Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2241–2246

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

DISCOVERY OF IRL 3461: A NOVEL AND POTENT ENDOTHELIN ANTAGONIST WITH BALANCED ET_A/ET_B AFFINITY

Junichi Sakaki,* Toshiki Murata, Yoko Yuumoto, Ikushi Nakamura, Thomas Frueh, Thomas Pitterna, Genji Iwasaki, Kyoko Oda, Takaki Yamamura, and Kenji Hayakawa

Takarazuka Research Institute, Novartis Pharma K.K., 10-66 Miyuki-cho, Takarazuka 665, Japan

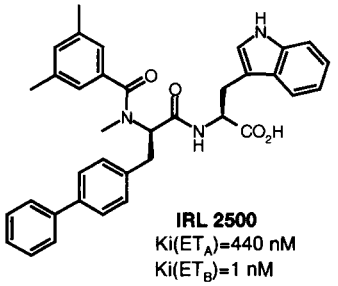
Received 20 March 1998; accepted 16 July 1998

Abstract: IRL 3461, N-butanesulfonyl-[N-(3,5-dimethylbenzoyl)-N-methyl-3-[4-(5-isoxazolyl)-phenyl]-alanyl]-(L)-valineamide, a potent and bifunctional (ET_A+ET_B) [Ki(ET_A)=1.8 nM, Ki(ET_B)=1.2 nM] antagonist was discovered by structural modification of IRL 2500, an ET_B selective antagonist. IRL 3461 was found to be stable on incubation with human, rat, mouse, and guinea pig plasmas.

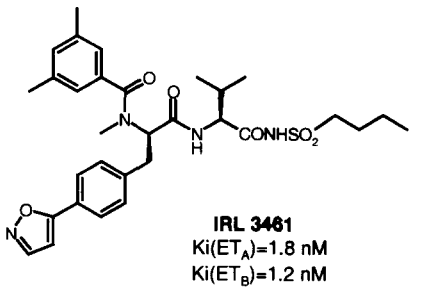
Since the discovery of endothelins in 1988[1], various types of potent and selective ET_A receptor antagonists have been identified[2]. Recently, it was demonstrated that not only ET_A but also ET_B receptors are involved in vasoconstriction[3]. Thus current research efforts have been directed toward development of potent orally active antagonists with a balanced profile of ET_A and ET_B activity.

We have already reported[4] a rational approach for the discovery of IRL 2500, a novel ET_B selective antagonist [Ki(ET_A)=440 nM, Ki(ET_B)=1.0 nM][5], by combination of sequence studies of an ET-1 analog and a homology study of the rhodopsin superfamily of seven transmembrane receptors. In this report we describe the development of IRL 3461, a potent and dual ET_A/ET_B antagonist based on the structural modification of IRL 2500 by increasing the ET_A receptor affinity [i) replacement of the biphenyl part with the 5-isoxazolyl phenyl group, ii) introduction of the sulfonamide moiety at the C-terminus] and by improving plasma stability [iii) replacement of tryptophan with valine].

Scheme 1



IRL 2500
Ki(ET_A)=440 nM
Ki(ET_B)=1 nM



IRL 3461
Ki(ET_A)=1.8 nM
Ki(ET_B)=1.2 nM

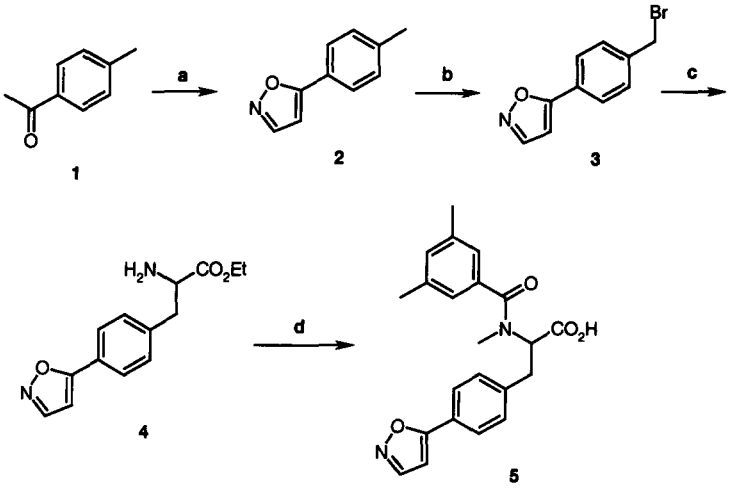
Chemistry

Synthesis of the left-hand fragment **5** is shown in Scheme 2. 4-(5-Isoxazolyl)benzyl bromide (**3**)[6] prepared from 4'-methylacetophenone (**1**) was converted to 4-(5-isoxazolyl)phenylalanine ethyl ester (**4**) by the

