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LINEAR PEPTIDE ET_A ANTAGONISTS: RATIONAL DESIGN AND PRACTICAL DERIVATIZATION OF N-TERMINAL AMINO- AND IMINO-CARBONYLATED TRIPEPTIDE DERIVATIVES[1]

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Abstract: Novel linear tripeptides possessing high endothelin antagonist activity were derived from endothelin antagonistic cyclic pentapeptides represented by BQ-123. The N-terminal urea moiety of the linear tripeptide derivatives was essential to show the strong antagonist activity. An easy method to prepare these peptides by treatment of the corresponding N-phenoxycarbonylated tripeptide esters with primary or secondary amines is described.

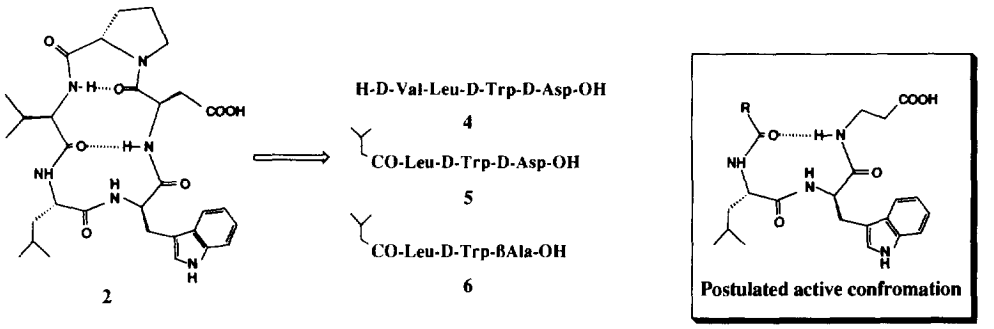
In the course of chemical modification of the endothelin (ET) antagonistic cyclic pentapeptide (CPP) BE-18257A (**1**) of microbial origin, we found that a series of novel CPPs represented by BQ-123 (**2**) showed antagonism that was potent and highly selective for ET_A receptors.[2] Detailed investigations of structure-activity relationships in these CPPs revealed that peptides possessing various kinds of the third amino acid residue (Xxx in **3**) all had similar ET_A antagonistic activity.[3] These results suggested that the residue did not directly interact with endothelin receptors but worked to maintain a suitable conformation and/or configuration of CPPs for exhibiting antagonistic activity. We therefore attempted to generate linear peptide ET antagonists which lack the third amino acid residue Xxx in **3**. In this communication we describe our investigated results directed toward

cyclo (-D-Trp-D-Glu-Ala- D-Val-Leu-)	**BE-18257A**	**(1)**
cyclo (-D-Trp-D-Asp-Pro- D-Val-Leu-)	**BQ-123**	**(2)**
cyclo (-D-Trp-D-Asp-Xxx-D-Val-Leu-)		**(3)**

the discovery of novel linear tripeptide ET_A antagonists derived from ET_A antagonistic CPPs. Furthermore, the practical derivatization of the N-terminal urea moiety and ET_A antagonistic activity of these derivatives is reported.

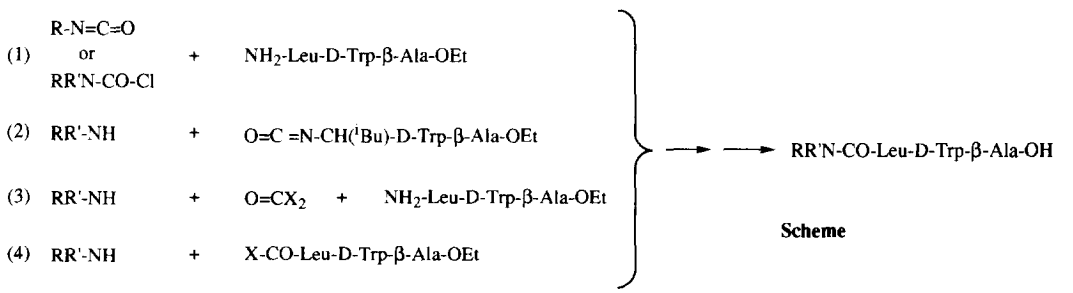
Based on the above-mentioned findings and prediction, we synthesized three linear peptide derivatives, *viz.*, H-D-Val-Leu-D-Trp-D-Asp-OH (**4**), isovaleryl-Leu-D-Trp-D-Asp-OH (**5**) and isovaleryl-Leu-D-Trp-β-Ala-OH (**6**) derived from formal deletion of the vicinity of the Pro residue in **2** (see Figure) and determined their binding affinity to ET_A receptors. Of these derivatives, the acylated tripeptide **6**, which was 100 times less potent than **2**, still had potency similar to that of **1** (IC_{50}: 32, 25 and 3.9 μM for **4**, **5** and **6**, respectively).[4] Since the potency of **6** was sufficient for another modification lead, we first carried out derivatization of its N-terminal acyl moiety. The structure-activity relationships of the corresponding amino acid residue in the CPPs suggested that the most favorable N-terminal acyl group for promising binding affinity to ET_A receptors is a

