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STEREOSELECTIVE SYNTHESIS OF A NOVEL AND BIFUNCTIONAL ENDOTHELIN ANTAGONIST, IRL 3630

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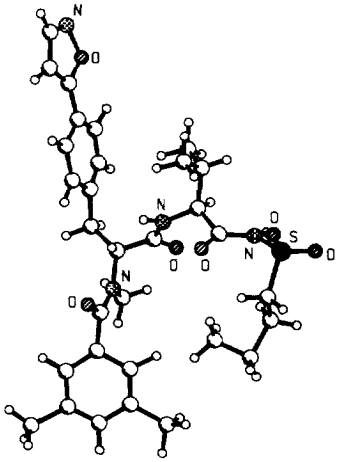
Abstract: IRL 3630 (**3**), a single enantiomer of IRL 3461 with more potency was identified. Coupling reaction of the racemic fragment (**1**) with the chiral (L)-valinesulfonamide (**2**) under a biphasic solvent system (CH_2Cl_2-H_2O) successfully led to the predominant formation of the desired isomer (**3**) with concomitant isomerization of **1**. IRL 3630, N-butanesulfonyl-[N-(3,5-dimethylbenzoyl)-N-methyl-3-[4-(5-isoxazolyl)-phenyl]-(D)-alanyl]-(L)-valineamide, is a highly potent and bifunctional (ET_A+ET_B) antagonist [Ki(ET_A)=1.5 nM, Ki(ET_B)=1.2 nM].

It has been demonstrated that the ET_A receptor, one of two distinct endothelin receptor subtypes, plays a major role in ET-1 induced constrictive and proliferative disorders[1]. Although therapeutic necessity of the ET_B receptor blockade is still unclear, endothelin antagonists displaying a balanced profile of ET_A and ET_B activity have attracted much attention and various types of dual antagonists have currently been under development[2].

In the preceding paper[3], we described the discovery of IRL 3461, a potent ET_A+ET_B antagonist which was a 7:3 mixture of two diastereoisomers, syn-(D,L) (**3**) and anti-(L,L) (**4**) (Scheme 1). The isomers were separated by HPLC[3] to give **3** (IRL 3630) as the major isomer with good potency [Ki(ET_A)=1.5 nM, Ki(ET_B)=1.2 nM][4] and **4** as the minor one with less potency. The absolute configuration of **3** was determined by X-ray analysis[5]. The unbalanced formation of the two isomers can be explained by considering a rapid equilibration between two intermediates (**A** and **B**) (Scheme 1). On treatment with WSCD [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide] and HOBt, the racemic left-hand fragment **1** is initially converted to the active esters (**A** and **B**) which are in a state of equilibrium. This equilibration is probably facilitated by the presence of the tertiary amine of WSCD.

Figure 1: Molecular structure of IRL 3630



In addition, it is well-known that N-acyl-amino acid is more easily prone to racemization than N-