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00387-4

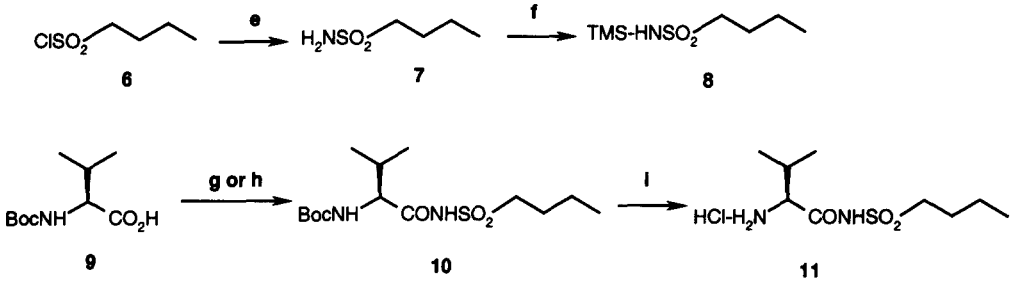
reaction with diphenylmethyleneglycine according to the reported procedure[7]. The biarylalanine (**4**) was then subjected to 3,5-dimethylbenzolylation, N-methylation, and hydrolysis to afford **5**, which was used for the following coupling reactions.

Scheme 2



(a) i. $Me_2NCH(OMe)_2$, reflux; ii. H_2NOSO_3H, MeOH, rt, 75% (2 steps); (b) NBS, dibenzoylperoxide, CCl_4, reflux, 93%; (c) i. $Ph_2C{=}NCH_2CO_2Et$, Bu_4NHSO_4, 2.5N NaOH, CH_2Cl_2, rt; ii. p-TsOH, H_2O, MeCN, rt, 57% (2 steps); (d) i. 3,5-dimethylbenzoic acid, HOBt, WSCD, DMF, rt; ii, NaH, MeI, DMF, rt; iii, LiOH, H_2O, MeOH, THF, rt, 67% (3 steps)

Scheme 3

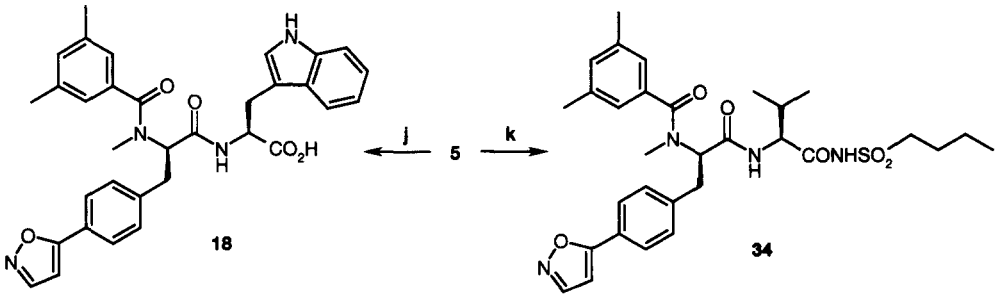


(e) NH_3, MeCN, 95%; (f) TMS-Cl, Et_3N, toluene, 87%; (g) i, cyanuric fluoride, pyridine; ii, **8**, DMAP, CH_2Cl_2, 72% (2 steps); (h) **8**, BOP, Et_3N, DMAP, CH_2Cl_2, 59%; (i) 4N-HCl, dioxane, 95%

The optically pure butanesulfonylvalineamide (**11**) was synthesized as shown in Scheme 3. The TMS-activated sulfonamide (**8**) [8] was successfully coupled with Boc-valine in two different ways without racemization [method 1: coupling via the acid fluoride of Boc-valine, method 2: direct coupling with Boc-valine in the presence of BOP (benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate)]. The resulting butanesufonyl Boc-valineamide (**10**) was deprotected to the HCl salt (**11**)[9].

Coupling reaction of racemic **5** with L-tryptophan methyl ester gave a 7:3 mixture of two diastereoisomers which could be separated by MPLC[10]. Saponification of the less polar isomer afforded the more potent isomer **18** (Scheme 4). Coupling reaction of **5** with the chiral **11** by using WSCD [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide] and HOBt in DMF gave a mixture of two diastereoisomers (**34**) in 7:3 ratio[11]. The major isomer was found to be a more potent isomer. A series of sulfonamide analogs (**20-37**) was synthesized by the similar procedure (Tables 2 and 3). In most cases, the asymmetric center of the phenylalanine moiety was isomerized, resulting in the unbalanced formation of two diastereoisomers in ca. 6:4 – 7:3 even though the racemic phenylalanine derivatives were used. The details are discussed in the following paper[12].

