Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 7, pp. 775-778, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00127-8

ANALOGS OF AN ENDOTHELIN ANTAGONIST RES-701-1: SUBSTITUTIONS OF C-TERMINAL AMINO ACID

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Abstract: C-terminal substituted analogs of an ET_B receptor specific antagonist RES-701-1 were prepared and evaluated of their receptor binding activities. C-terminal Trp deletion or substitutions for other aromatic amino acids, containing D-configurational ones, proved to be possible without reducing the receptor binding activity, which differs from the case of ET-1 previously reported on its Trp^{21} for its biological activities.

Introduction: Endothelins (ETs) are the family of potent vasoactive peptides[1], of which the first member, ET-1[2], has been shown to be one of the most potent vasocontractors known. Their versatile physiological actions, such as, for example, vasoconstriction, release of endothelium-derived relaxing factors, mitogenesis and bronchoconstriction, are mediated through their receptors, ET_A and ET_B, which have been distinguished based on their differential sensitivity for ET-1 and ET-3[3]. ETs have been suggested to play a possible role in many disease states, such as cardiovascular diseases, asthma, myocardinal infarction and acute renal failure. Therefore compounds specifically block the effects of ETs have been thought to have some therapeutic potential in endothelin-related diseases[3a, 4].

In the course of screening research to obtain ET-receptor antagonists of microbial origin, we found an ET_B receptor-specific antagonist RES-701-1[5, 6] and its three analogs[7], novel peptides consist of 16 amino acids with "sidechain-to-head" cyclic structure by an amide bond between Gly^1 α-NH_2 and Asp^9 β-COOH (Fig. 1). Each of them has a tryptophan or analogous residue at the C-terminal as all ETs (ET-1, 2, 3, and sarafotoxins etc.) do. From the previous reports on the structure-activity relationships of ET-1, C-terminal tryptophan has been shown to be necessary for its biological actions containing the receptor binding[8]. To elucidate the role of the C-terminal Trp residue of RES-701-1, we have investigated the receptor binding activities of C-terminal-substituted RES-701-1 analogs.

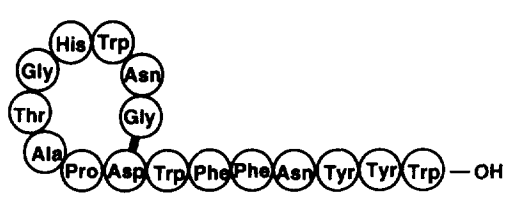


Fig. 1: Structure of RES-701-1

Experimental Procedure: RES-701-1 (**1**) and RES-701-2 were isolated from the supernatant of microbial fermentation as reported[5, 7]. RES-701-1(1-15) (**2**) was prepared by enzymatic digestion of C-terminal residue of RES-701-1 or RES-701-2 with carboxypeptidase-A[7] followed by reverse phase (RP)-HPLC purification.

Preparation of RES-701-1 analogs reported here were carried out with standard liquid phase condensations[9] between **1** or **2** and amino acids of which C-terminal were protected with appropriate esters. For representation, [Ala[16]]RES-701-1 (**3**) was prepared as follows: Compound **2** was coupled with alanine benzylester-tosylate (5 equiv. to **2**) by benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP)/1-hydroxybenzotriazole (HOBt)/N-methylmorpholine (3, 3 and 5 equiv. to the alanine benzylester, respectively) activating system in DMF at 4°C. After 15h, 60% of **2** was converted to the benzylester of **3** on HPLC analysis. The benzylester of **3** was isolated by semi-preparative RP-HPLC, followed by the benzyl group removal by catalytic hydrogen transfer with ammonium formate and 10% palladium on carbon in methanol[10]. Compound **3** was isolated by semi-preparative RP-HPLC (isolated yield was 12% calculated from **2**)[11], followed by confirming its structure with fast atom bombardment mass spectrum (FAB-MS) and amino acid analysis (AAA). Compounds **4~11** were prepared as above using corresponding amino acids esters. Compound **4~7** were obtained in almost the same yields as **3** (10~15%)[11]. Compounds **8** and **9** or **10** and **11** were generated at the same time in the course of benzyl-deprotection after condensation reactions. The molecular weights and amino acid components of **8** or **10** were proved to be the same as **9** or **11**, respectively. Compounds **8** or **10** were first eluted from the HPLC column and **9** or **11** were second. They were isolated in yields of 4.7 and 4.7% (**8** and **9**, respectively), and 5.2 and 3.7% (**10** and **11**, respectively)[11]. All compounds were purified to homogeneity by RP-HPLC (>95%) and confirmed their structure by fast atom bombardment (FAB) -MS analyses and amino acid analyses (AAA).

Receptor binding assay was performed as previously described[12]. Analogs were tested using bovine lung membrane, in which ET_B receptor was masked by RES-701-1 (5 μM), for ET_A receptor and bovine cerebellum membrane for ET_B receptor.