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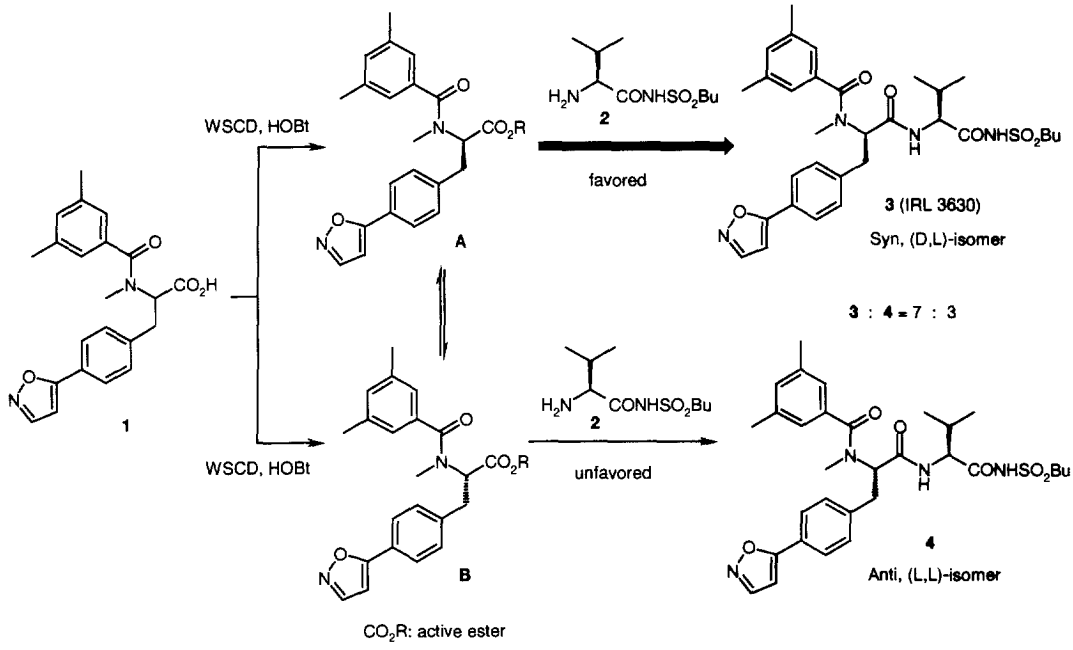
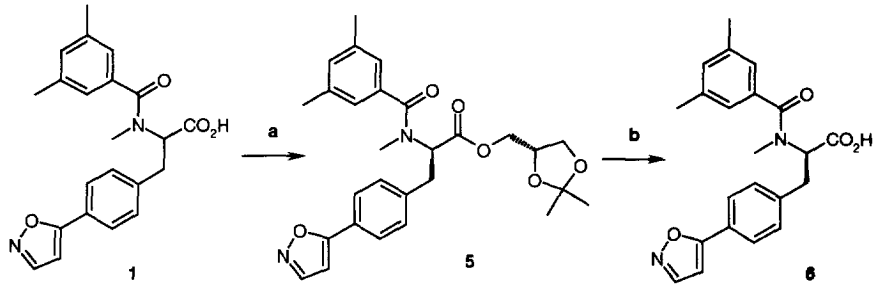
alkoxycarbonyl- [ex) Boc[6] or Cbz protected] amino acid. Therefore, the degree of easiness (match/mismatch) of the coupling reaction with L-valinesulfonamide (**2**) plays an important role in determining their diastereoselectivity. The D-isomer (**A**) seems to be diastereomerically more favored than the (L)-isomer (**B**) in this coupling reaction. As a result, the syn-isomer (**3**) was predominantly formed over the anti-isomer (**4**). It was confirmed that both **3** and **4** are stable and no interconversion occurs under the reaction conditions.

Scheme 1



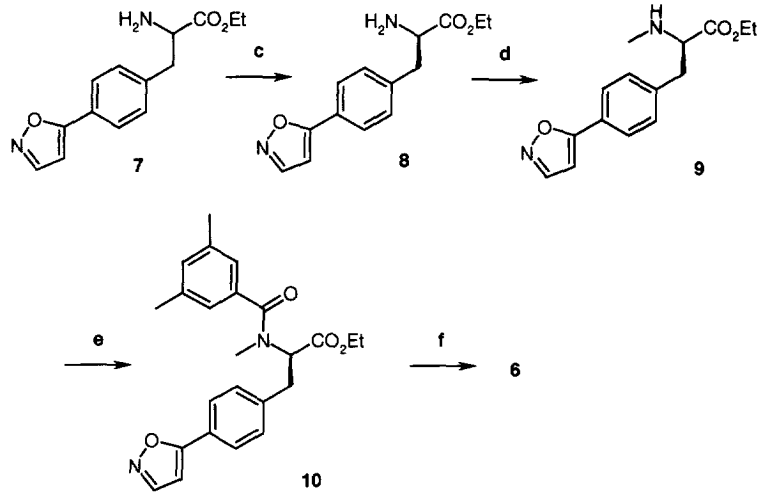
In order to synthesize IRL 3630 (**3**) more selectively, we planned to prepare 4-(5-isoxazolyl)phenylalanine (**6**) in an enantiomerically pure form and to couple with the chiral right-hand fragment **2**. The isoxazol-phenyl-(D)-alanine (**6**) was prepared by following two methods. The first method was due to HPLC separation of a diastereomeric mixture of esters formed in the reaction with (R)-2,2-dimethyl-1,3-dioxolane-4-methanol as a chiral auxiliary[7] (Scheme 2). The second method was chiral resolution of the isoxazolylphenylalanine ethyl ester (**7**) by selective crystallization with a chiral acid[8] (Scheme 3). After screening of various chiral acids such as 10-camphorsulfonic acid, (R)-mandelic acid, and (L)-pyroglutamic acid, we finally found that one equivalent of (R,R)-dibenzoyltartaric acid was co-crystallized selectively with two equivalents of 3-[4-(5-isoxazolyl)phenyl]-(D)-alanine ethyl ester (**8**) in a modest chemical yield[9]. After removal of the chiral auxiliary by neutralization, **8** was methylated according to Grieco's procedure[10] to give the N-methyl amino acid (**9**) which was further converted to the chiral left-hand fragment **6** by acylation and hydrolysis without racemization[11].

