Pyrrolidine-3-carboxylic Acids as Endothelin Antagonists. 5. Highly Selective, Potent, and Orally Active ET_A Antagonists

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The synthesis and structure−activity relationships (SAR) of a series of pyrrolidine-3-carboxylic acids as endothelin antagonists are described. The data shows an increase in selectivity when the methoxy of Atrasentan (ABT-627) is replaced with methyl, and the benzodioxole is replaced with dihydrobenzofuran. Adding a fluorine further increases the binding activity and provides a metabolically stable and orally bioavailable ET_A-selective antagonist.

Introduction

Endothelins (ET-1, ET-2, and ET-3) are 21-amino acid bicyclic peptides. ET-1 is one of the most powerful pressor peptides isolated to date,[1,2a,b] a long-acting constrictor of vascular smooth muscle, and a potent mitogen.[3]

The production of ET-1 is enhanced by stimuli such as hypoxia, shear stress, and various neurohumoral factors, which induce the transcription of ET-1 RNA and subsequent ET-1 synthesis. The biological effects of the ETs are mediated by G-protein-linked receptors. Two types of receptors, ET_A and ET_B, have been cloned and are approximately 55% homologous in their amino acid sequences.[4] The ET_A receptor has much greater affinity for ET-1 and ET-2 than for ET-3, and it is abundantly expressed in vascular smooth muscle cells, cardiomyocytes, and fibroblasts. The ET_B receptor has equal affinity for ET-1 and ET-3. ET_B is considered the vasodilator receptor due to its mediation of nitric oxide release.[5] It now appears that ET_B receptors are involved in smooth muscle contraction in blood vessels such as the rabbit saphenous vein[6] and guinea pig bronchus.[7] These diverse actions imply a role for endothelin in various constrictive and/or proliferative disorders.

Our group has reported several families of selective or balanced endothelin receptor antagonists,[10,13,14,15] along with data to suggest that they may be of benefit in diseases[8] such as congestive heart failure,[16] acute myocardial infraction,[17] pulmonary hypertension,[18] renal failure,[19] asthma,[20] and restenosis[21] and as a therapeutic agent for cancers.[22] In particular we have focused on the development of highly ET_A-selective agents. The conclusion of these studies on ET_A-selectivity is reported herein.

History of ET_A-Selective Antagonists at Abbott

Since the selection of **1** (Atrasentan)[10a] as a clinical candidate, we have pursued greater selectivity for the ET_A receptor and increased metabolic stability in a back-up compound. Our efforts focused in particular on the C_2- and C_4-aromatic substituents. Metabolic studies of **1** showed that the *p*-CH_3O- group on the C_2-anisyl group is metabolized to *p*-OH- by demethylation. Others have shown that a benzodioxole group can inhibit the cytochrome P450 enzyme,[9] though this effect seems to be minor with **1**.[11] We found one solution to this first problem through replacing the *p*-anisyl group with a 2,2-dimethylpentyl, **2** (ABT-546), which gave us a highly ET_A-selective (>26,000-fold) antagonist.[15a]

Intrigued by these observations, we pursued additional SAR studies focusing on modification on the 2-aryl group and replacing the benzodioxole group to increase the selectivity and the binding affinity. This paper provides the results, chemistry, and pharmacology (Figure 1).

Chemistry

The general procedures for preparation of most compounds in this paper were described in previous reports.[10a,b] The new compounds are synthesized according to Schemes 1 and 2. The core pyrrolidines employed in this study have been assembled (Scheme 1) by direct analogy to our earlier work. The β-ketoesters **3** were prepared by reacting an imidazolide of various benzoic acids with ethyl malonate potassium salt followed by decarboxylation (ca. 90% yield). The Michael addition reaction between β-ketoester **3** and nitrostyrene **4** with potassium *tert*-butoxide catalyst provides a mixture of diastereomeric nitroketones **5**. These nitrostyrenes are derived from corresponding benzaldehydes[11] by Henry reaction.