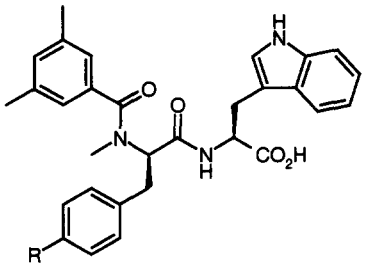
Scheme 4



(j) i, L-Trp-OMe, HOBt, WSCD, DMF. rt, 90%; ii, MPLC separation of diastereoisomers, iii, LiOH, H_2O, MeOH, THF, rt, 91%; (k) **11**, WSCD, HOBt, DMF, 90%

Structure Activity Relationship

Table 1: SAR for the 4-Substituted Phenylalanyltryptophan Derivatives



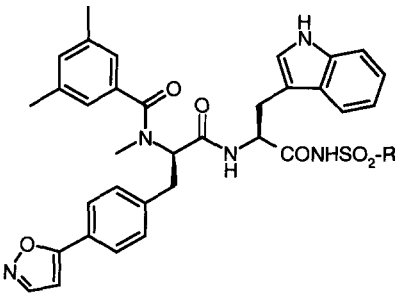
No	R	Ki(ET_A) (nM)	Ki(ET_B) (nM)	Ratio A/B
12	H	11000	36	306
13[a]	phenyl	440	1	440
14[b]	2-pyridyl	2300	4.4	523
15	2-furyl	250	0.76	329
16	2-thienyl	87	0.6	145
17	3-thienyl	54	0.23	235
18	3-isoxazolyl	130	0.79	165
19	5-isoxazolyl	45	0.21	214

a) IRL 2500 b) racemic phenylalanine analog

In the case of tryptophan derivatives (Table 1), an aromatic substituent at the 4-position of the phenylalanine moiety shows a significant effect in changing the binding affinity for the endothelin receptors. Among several derivatives, replacement of this position with 3-thienyl or 5-isoxazolyl group most remarkably contributed to the increase in binding affinity for both ET_A and ET_B receptors (Table 1: **17** and **19**).

A great breakthrough was achieved in the course of a systematic SAR study for replacement of carboxylic acid. It was found that the ET_A receptor binding was dramatically improved (10-50 times) by attaching a sulfonamide group to the C-terminus of tryptophan with retaining the ET_B receptor binding, resulting in the more balanced binding affinity (A/B ratio 4-26) (Table 2: **20-29**).

Table 2: SAR for the 4-Isoxazolylphenylalanyltryptophan Derivatives



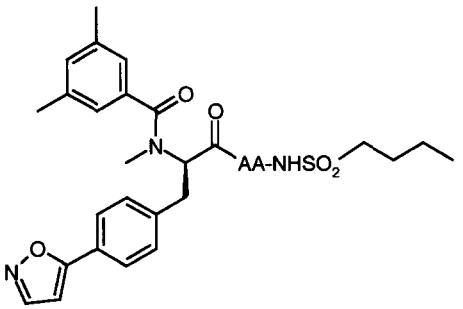
No	R	Ki(ET_A) (nM)	Ki(ET_B) (nM)	Ratio A/B
20	ethyl	5.3	0.38	13.9
21	vinyl	1.9	0.14	13.6
22	propyl	2.2	0.24	9.2
23	1-propenyl	3.9	0.39	10
24	2-propenyl	0.89	0.23	3.9
25	i-propyl	10	0.38	26.3
26	butyl	2.9	0.24	12.1
27	ethoxyethyl	6.0	0.23	26.1
28	phenyl	2.3	0.34	6.8
29	benzyl	5.4	0.50	10.8

Then we investigated replacement of the tryptophan moiety with various amino acids (Table 3). It is of great interest to find that the butylsulfonamides of β-branched-α-amino acids (**34-37**) have binding affinities well balanced between ET_A and ET_B receptors. These compounds are also completely resistant to rat and mouse plasmas[13]. IRL 3461 (**34**) shows the highest binding affinity with good stability against plasma of different

species.

In summary, we have discovered a highly potent and well-balanced ET_A/ET_B receptor antagonist, IRL 3461, which is resistant to degradation by plasma of rat, mouse, guinea pig as well as human.

Table 3: SAR for the 4-Isoxazolylphenylalanine sulfonamide Derivatives



No	AA[a]	Ki(ET_A) (nM)	Ki(ET_B) (nM)	Ratio A/B
30	2-naphthyl-alanine	3.1	0.59	5.3
31	ethylglycine	5.5	0.7	7.9
32	methionine	5.2	0.49	10.6
33	leucine	8.0	1.3	6.2
34[b]	valine	1.8	1.2	1.25
35	isoleucine	6.9	3.5	2.0
36	cyclohexyl glycine	8.6	3.1	2.8
37	threonine	9.1	3.2	2.8

a) (L)-isomer b) IRL 3461

References and Notes

1. Yanagisawa, M.; Kurihara, H.; Kimura, H.; Tomobe, Y.; Kobayashi, M.; Mitsui, Y.; Goto, K.; Masaki, T. *Nature* **1988**, *332*, 411.