Scheme 2



(a) i, (R)-2,2-dimethyl-1,3-dioxolane-4-methanol, DICD, DMAP, CH_2Cl_2, ii, HPLC separation, 43% (2 steps); (b) LiOH, MeOH, THF, H_2O, 98%

Scheme 3



c) i, (2R,3R)-O,O'-dibenzoyltartaric acid, EtOH; ii, aq. $NaHCO_3$, 14% (2steps); d) i, HCl-dioxane; ii, cyclopentadiene, formalin, THF, H_2O; iii, TFA, triethylsilane, $CHCl_3$; e) 3,5-dimethylbenzoyl chloride, Na_2CO_3, $CHCl_3$, H_2O, 43% (4 steps); f) LiOH, MeOH, THF, H_2O, 97%

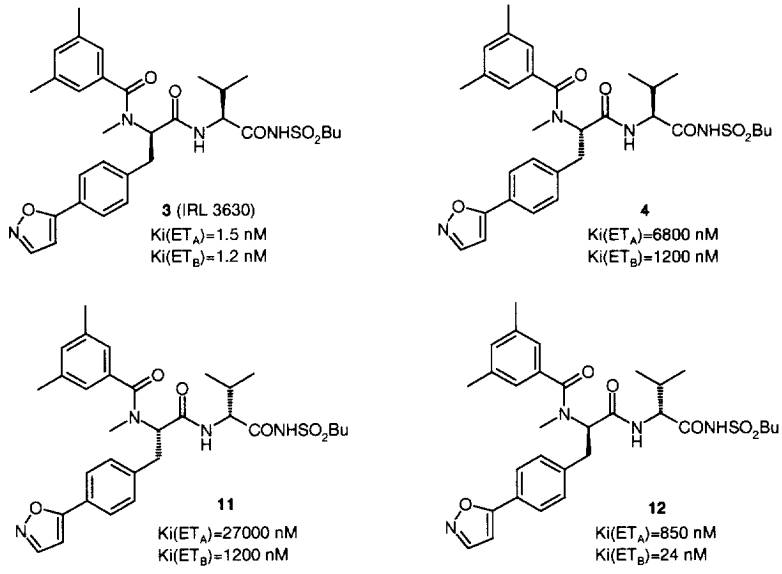
With having the optically pure 4-(5-isoxazolyl)phenylalanine (**6**) in hand, we then attempted coupling reaction with the chiral valinesulfonamide (**2**) under the same conditions (WSCD+HOBt in DMF) used for the synthesis of IRL 3461[3]. Contrary to our expectations, the reaction resulted in the formation of a mixture of the two diastereoisomers (**3** and **4**) in 9:1 due to the undesired isomerization of the left-hand fragment **6**. In the course of examining the reaction conditions to improve the diastereoselectivity, it was found that a biphasic solvent system (CH_2Cl_2-H_2O) remarkably accelerated the isomerization of the isoxazolylphenylalanine chirality.

Although it was disappointing, it caused us to change the way of our thinking and led us to finding a more practical way for the selective preparation of **3**. When this biphasic solvent system was applied to the reaction of the racemic **1** and the chiral **2**, the diastereoisomers, **3** and **4** were formed in a 12:1 ratio in 82% yield[12]. A single crystallization of the mixture from i-propanol gave the pure **3**, IRL 3630, which was identical to the more potent isomer separated from IRL 3461.

Table: Diastereoselectivity in the Coupling Reaction using WSCD and HOBt

No	acid	amine	solvent	Ratio of diastereoisomers **3** : **4**
1	**1**	**2**	DMF	7 : 3
2	**6**	**2**	DMF	9 : 1
3	**1**	**2**	CH_2Cl_2 - H_2O	12 : 1

Figure 2



All four isomers (**3**, **4**, **11**, and **12**) obtained in the above synthetic studies were isolated and characterized[13]. Their structure and binding potency are summarized in Figure 2, confirming that IRL 3630 is the isomer with highest binding potency. These results indicate that the stereochemistry of the isoxazolylphenylalanine moiety is more important than that of the valinesulfonamide for binding affinity.

The potent antagonist IRL 3630 (**3**) could be converted to the water soluble sodium salt by treating with 1N NaOH in MeOH and H_2O.

In conclusion, we identified a potent and bifunctional ET_A/ET_B antagonist, IRL 3630 (**3**). The asymmetric center of the fragment (**1**) was found to be readily isomerized under the coupling conditions in the biphasic solvent system (CH_2Cl_2-H_2O). The unique profile enabled us to synthesize IRL 3630 stereoselectively starting with recemic **1**. The procedure is applicable to a large scale preparation.

Acknowledgement

The authors would like to thank Ms. Rihs Grety, Novartis Pharma AG for the X-ray analysis of IRL 3630.