These nitroketones **5** are reductively cyclized over Raney nickel to provide cyclic imines, which upon further hydrogenation of the resulting cyclic iminium TFA salts provide almost exclusively the *cic*,*cis*-isomers of the pyrrolidines **6**. These were epimerized with DBU to afford the pure *trans*,*trans*-pyrrolidines **7** in ca. 80% overall yield from **3** and **4**. Alkylation of pyrrolidine **7** with *N*,*N*-dibutylbromoacetamide and subsequent hydrolysis of the ethyl ester furnished final compound **8** in good yield. Ethyl-3-fluoroacetophenone is not available, so it was synthesized[12] from 3-fluoro-4-hydroxyacetophenone by treatment with trifluoromethanesulfonic anhydride in TEA and CH_2Cl_2, and further reaction

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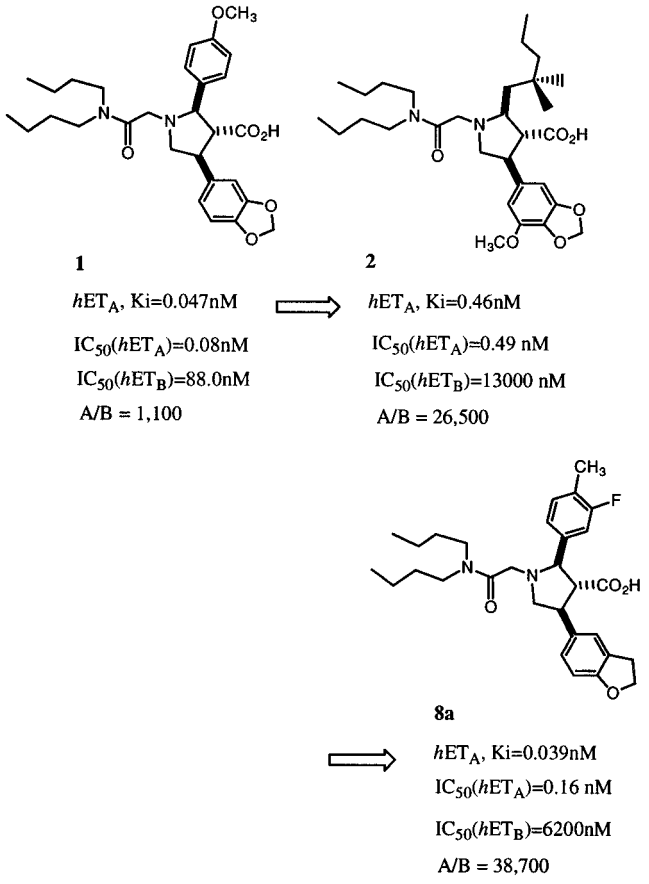


Figure 1.

of palladium-catalyzed coupling of the triflate with tetraethyltin in the presence of LiCl. This pyrrolidine was assembled as described in Scheme 1 and subsequently hydrolyzed to get the final product **9**.

The preparation of optically pure **8a** as a single enantiomer is accomplished by resolution of core pyrrolidine **7** (Scheme 2). In practice, the racemic pyrrolidine ester is converted in two steps to the corresponding Boc acid; this hydrolysis step selects for the *trans,trans* isomer. Formation of a chiral salt of racemic acid **7a** using *R*-(+)-α-methylbenzylamine produces product of >99.5% ee after a single recrystallization as evaluated by chiral HPLC. The resultant optically active acid is reconverted to amino ester **7b** under Fischer esterification conditions. Optically pure antagonist **8a** is prepared by the steps already described in Scheme 1.