cyclopentylacetyl or a 2-thienylacetyl group. In fact, tripeptide derivatives possessing these acetyl groups (**7** and **8**) exhibited activity 4 to 7 times higher than that of **6**, while their potencies were still around the submicromolar order (IC_{50}: 0.97 and 0.54 μM for **7** and **8**, respectively). However, a synthetic intermediate Boc-Leu-D-Trp-β-Ala-OH (**9**) exhibited fairly potent activity (IC_{50}: 0.42 μM). The potency of the urethane derivative **9** was over 10-fold higher than that of the corresponding amide derivative (**10**), which had an IC_{50} value of only 6.7 μM. We interpreted these data as follows: in general, a carbonyl group of a urethane is a higher hydrogen bonding acceptor than that of an amide. Therefore, a stronger intramolecular hydrogen bond between the N-terminal carbonyl oxygen and the C-terminal amide hydrogen will make it easier to turn the linear peptide into the restricted form; in other words, the β-turn structure of the peptide backbone is more suitable for the peptide to interact with ET_A receptors as an active conformation, which is presumably because the urethane **9** showed higher activity than the amide **10** (the postulated active conformation is described in the Figure).[5] If this interpretation is correct, the corresponding urea derivative is expected to be more potent than **9**. The prepared urea **11** did inhibit ET-1 binding more potently than **9** with an IC_{max50} of 0.15 μM.



Figure

We next focused on the optimization of the N-terminal urea moiety and tried to efficiently synthesize a wide variety of urea analogues. The urea derivatives were usually prepared by the following methods (see Scheme): (1) reaction of an N-terminal free tripeptide with an isocyanate or a carbamoyl chloride; (2) reaction of an N-terminal isocyanato-derivative with a primary or a secondary amine; (3) condensation of an N-terminal free tripeptide with a primary or a secondary amine in the presence of a condensation agent such as phosgene or 1,1'-carbonyldiimidazole; and (4) reaction of an N-terminal activated tripeptide derivative with a primary or a secondary amine.[6] With respect to method (1), a wide variety of isocyanates and carbamoyl chlorides are not commercially available; in addition, a carbamoyl chloride was only reactive enough to give the desired urea

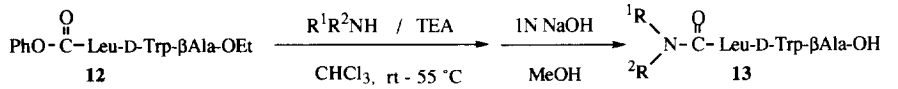


Scheme

derivatives in low to moderate yields. In method (2), preparation of the N-terminal isocyanato-derivative was laborious and occasionally racemization of the α-position of Leu was detected under the reaction conditions. Using method (3), a primary amine occasionally gave mainly the corresponding symmetrical urea depending upon the reactivity of the amine and/or reaction conditions, and the desired urea tripeptide derivatives were obtained concomitantly, whereas in method (4), if an appropriate activating group was selected, a wide variety of commercially available amines seemed to be usable as a starting material to effect the desired urea derivatives easily.

We chose a phenoxycarbonyl group as an activating group and treated an N-terminal phenoxycarbonylated linear tripeptide ester with a primary or secondary amine. PhO-CO-Leu-D-Trp-β-Ala-OEt (**12**),[7] which was prepared from phenyl chloroformate and H-Leu-D-Trp-β-Ala-OEt in the presence of triethylamine, was treated with 5 - 10 equimolecular amounts of various amines in the presence of triethylamine (5 - 20 equiv) at room

Table 1. Reaction of phenoxycarbonylated tripeptide esters with various amines[a]

