **11**, the tripeptide derivative phenylacetyl-Leu-D-Trp(Me)-D-Glu(OBzl)-OH **12** was found to be more potent than the original cyclic pentapeptide **2**. We therefore selected **12** as a new lead and extensively modified this compound.

3. A Highly Potent and Selective ETA Receptor Antagonist FR139317

In order to discover a more potent antagonist, we attempted to optimize the new lead tripeptide derivative **12** (12). First, the D-Glu(OBzl) part was converted into other amino acid residues (Table 2). To investigate whether the hydrophobicity of the Bzl ester group had an important role on binding affinity, we prepared substituted derivatives with an achiral amino acid, β-Ala **13**, an aliphatic amino acid, D-Leu **14** and an aromatic amino acid, D-Phe **15**. The activity of β-Ala **13** was to some extent attenuated, whereas **14** and **15** were more potent than **12**. Replacement of D-Phe in **15** with D-2-pyridylalanine (D-2-Pya) **16** resulted in a remarkable increase in potency with an IC50 value of 7.0 nM, and in water solubility. Second, the Leu part was converted into other amino acid residues (Table 3). However, the substitution of Leu in **13** with Ile **17** and Nle **18**, which are structural isomers of Leu, gave compounds with much lower activity compared with **13**.

TABLE 2. Effect of Replacement of D-Glu(OBzl) in **12** on ETA Receptor Binding



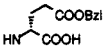
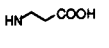
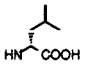
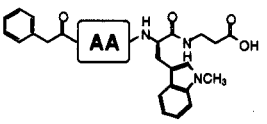
Compd.	AA	IC50 (nM)	Compd.	AA	IC50 (nM)
12		59	15		38
13		120	16		7
14		24			

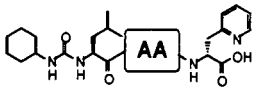
TABLE 3. Effect of Replacement of Leu in **13** on ETA Receptor Binding



Compd.	AA	IC50 (nM)	Compd.	AA	IC50 (nM)
13		390	18		800
17		540			

Effects on the substitution of D-Trp(Me) are listed in Table 4. Among the evaluated compounds, the cyclohexylaminocarbonyl-Leu-D-Trp(Me)-D-2-Pya-OH **19** was indicated to be the most potent compound. The replacement of the Me group with the Et group, i.e., **20** and the removal of the Me group, i.e., **21** resulted in a 60-fold and 5-fold loss of activity in comparison with **19**, respectively. The substitution of D-Trp(Me) with D-Phe **22** exhibited a 130-fold decrease in binding affinity.

TABLE 4. Effect of Replacement of D-Trp(Me) in **19** on ETA Receptor Binding



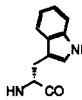
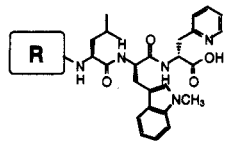
Compd.	AA	IC50 (nM)	Compd.	AA	IC50 (nM)
19		2.3	21		12
20		140	22		300

TABLE 5. Effect of Replacement of Acyl group in**16** on ETA Receptor Binding



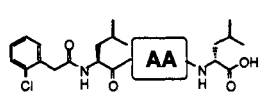
Compd.	R	IC50 (nM)	Compd.	R	IC50 (nM)
16		7.0	25		2.7
23		2.7	26		1.9
24		2.3	27 (FR139317)		0.53

Finally, we attempted to optimize the N-terminal acyl group moiety in **16** (Table 5). Replacement of the phenylacetyl group in **16** by a phenylaminocarbonyl group, as in **23** resulted in a 3-fold increase in binding affinity. This demonstrated that the incorporation of the urea group resulted in the higher activity than the corresponding amide derivative **16**. Based on this data, a variety of urea analogues were synthesized. The compounds substituted with cyclohexylaminocarbonyl **24**, N-cyclohexyl-N-methyl-aminocarbonyl **25** and N,N-di-n-propylaminocarbonyl **26** maintained more potent binding affinity. Among these compounds, the cyclic iminocarbonyl derivative, hexamethyleneiminocarbonyl **27** (FR139317) exhibited significantly higher affinity with an IC50 value in the 10^{-10}M range. Therefore, we selected the most potent and highly water soluble tripeptide derivative **27** (FR139317) (IC50: 0.53 nM for ETA and 3200 nM for ETB; solubility: > 100 mg/ml as the sodium salt) for further pharmacological evaluation.
From the structure-activity relationships on **27** FR139317 and its related derivatives, it may be concluded that the Leu-D-Trp(Me) part in the β-turn region probably contributes to the ETA receptor binding and that both the N-terminal hexamethyleneiminocarbonyl group and the C-terminal D-2-Pya residue may play important roles for stabilizing the β-turn structure.