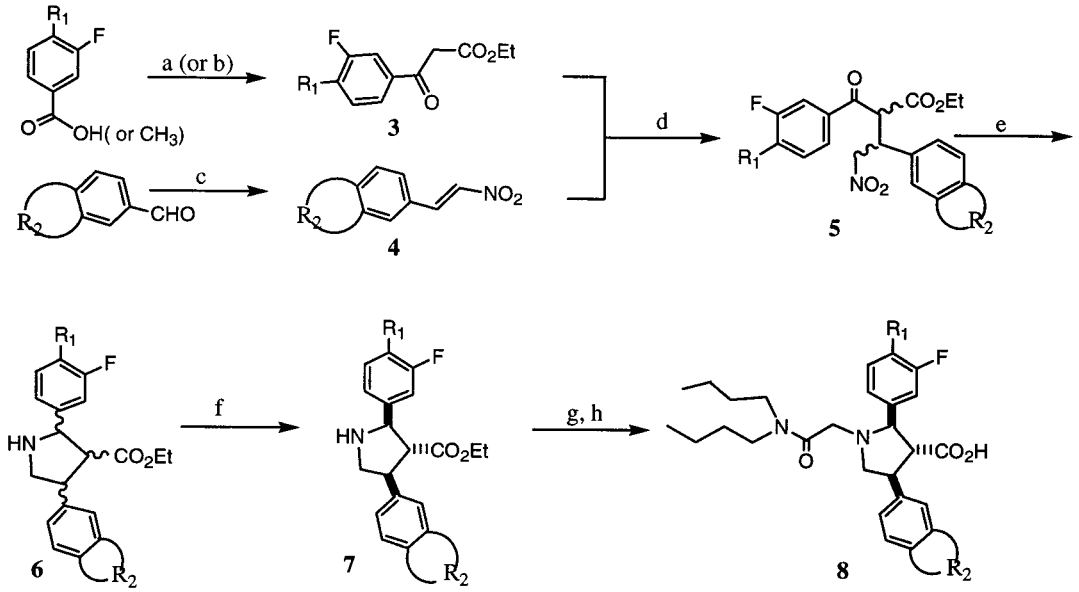
Structure−Activity Relationships

The previous studies by our group have indicated that the N-linked side chain amide carbonyl of **1** is important to determine the receptor binding and selectivity. 2,6-Dialkylphenylacetamides[13] and diarylalkylacetamides[14] exhibit high affinity and selectivity for ET_B (IC_{50} = 1.1∼1.4 nM), while the corresponding dialkylacetamides are ET_A-selective.[13,14] The *N*,*S*-dialkylsulfonamides retain high affinity for ET_A and simultaneously bind to ET_B receptors as well.[10b] However, our work also indicated that the presence of both alkyl groups (e.g., *n*-dibutyl) are required to maintain high ET_A selectivity, thus only *N*,*N*-dibutylacetamide side chain compounds will be discussed in this paper.

Our success replacing the C-2 aryl substituent with an alkyl group led us to explore aryl groups with increased hydrophobicity. When the *p*-anisyl substituent of **1** was changed to tolyl **1a**, a decrease of ET_B affinity was observed while retaining the ET_A affinity (Table 1), resulting in an increase in selectivity. Furthermore, as the *p*-alkyl group increased in size to *p*-ethylphenyl **1b** and *p*-propylphenyl **1c**, the ET_A affinity was decreased slightly and ET_A/ET_B selectivity was less than *p*-tolyl **1a**. Placing a substituent adjacent to the methyl group (**1e**−**f**) improved potency but decreased selectivity for ET_A.

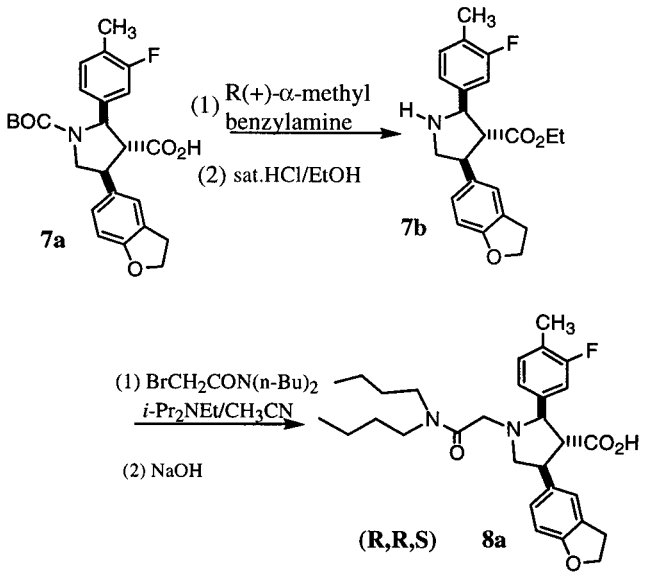
To avoid possible complications arising from cytochrome P450 (3A4) inhibition, we studied a number of compounds with benzodioxole replacements at C_4.[11]

Scheme 1[a]



[a] (a) CDI, THF, $K^+O_2CCH_2CO_2Et/MgCl_2$, on carboxylic acid; (b) NaH, $(EtO)_2CO$, THF, on acetophenone; (c) CH_3NO_2, NH_4OAc, HOAc, heat; (d) cat. *t*-BuOK, THF, rt; (e) H_2, Raney Ni, AcOH, THF, rt, then TFA, rt; (f) DBU, toluene, reflux; (g) $BrCH_2CO(n\text{-}Bu)_2$, *i*-Pr_2NEt, CH_3CN, rt; (h) aq NaOH, EtOH.