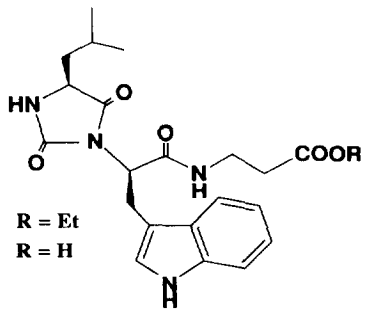


13	R^1	R^2	Yield (%)[b] step 1	Yield (%)[b] step 2	13	R^1	R^2	Yield (%)[b] step 1	Yield (%)[b] step 2
a	1-adamantyl	H	87	82	**i**	$-(CH_2)_4-$		99	95
b	Me_3CCH_2-	H	quant.	74	**j**	$-(CH_2)_5-$		quant.	72
c	$PhCH_2-$	H	quant.	60	**k**	$-CHMe(CH_2)_3CHMe-$		98	59
d	^iPr	^iPr	77	92	**l**	$-CMe_2(CH_2)_3CMe_2-$		0 (89)[c]	-
e	^tBu	$HO-(CH_2)_2-$	44 (17)[c]	27[e]	**m**	$-(CH_2)_2-NMe-(CH_2)_2-$		92	98
f	3-MePh	H	36 (64)[c]	84	**n**	 $-CH_2(CH_2)_2-$		96	76
g	3-ClPh	H	8[d]	47	**o**	$-(CH_2)_6-$		94	88
h	2,4,6-Me_3Ph	H	0[d]	-	**p**	$-(CH_2)_7-$		83	68

a) All linear peptides showed satisfactory 300 MHz ^1H NMR, IR and high resolution FAB-MS spectra supporting the described structures. b) Isolated yield, not optimized. c) Yield showed in parentheses is that of the hydantoin ester. d) A major product was the hydantoin ester which was not isolated. e) The hydantoin acid was also formed.

temperature to 55 °C to furnish the N-terminal urea moiety. As shown in Table 1, a phenoxy group was formally replaced with most of the amines under the above-mentioned reaction conditions with high efficiency. In the case of amines with low nucleophilicity due to steric hindrance and/or electrostatic effects (**13f-h** and **13l**), the intended reaction rarely occurred and the major product was a hydantoin ester (**14**) that was formed by intramolecular attack of the amide nitrogen of D-Trp on the carbonyl carbon of the phenoxycarbonyl group (**13l**, which could not be obtained according to the present method, was finally prepared from phosgene, 2 equi-

molecular amounts of 2, 2, 6, 6-tetramethylpiperidine and NH_2-Leu-D-Trp-β-Ala-OEt using the same procedures described in the literature).[8] In the case of **13e**, the urea ester was predominantly obtained; however, hydrolysis of this ester gave the desired acid in only a 27% yield, accompanied by a C-terminal carboxylic acid (**15**). This is due to instability of the N-*tert*-butyl-N-(2-hydroxyethyl)aminocarbonyl group under basic conditions in the second step: indeed, protection of the hydroxy group almost prevented the hydantoin from forming; that is, the corresponding O-*tert*-butyldimethylsilylated ester gave the O-*tert*-butyldimethylsilylated carboxylic acid in a 60% yield under the same reaction conditions, accompanied by **15** in a 6% yield. We presumed that an intramolecular nucleophilic attack of the terminal hydroxy group on the ureido carbonyl carbon was a driving force and that the successive intramolecular nucleophilic attack of the amide hydrogen of D-Trp residue toward the resulting 2-(*tert*-butylamino)ethoxycarbonyl group caused the unusual hydantoin formation, or that an intramolecular attack of the terminal hydroxy group under basic conditions on the amide hydrogen of the Leu residue generated the N-terminal isocyanate (with eliminating N-*tert*-butyl-N-(2-hydroxyethyl)amine), which the amide hydrogen of the D-Trp residue attacked intramolecularly.



14: R = Et
15: R = H

Although there are some limitations caused by serious steric hinderance and certain of electronic effects, the present method was found to be very powerful to produce a small quantity of product with minimum effort and to be a very useful procedure from the view point of medicinal chemistry. The intermediate **12** is easily prepared as a crystalline powder and is stable under storage at 4 °C or below despite good reactivity during reaction conditions, which is an additional merit of this method. Compared with the results cited in Table 1, the formal replacement of the phenoxy group did not occur easily using PhO-CO-OCH(^{i}Bu)CO-D-Trp-β-Ala-OEt as a starting material in place of **12**, even after treatment with a reactive amine such as 2,6-dimethylpiperidine. This fact suggests that the substitution reaction described here may proceed via an isocyanate intermediate formed *in situ*. Detailed mechanistic investigations are now under consideration.