4. A Highly Potent and Selective ETB Receptor Antagonist FR164343

During modification of the lead tripeptide antagonists, we encountered an antagonist **28** [2-chlorophenylacetyl-Leu-D-Trp(Me)-D-Leu-OH] that had relatively high affinity for the ETB receptor (IC50: 32 nM for ETA and 210 nM for ETB). On the basis of this observation, we started to design selective ETB receptor antagonists (13).

TABLE 6. Effect of Replacement of D-Trp(Me) in **28** on ET Receptor Binding



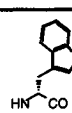
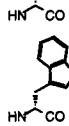
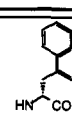
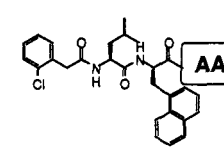
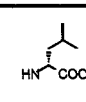
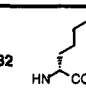
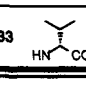
Compd.	AA	IC50 (nM) ETA	IC50 (nM) ETB
28		32	210
29		340	3400
30		1200	310

TABLE 7. Effect of Replacement of D-Leu in **30** on ET Receptor Binding

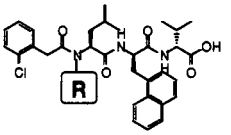


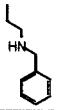
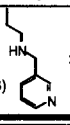
Compd.	AA	IC50(nM) ETA	IC50 (nM) ETB
30		1200	310
31		350	1400
32		910	100
33		1300	65

First, the D-Trp(Me) part in **28** was modified (Table 6). Replacement with D-Trp **29** resulted in 10-fold decreased affinities for both the ETA and ETB receptors. On the other hand, the substitution with D-1-naphthylalanine (D-1-Nal) residue **30** retained ETB receptor affinity but significantly decreased ETA receptor affinity. The replacement of the 1-methylated indole ring by a naphthalene ring led to the discovery of the first tripeptide derivative **30** having a higher affinity for the ETB than the ETA receptor. The C-terminal D-Leu part in **30** was converted to other aliphatic amino acid residues, such as D-Ala **31**, D-Nle **32** and D-Val **33** (Table 7). Among these compounds, the replacement with D-Val **33** considerably increased ETB affinity, but retained weak ETA affinity. From these results, we speculated that the increased ETB selectivity might be due to a conformational change in the β-turn region by the N-terminal 2-chlorophenylacetyl group and the β-branched alkyl side chain of the C-terminal D-Val residue. We therefore introduced an N-methyl group to the N-terminal Leu residue in order to disturb the formation of an intramolecular hydrogen bond in the β-turn region. As predicted, 2-chlorophenylacetyl-N-MeLeu-D-1-Nal-D-Val-OH **34** retained high ETB affinity and completely abolished ETA affinity (Table 8). While its selectivity and potency were very attractive, the poor water solubility of compound **34** was found to limit its usefulness in further pharmacological evaluation.

Finally, we tried to optimize the N-Me moiety in **34** to improve water solubility (Table 8). Replacement with the N-(2-N-methylaminoethyl) group **35**, as a hydrophilic substituent resulted in a 6-fold decrease in ETB binding affinity, whereas its solubility was remarkably increased. The compound with an N-(2-N-benzylaminoethyl) group **36** was more active than the corresponding N-MeLeu analogue **34**, whereas its solubility was slightly decreased. Replacement with the N-[2-N-(3-pyridylmethyl)aminoethyl] group **37** (FR164343) resulted in increases in both ETB receptor binding affinity and water solubility. The desirable conformational change in the β-turn region caused by the introduction of 2-chlorophenylacetyl group and 2-N-(3-pyridylmethyl)aminoethyl group to the amino group of Leu residue led to the discovery of a highly potent and selective ETB receptor antagonist **37** (FR164343) with highly water solubility (> 100 mg/ml as the sodium salt).