References and Notes

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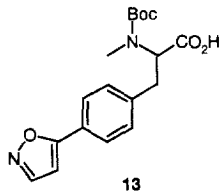
4. The binding affinity for the ET receptors was determined by using human ET_A and ET_B receptors expressed in CHO (Chinese hamster ovary) cells. For a detailed description of the binding assay: Sakaki, J.; Murata, T.; Yuumoto, Y.; Nakamura, I.; Okada, T. WO97/11960.
5. X-ray structure analysis of IRL 3630: The relative stereochemistry was determined by a single crystal structure analysis. A platelet shaped crystal measuring 0.32 x 0.38 x 0.01 mm^3 was investigated on a Enraf-Nonius CAD4 diffractometer with graphite monochromated CuKα radiation. The crystallographic data are summarized as follows: $C_{31}H_{40}N_4O_6S$, tetragonal, space group $P4_122$, a=8.459(1) Å, c=89.535(5) Å, V=6406(2) $Å^3$, Z=8, Dcalc = 1.237 g/cm^3. A total of 4719 independent intensities were measured of which 1011 were classified as observed with $I>3\sigma(I)$. The structure was solved by direct methods (Program SHELXS86). The structure was refined using full matrix least squares calculations with isotropic displacement parameters for S and anisotropic ones for all the other non-hydrogen atoms (Program CRYSTALS). The positions of the hydrogen atoms were calculated and not refined. The final R-factor for 157 variables was 0.086. The authors will deposit the atomic coordinates with the Cambridge

Crystallographic Data Centre. They can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EW, UK.

6. When the same coupling reaction was carried out using the Boc protected fragment (**13**) with the right hand fragment (**2**), the isomerization of the asymmetric center was surpressed to give the mixtures in 1:1-3:2 ratio.



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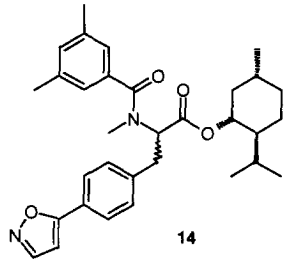

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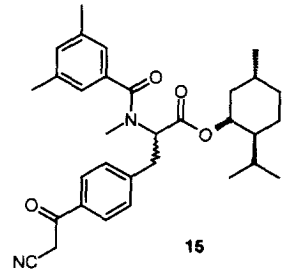

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7. l-Menthol was also applied to the isomer separation as the chiral auxiliary. Although separation of the diastereoisomers (**14**) by HPLC was easier than **5**, removal of the chiral secondary alcohol by hydrolysis was unsuccessful. Drastic conditions resulted in degradation of the isoxazol ring (**15**).
8. Chiral amines such as (D)-(+)-phenylethylamine, (-)-brucine, cinchonidine, or quinine were also tried to use for the chiral resolution of the acid (**1**) without success
9. Resolution for **8**: To a solution of **7** (1 g, 3.84 mmol) in EtOH (2 ml) was added (2R,3R)-(-)-O,O'-dibenzoyltartaric acid monohydrate (361 mg, 0.96 mmol) and dissolved at 60 °C. The mixture was allowed to stand for one day. Colorless needles were collected and washed with cold EtOH (1 ml). The crystals were dissolved in saturated sodium bicarbonate (10 ml) and extrated with ethyl acetate (2x10 ml). The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **8** (140 mg). $[\alpha]_D$ -24.6 ° (c=1, $CHCl_3$).
10. Grieco, P. A.; Bahsas, A. *J. Org. Chem.* **1987**, *52*, 5746.
11. When acylation of **8** with 3,5-dimethylbenzoyl chloride was performed prior to methylation, the chiral center was racemized to some extent by the base (NaH) used for methylation.
12. Experimental procedure for the preparation of IRL 3630 (**3**): WSCD (1.97 ml, 10.75 mmol) was added dropwise to a cooled (0 °C) solution of HOBt (2.57 g, 19.02 mmol), **1** (4.10 g, 10.82 mmol) and **2**-HCl (2.94 g, 10.76 mmol) in CH_2Cl_2 (30 ml) and water (30 ml). The mixture was stirred for 2h at 0 °C, then slowly warmed up to room temperature and further stirred overnight. The organic layer was separated and the aqueous layer was washed with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was recrystallized from i-propanol to give **3** (5.27 g). mp 194-196 °C. $[\alpha]_D$ +104.5 ° (c=1, $CHCl_3$).
13. Chiralpak AD (Daicel): hexane : EtOH : TFA=85 : 15 : 0.5 (1.0 ml/min). **11** [(L,D)-isomer], 33.1 min; **4** [(L,L)-isomer], 41.6 min; **3** [(D,L)-isomer], 64.7 min; **12** [(D,D)-isomer], 127.0 min.