The inhibitory activities of the thus obtained **13a-p** (except for **13h**, which was not finally synthesized) against ET_A receptors together with those of **6-10** are summarized in Table 2. In contrast to the urea derivatives arising from primary amines, those derived from some of the secondary amines (**13d** and **e**) or some of the cyclic imines (**13j**, **k**, **o**[9] and **p**) were revealed to be more potent than **11**. In the case of non-substituted cyclic imines, the least hydrophobic pyrrolidine gave the least potent urea derivatives, whereas in the case of 6-membered cyclic imines, 2, 6-dimethylpiperidine gave the most potent urea derivative **13k**. Ureas derived from 2, 2, 6, 6-tetramethylpiperidine, 4-methylpiperadine and 1, 2, 3, 4-tetrahydroisoquinoline were less potent than those from the non-substituted piperidine. The ureas derived from benzylamine and 3-substituted anilines showed poor affinity to ET_A receptors. Although the potency of **13d, e, j, k, o** and **p** is still around the 50 - 80 nanomolar order, it approached that of **2** and selectivity against ET_A receptors still remained (the affinity of these compounds for ET_B receptors is 500 - 2000 times weaker than that for ET_A receptors). A representative derivative **13o** strongly antagonized ET-1-induced contraction in porcine coronary arteries with a pA_2 value of 7.22.[10]

In conclusion, we accomplished the following through the present investigation: 1) generation of a new lead tripeptide derivative derived from **2** based on the structure-activity relationships of CPP ET_A antagonists; 2)

discovery of a prototype linear ET_A antagonist by enhancement of the intramolecular hydrogen bond with replacement of the N-terminal acyl moiety from amide to urea; 3) development of an easy way to prepare the N-terminal urea moiety by treating an amine with the tripeptide ester activated by the N-terminal phenoxycarbonyl group, a method that is widely applicable in the preparation of peptide derivatives possessing an N-terminal urea moiety; and 4) demonstrated the efficacy of the N-terminal urea moiety derived from secondary amines or some kinds of cyclic imines for the ET_A antagonists. Detailed structure-activity relationships of the N-terminal acyl moiety and further optimization of each amino acid residue directed towards the potent ET_A antagonists BQ-485 and BQ-610 or the potent ET_B antagonist BQ-788 will be reported elsewhere.

Table 2. Effects of N-terminal acyl moiety on ET_A affinity[a)]

R-CO-Leu-D-Trp-β-Ala-OH

No.	R	IC_{max50} μM	(IC_{50}) μM	No.	R	IC_{max50} μM	(IC_{50}) μM
6	isobutyl		(3.9)	**13f**	3-methylphenylamino		(2.1)
7	cyclopentylmethyl	0.62	(0.97)	**13g**	3-chlorophenylamino		(2.1)
8	2-thienylmethyl	0.42	(0.54)	**13i**	pyrrolidino	0.31	
9	*tert*-butoxy	0.27	(0.42)	**13j**	piperidino	0.081	
10	neopentyl		(6.7)	**13k**	2,6-dimethylpiperidino	0.051	
11	*tert*-butylamino	0.15	(0.23)	**13l**[b)]	2,2,6,6-tetramethyl-piperidino	0.19	
13a	1-adamantylamino	0.33		**13m**	4-methylpiperadino	0.58	
13b	neopentylamino	0.56		**13n**	1,2,3,4-tetrahydro-isoquinolin-2-yl	0.53	
13c	benzylamino		(3.2)	**13o**	perhydroazepino	0.061	
13d	diisopropylamino	0.057		**13p**	perphydroazecino	0.055	
13e	N-(2-hydroxyetnyl)-*tert*-butylamino	0.053					

a) Porcine aortic smooth muscle membtanes. b) Prepared according to the procedures described in the literature. See ref. 8.