TABLE 8. Effect of Replacement of N-Alkyl group in **33** on ET Receptor Binding



Compd.	R	IC50 (nM) ETA	IC50 (nM) ETB
33	H	1300	65
34	CH_3	>10000	89
35		>10000	570
36		>10000	25
37 (FR164343)		>10000	14

5. Pharmacological Profile of ET Receptor Antagonists

Our efforts to optimize the natural cyclic pentapeptide WS 7338 B **2** have led to the discovery of a novel linear tripeptide ETA antagonist FR139317 and a novel linear tripeptide ETB antagonist FR164343. We next investigated the pharmacological profile of these antagonists. Binding to human ETA and ETB receptors expressed in CHO cells was determined (Table 9). From these results it has been confirmed that FR139317 is a highly potent and selective ETA receptor antagonist, whereas FR164343 is a highly potent and selective ETB antagonist. We examined the effect of FR139317 on the changes in blood pressure induced by ET-1 in conscious rat. An intravenous bolus injection of ET-1 induced a biphasic response consisting of a transient fall in blood pressure followed by a sustained pressor response. FR139317 dose-dependently inhibited the pressor response (Figure 3). In contrast, even at the highest dose it did not show any effect on the initial depressor response mediated by the ETB receptor. This data demonstrated that FR139317 is effective in blocking ETA receptor-mediated responses (14).

TABLE 9. Binding to Human Receptors in CHO Cells

Compd.	ETA IC50 (nM)	ETB IC50 (nM)
FR139317	0.17	>1000
FR164343	>1000	4.9

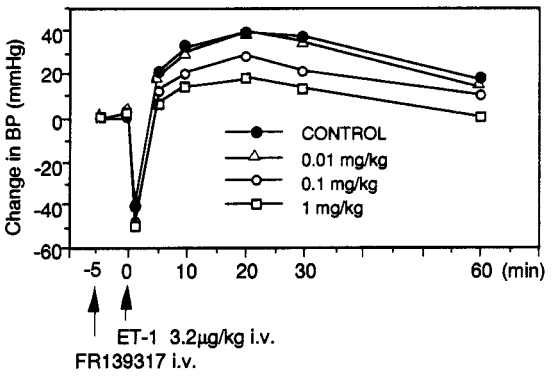


Figure 3. Effect of FR139317 on Pressor Response Induced by ET-1 in Conscious Rats

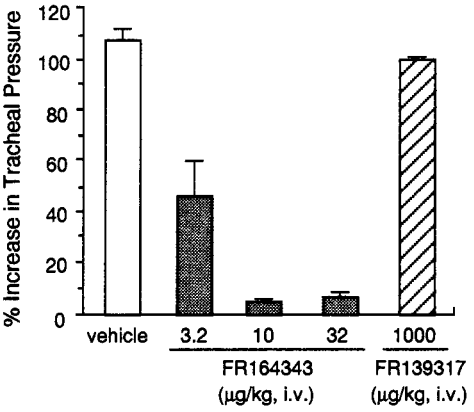


Figure 4. Effect of FR164343 on Bronchoconstriction Induced by ET-1 in Anesthetized Guinea Pigs

Furthermore, we evaluated the effect of FR164343 on bronchoconstriction induced by ET-1 in anesthetized guinea pigs. An intravenous bolus injection of ET-1 induced a transient bronchoconstriction. This response is mainly mediated by the ETB receptor (15). FR164343, a highly potent ETB receptor antagonist, inhibited the bronchoconstrictor response in a dose-dependent manner (Figure 4). In the same model, FR139317 (1 mg/kg, i.v.) did not show any effect on bronchoconstrictor response. These results demonstrate that FR164343 is effective in blocking ETB receptor mediated bronchoconstriction in guinea pigs.

6. Conclusion

Through extensive modification of the cyclic pentapeptide **2** on the basis of its conformation analysis, we have discovered a series of tripeptide ET receptor antagonists with ETA and ETB subtype-selectivity. Further *in vivo* characterization demonstrated that FR139317 is a highly potent and selective ETA receptor antagonist, whereas FR164343 is a highly potent and selective ETB receptor antagonist. It may be concluded that FR139317 and FR164343 are useful pharmacological agents for investigating the pathophysiological roles of the ET system.

Acknowledgments

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