DL-AAA was performed using vapor phase hydrolysis with 6 N HCl followed by GC detection of N-trifluoroacetyl-isopropyl esters of amino acids (only His was detected as N-(imidazole)-ethoxycarbonyl derivative) using a chiral column[13].

Results and Discussion: Receptor binding activities of prepared RES-701-1 C-terminal substituted analogs are presented in Table 1 as their IC_{50} values.

Compared to the importance of C-terminal Trp residue of ETs[8], it is interesting to note that Trp-truncated analog **2** retains relatively high affinity for ET_B receptor. In the case of RES-701-1, C-terminal-Trp truncation is suspected not to disorder the whole molecular structure around the C-terminal, important for its biological activity, because RES-701-1 has a unique "tale through ring" rigid structure (Fig. 2)[14].

Trp^{16} substitution for other amino acids clearly showed some characteristic results. Analogs substituted for aromatic amino acids (**5**, **6** and **7**) showed relatively high affinity for ET_B receptor comparable to **1**, especially the sterically hindered naphtyl group substitutant **7** exhibited slightly higher affinity than **1**. Contrary to these results, substitution for aliphatic amino acids not sterically hindered, such

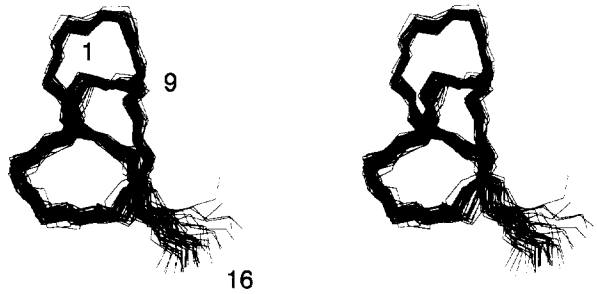
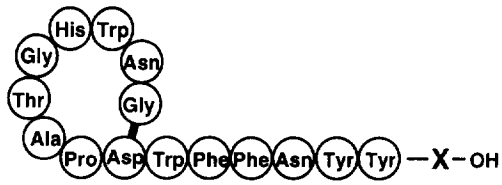
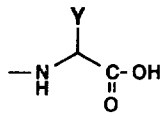
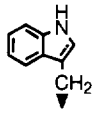
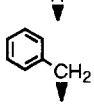
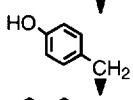
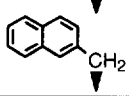


Fig. 2: Stereoview of the backbone 3D-structure of RES-701-1 (cited from ref. 13).

Table 1: Receptor binding activities of RES-701-1 C-terminal analogs



X: 

No.	X	Y	IC_{50} (nM) ET_A[a)]	IC_{50} (nM) ET_B[b)]	No.	X	IC_{50} (nM) ET_A[a)]	IC_{50} (nM) ET_B[b)]
1	Trp (RES-701-1)		>1000	10	**8**	D-Trp (1st eluted) (D-Tyr[15]-D-Trp[16])	>98	>98
2	none	——	n.d.[c)]	54	**9**	D-Trp (2nd eluted) (L-Tyr[15]-D-Trp[16])	>98	7.3
3	Ala		n.d.	470				
4	Gly		>520	120	**10**	Trp-Trp (1st eluted) (L-Trp[16]-L-Trp[17])	n.d.	450
5	Phe		n.d.	12				
6	Tyr		n.d.	45	**11**	Trp-Trp (2nd eluted) (D-Trp[16]-L-Trp[17])	n.d.	36
7	Nal[d)]		>440	6.3				

a) bovine lung membrane preparation, containing ET_B masked with RES-701-1. b) bovine celleberum membrane preparation. c) not determined. d) β-2-naphtyl-L-alanine

as Gly (**3**) or Ala (**4**), resulted in much reducing the affinity for ET_B receptor. These results also indicate the importance of C-terminal residue's aromaticity and/or steric hindrance, not only indole groups but phenyl or naphtyl groups, for increasing their affinity for ET_B receptor than the Trp-truncated analog **2**. It may be considered that small residues at C-terminal disrupt the receptor binding by free changing rotation of side chains or carboxyl groups because of no interaction between C-terminal and other residue that restricts their conformations[15].

To investigate the role of stereochemistry of the residue at position 16, we prepared the substitutants of L-Trp[16] to D-configuration. We obtained two product of same molecular weight (FAB-MS), of which first eluted from RP-HPLC is **8** and second is **9**. From the DL-AAA, only in the case of **8** was observed D-Tyr with the same amount of L-Tyr. So epimerization at condensation site, position 15, was considered to occur, resulted that **8** has D-Tyr[15]-D-Trp[16] and **9** has L-Tyr[15]-D-Trp[16] component[16]. Substitution only at position 16 was shown not to reduce the affinity (**9**), differently from the case of ETs, however the configuration of position 15 is proved to be much important (**8**). In the case of a Trp residue addition, the same phenomenon was observed, that is, two product of the same mass, **10** and **11**, first and second eluted from RP-HPLC, respectively, were obtained and the second eluted **11** showed higher affinity. From the DL-AAA, only in the

case of **11**, surprisingly, was observed D-Trp. So epimerization at condensation site, position 16, was considered to occur. In this case, for ET_B binding, the favorable configuration at position 16 and 17 is considered to be D- and L-, respectively. These results are very interesting in connection with the structure-activity relationships of ET antagonists.