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References and Notes

1. Part of this work was presented at the 2nd Japan Symposium of Peptide Chemistry, November, 1992: Nagase, T.; Fukami, T.; Hayama, T.; Kumagai, U.; Urakawa, Y.; Nagasawa, Y.; Ishikawa, K. A facile synthesis of endothelin antagonistic linear peptides; and at the 2nd Annual Meeting of Division of Medicinal Chemistry, the Pharmaceutical Society of Japan, December, 1992: Mase, T.; Fukami, T.; Nagase, T.; Niiyama, K.; Hayama, T.; Takahashi, H.; Urakawa, Y.; Kumagai, U.; Amano, Y.; Katsuki, K.; Saeki, T.; Ihara, M.; Ishikawa, K. Generation of a novel, linear peptide endothelin antagonist lead and its modification.
2. a) Ishikawa, K.; Fukami, T.; Nagase, T.; Fujita, K.; Hayama, T.; Niiyama, K.; Mase, T.; Ihara, M.; Yano, M. *J. Med. Chem.* **1992**, *35*, 2139; b) Nagase, T.; Kumagai, U.; Niiyama, K.; Mase, T.; Ishikawa, K.

Tetrahedron Lett. **1993**, *34*, 1173.

3. Publication of detailed SAR studies on these ET_A antagonistic CPPs are now in progress. The antagonistic activity of **3a** - **i** (Xxx, $IC_{max\ 50}$ for ET_A (porcine aorta membrane, nM)) is as follows: **3a** (Gly, 160); **3b** (Ala, 110); **3c** (Ser,190); **3d** (Gln,160); **3e** (Asp, 180); **3f** (Lys, 200); **3g** (Arg, 150); **3h** (Trp, 150); and **3i** (His, 240).
4. Details of experimental methods: Ihara, M.; Fukuroda, T.; Saeki, T.; Nishikibe, M.; Kojiri, K.; Suda, H.; Yano, M. *Biochem. Biophys. Res. Commun.* **1991**, *178*, 132.
5. Unfortunately, spectral and/or physiological data supporting the presence of the β-turn structure of the linear peptide backbone in the solution phase have not been detected yet.
6. Specific examples of urea synthesis using a 5-norbornen-2,3-dicarboximidoxycarbonyl group or a 2,4-dinitrophenylcarbonyl group as an activating group are already described in the literature: a) Henklein, P.; Jahrling, R.; Teubner, H.; Tietze, H.; Ott, T. *Pharmazie* **1989**, *44*, 225; b) Gray, C. J.; Quibell, M.; Jiang, K. -L.; Baggett, N. *Synthesis* **1991**, 141.
7. **PhO-CO-Leu-D-Trp-β-Ala-OEt (12)**: mp: 159 - 160 °C; ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 0.88 (3H, d, J = 4.7 Hz, CH_3 of Leu), 0.90 (3H, d, J = 5.1 Hz, CH_3' of Leu), 1.12 - 1.36 (3H, m, β-CH_2 and γ-CH of Leu), 1.18 (3H, t, J = 7.2 Hz, CH_3 of OEt), 2.19 (1H, dt, J = 17.0 Hz, 6.7 Hz, CHH'-COOEt), 2.31 (1H, dt, J = 17.0 Hz, 6.3 Hz, CHH'-COOEt), 3.16 (1H, dd, J = 6.3 Hz, 14.6 Hz, β-CHH' of D-Trp), 3.24 - 3.46 (2H, m, NH-CH_2 of βAla), 3.39 (1H, dd, J = 6.3 Hz, 14.6 Hz, β-CHH' of D-Trp), 3.93 - 4.13 (1H, m, α-CH of Leu), 4.02 (2H, q, J = 7.2 Hz, CH_2 of OEt), 4.72 (1H, dt, J = 7.3 Hz, 6.3 Hz, α-CH of D-Trp), 5.46 (1H, d, J = 7.6 Hz, NH of Leu), 6.35 - 6.44 (1H, m, NH of βAla), 6.48 (1H, d, J= 7.3 Hz, NH of D-Trp), 7.02 - 7.10 (2H, m, o-Ph), 7.06 (1H, d, J = 2.4 Hz, indole-2), 7.13 (1H, t, J = 7.6 Hz, indole-5), 7.17 - 7.24 (2H, m, p-Ph and indole-6), 7.29 - 7.39 (3H, m, m-Ph and indole-7), 7.66(1H, d, J = 7.6 Hz, indole-4), 8.06 (1H, d, J = 2.4 Hz, indole-1); IR (KBr, cm^{-1}): 3328, 2956, 2926, 1728, 1656, 1536, 1497, 1209, 1161, 1098, 1071, 1026, 741; High Resolution FAB-MS (m/e for $(C_{29}H_{36}N_4O_6 + H)^+$): Calcd: 537.2713; Found: 537.2725.