2. Recent reviews: a) Cheng, X. M.; Nikam, S. S.; Doherty, A. M. *Cur. Med. Chem.* **1995**, *1*, 271. b) Doherrty, A. M. *Drug Discovery Today*, **1996**, *1*, 60.

3. a) Clozel, M.; Gray, G. A.; Breu, V.; Loeffler, B.-M.; Osterwalder, R. *Biochem. Biophys. Res. Comm.* **1992**, *2*, 867. b) Dagassan, P. H.; Breu, V.; Clozel, M.; Kuenzli, A.; Vogt, P.; Turina, M.; Kiowski, W.; Clozel, J.-P. *J. Cardiovasc. Pharmacol.* **1996**, *27*, 147. c) Seo, B.; Luescher, T. *Hypertension* **1995**, *25*, 501.

4. Frueh, Th.; Saika, H.; Svensson, L.; Pitterna, Th.; Sakaki, J.; Okada, T.; Urade, Y.; Oda, K.; Fujitani, Y.; Takimoto, M.; Yamamura, T.; Inui, T.; Makatani, M.; Takai, M.; Umemura, I.; Teno, N.; Toh, H.; Hayakawa, K.; Murata, T. *Bioorg. Med. Chem. Lett* **1996**, *6*, 2323.
5. The binding affinity for the two subtypes of ET receptors, ET_A and ET_B were examined in porcine lung membranes. For a detailed description of the binding assay, see: Takai, M.; Umemura, I.; Yamasaki, K.; Watanabe, T.; Fujitani, Y.; Oda, K.; Urade, Y.; Inui, T.; Yamamura, T.; Okada, T. *Biochem. Biophys. Res. Commun.*, **1992**, *184*, 953.
6. Preparation of 4-(5-isoxazolyl)toluene: Lin, Y.i, Lang, S. A., Jr. *J. Org. Chem.* **1980**, *45*, 4857.
7. O'Donnell, M.; Bennett, W. D.; Wu, S. *J. Am. Chem. Soc.* **1989**, *111*, 2353.
8. Trimethylsilylation of sulfonamide: Roy, A. K. *J. Am. Chem. Soc.* **1993**, *115*, 2598.
9. Synthetic procudures for **10**. Method 1) A solution of **8** (1.72 g, 8.28 mmol), Boc-valine fluoride[14] (1.01 g, 4.6 mmol), and DMAP (220 mg, 1.84 mmol) in THF (25 ml) was stirred at room temperature for 1h. The mixture was diluted with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (hexane : ethyl acetate=3:1) to give **10** (1.11 g, 72%). Method 2) Et_3N (6.75 ml, 48.4 mmol) was added dropwise to a mixture of Boc-valine (10.18 g, 44.0 mmol), BOP (23.05 g, 52.1 mmol), and CH_2Cl_2 (180 ml) at room temperature. After being stirred for 15min, to the mixture were added **8** (9.96 g, 47.6 mmol) and DMAP (1.62g, 13.3 mmol) at room temperature. The reaction mixture was stirred overnight and concentrated in vacuo. The residue was diluted with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude material was purified by flash column chromatography with ethyl acetate/hexane (3:1) to give **10** (8.73 g, 59%).
10. Kusano (KHLC-201-43 type III, CPS-223L-1), hexane : ethyl acetate=1 : 1.
11. Two isomers were separated into pure isomers by HPLC [Shim-pack PREP-SIL(H), hexane : isopropanol : TFA=90 : 10 : 0.5 (1.0 ml/min); the major isomer: 38.6 min, the minor one: 43.8 min (1.0 ml/min).
12. Sakaki, J.; Murata, T.; Yuumoto, Y.; Nakamura, I.; Hayakawa, K. *Bioorg. Med. Chem. Lett* accepted.
13. Plasma stability test: A compound (100 μM) was incubated with plasma (80 μl) at 37 °C for two hours. After acidification with HCl, the compound was extracted with ethyl acetate and analyzed by HPLC (TOSO ODS120T, 0.1%TFA/H_2O – 0.1%TFA/CH_3CN).
14. a) Carpino, L.; Sadat-Aalaee.; Chao, H. G.; DeSelms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 9651. b) Bertho, J.-N.; Loffet, A.; Pinel, C.; Reuther, F.; Sennyey, G. *Tetrahedron Lett.* **1991**, *32*, 1303.