In summary, our results in this report showed some characteristic properties of ET_B receptor specific antagonist RES-701-1 by estimating the receptor binding activities of C-terminal Trp substitutants. The results are quite different from those about ETs. The three dimensional structure analysis of some peptides reported here, which thought to be very attractive, are now under investigation.

Acknowledgment: We thank Ms. Yumiko Aotani for FAB-MS analyses and Mr. Kiyotoshi Honda for DL-AA analyses.

References and Notes:

1. For reviews, see; a) Simonson, M. S.; Dunn, M. J. *Hypertension* **1991**, *17*, 856. b) Rubanyi, G. M.; Parker Botelho, L. H. *FASEB J.* **1991**, *5*, 2713.
2. Yanagisawa, M.; Kurihara, H.; Kimura, S.; Tomobe, Y.; Kobayashi, M.; Mitsui, Y.; Yazaki, Y.; Goto, K.; Masaki, T. *Nature* **1988**, *332*, 411.
3. For reviews, see; a) Miller, R. C., Pelton, J. T.; Huggins, J. P. *Trends Pharmacol. Sci.* **1993**, *14*, 54. b) Bax, W. A.; Saxena, P. R. *ibid.* **1994**, *15*, 379.
4. Haynes, W. G.; Davenport, A. P.; Webb, D. J. *ibid.* **1993**, *14*, 225.
5. Morishita, Y; Chiba, S.; Tsukuda, E.; Tanaka, T.; Ogawa, T.; Yamasaki, M.; Yoshida, M.; Kawamoto, I.; Matsuda, Y. *J. Antibiot.* **1994**, *47*, 269.
6. Yamasaki, M.; Yano, K.; Yoshida, M.; Matsuda, Y.; Yamaguchi, K. *J. Antibiot.* **1994**, *47*, 276.
7. RES-701-2; [7-hydroxy-Trp^{16}]RES-701-1, RES-701-3; [Ser^{7}]RES-701-1, RES-701-4; [Ser^{7}, 7-hydroxy-Trp^{16}]RES-701-1. a) Ogawa, T.; Ochiai, K.; Tanaka, T.; Tsukuda, E.; Chiba, S.; Yano, K.; Yamasaki, M.; Yoshida, M.; Matsuda, Y. *J. Antibiot.* **1995**, *48*, 1213. b) Yano, K.; Yamasaki, M.; Yoshida, M.; Matsuda, Y.; Yamaguchi, K. *J. Antibiot.* **1995**, *48*, 1368.
8. Kimura, S.; Kasuya, Y.; Sawamura, T.; Shinmi, O.; Sugita, Y.; Yanagisawa, M.; Goto, K.; Masaki, M. *Biochem. Biophys. Res. Commun.* **1988**, *156*, 1182.
9. *The Peptides. Analysis, Synthesis, Biology* ; Gross, E.; Meienhofner, J., Ed.; Academic Press: New York, 1979.
10. Anwer, M. K.; Spatola, A. F. *Synthesis* **1980**, 929.
11. Because of very small scale preparations (~100μg), absorption of the products to palladium on carbon let the yields lower.
12. Tanaka, T.; Tsukuda, E.; Nozawa, M.; Nonaka, H.; Ohno, T.; Kase, H.; Yamada, K.; Matsuda, Y. *Mol. Pharmacol.* **1994**, *45*, 724.
13. a) Nicholson, G.; Frank, H. *J. High Resolut. Chromatogr. and Chromatogr. Commun.* **1979**, *2*, 411. b) Abe, I.; Musha, S. *J. Chromatogr.* **1980**, *200*, 195.
14. RES-701-1 has a extraordinary folded rigid structure, which tail portion passes through inside of the ring. Katahira, R.; Shibata, K.; Yamasaki, M.; Matsuda, Y.; Yoshida, M. *Bioorg. Med. Chem.* **1995**, *3*, 1273.
15. Although Trp^{16} of RES-701-1 interacts with Tyr^{14}, no interaction was seen between C-terminal Ala and Tyr^{14} in [Ala^{16}] analog (ref. 13 and unpublished result).
16. Compounds **3~7** were generated as exclusively single products by HPLC analyses. It can not be proved why so evident epimerizations occurred only in the case of Trp-condensations. It is presumed for one reason that the Trp-condensation reactions were very slow compared to other amino acids used here.

(*Received in Japan* 6 *December* 1995; *accepted* 21 *February* 1996)