8. Hassel, T.; Seebach, D. *Helv. Chim. Acta* **1978**, *61*, 2337 and the references cited therein.
9. Spectral data of a representative linear ET_A antagonist and its ester are as follows: **perhydroazepino-CO-Leu-D-Trp-β-Ala-OEt**: ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 0.83 (3H, d, J = 6.2 Hz, CH_3 of Leu), 0.84 (3H, d, J = 6.2 Hz, CH_3' of Leu), 1.21 (3H, t, J = 7.2 Hz, CH_3 of OEt), 1.40 - 1.75 (11H, m, β-CH_2 and γ-CH of Leu, and β- and γ-CH_2 of perhydroazepino group), 2.35 - 2.55 (2H, m, α-CH_2 of β-Ala), 3.15 - 3.55 (8H, m, β-CH_2 of D-Trp, α-CH_2 of perhydroazecino group and β-CH_2 of β-Ala), 3.81 (1H, q, J = 6.8 Hz, α-CH of Leu), 4.07 (2H, q, J = 7.2 Hz, CH_2 of OEt), 4.58 (1H, d, J = 6.8 Hz, NH of Leu), 4.75 - 4.85 (1H, m, α-CH of D-Trp), 6.22 (1H, d, J= 8.8 Hz, NH of D-Trp), 7.07 (1H, d, J = 2.6 Hz, indole-2), 7.10 (1H, t, J = 7.4 Hz, indole-5), 7.19 (1H, dd,J = 1.1 Hz, 7.4 Hz, indole-6), 7.30 - 7.40 (1H, m, NH of β-Ala), 7.36 (1H, d, J = 7.4 Hz, indole-7), 7.61 (1H, dd, J = 1.1 Hz, 7.4 Hz, indole-4), 8.11 (1H, brs, indole-1); IR (KBr, cm^{-1}): 3418, 2932, 1728, 1632, 1539, 1464, 1413, 1377, 1260, 1191, 741; High Resolution FAB-MS (m/e for $(C_{29}H_{43}N_5O_5 + H)^+$): Calcd: 542.3342; Found: 542.3369; **13p**: mp: 110 - 115 °C; ^{1}H-NMR (300 MHz,DMSO-d_6, δ ppm): 0.71 (3H, d, J = 5.6 Hz, CH_3 of Leu), 0.78 (3H, d, J = 5.6 Hz, CH_3' of Leu), 1.15 - 1.35 (3H, m, β-CH_2 and γ-CH of Leu), 1.35 - 1.65 (8H, m, β- and γ-CH_2 of perhydroazepino group), 2.30 - 2.40 (2H, m, α-CH_2 of β-Ala), 2.86 (1H, dd, J = 10.1 Hz, 14.1 Hz, β-CHH' of D-Trp), 3.15 - 3.40 (7H, m, α-CH_2 of perhydroazepino group, β-CH_2 of β-Ala and β-CHH' of D-Trp), 3.90 - 4.05 (1H, m, α-CH of Leu), 4.25 - 4.40 (1H, m, α-CH of D-Trp), 6.11 (1H, d, J = 6.3 Hz, NH of Leu), 6.95 (1H, t, J = 7.4 Hz, indole-5), 7.04 (1H, t, J = 7.4 Hz, indole-6), 7.07 (1H, s, indole-2), 7.30 (1H, d, J = 7.4 Hz, indole-7), 7.54 (1H, d, J = 7.4 Hz, indole-4), 8.05 - 8.15 (1H, m, NH of β-Ala), 8.14 (1H, d, J = 8.7 Hz, NH of D-Trp), 10.78 (1H, brs, indole-1); IR (KBr, cm^{-1}): 3406, 2932, 2866, 1719, 1629, 1536, 1446, 1419, 1362, 1212, 741; High Resolution FAB-MS (m/e for $(C_{27}H_{39}N_5O_5 + H)^+$): Calcd: 514.3029; Found: 514.2983.
10. Researchers from Fujisawa Pharmaceutical Company have identified a similar peptidyl potent and selective tripeptide ET_A receptor antagonist represented by FR-139317 through chemical modification of the same cyclic pentapeptide leads: Sogabe, K.; Nirei, H.; Shoubo, M.; Nomoto, A.; Ao, S.; Notsu, Y.; Ono, T. *J. Pharmacol. Exp.Ther.***1993**, *264*, 1040; also see Hemmi, K.; Neya, M.; Fukami, N.; Hashimoto, M.; Tanaka, H.; Kayakiri, N. *Eur. Patent No.* 457195 (1993).

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