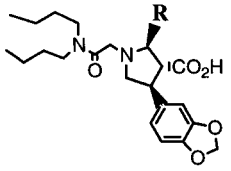
Scheme 2. Resolution of **8a**

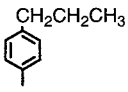
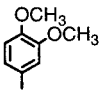


Our initial studies focused on replacing the benzodioxole with simple carbocyclic analogues. An isosteric 5-indan substitution **2c** which has no hydrogen-binding capability, showed 10-fold less potency than benzodioxole **1** but greater selectivity for ET_A. Simple deletions of each oxygen atom gave 5-substituted dihydrobenzofuran **2a** and the 6-isomer **2b**. Both are very potent ET_A receptor antagonists with IC_{50s} of 0.36 and 1 nM, respectively. Interestingly, the 6-substituted analogue **2b** is 7-fold less selective than 5-isomer **2a**. In contrast to the saturated analogue, the unsaturated 5-benzofuran **2d** has basically the same binding profile. The benzodioxan **2e** follows the same pattern, showing increased affinity for both receptor subtypes.

Combinations of C-2 and C-4 substituents were prepared in the hope of achieving simultaneous improvements in potency and selectivity. As a result, the tolyl/dihydrobenzofuran combination **2f** gave a highly potent antagonist (ET_A = 0.78 nM) and improved selectivity by nearly a factor of 3 (A/B = 15 300)

Table 1. SAR of Pyrrolidine-C-2 Aryl Modifications: Radioligand Binding



		IC_{50}(nM)[a]		
Compd	R	rET_A binding	pET_B binding	A/B ratio[d]
1 (racemic)		0.36 (±0.04)[b]	515 (±52)[b]	1,400
1a		0.12 (0.11-0.14)	2140 (1940-2360)	17,800
1b		0.17 (0.074-0.4)	1300 (875-1930)	7,650
1c		1.06 (0.81-2.36)	1130 (1110-1150)	3,100
1d		3.28 (2.5-4.1)	14300 (13800-14800)	4,350
1e		0.11 (0.09-0.14)	300 (160-610)	2,700
1f		0.07 (0.003-0.17, n= 3)	30 (16-46, n=3)	430
1g		1.38 (0.81-2.36)	1040 (850-1270)	750

[a] IC_{50} calculated using a mean of two measurements for 11 concentrations from 10^{-10} to 10^{-5} M unless otherwise noted. [b] Standard error of the mean. [d] Expressed as $IC_{50}(ET_A)/IC_{50}(ET_B)$.

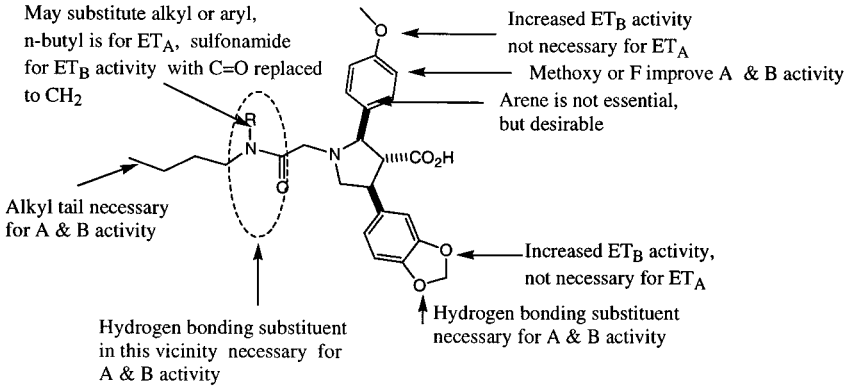
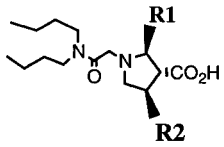


Figure 2.

compared to **2a**. Adding a fluorine to 3-aryl of **2f** provides compound **8**, a highly potent ET-A antagonist (ET_A = 0.18 nM) which displays increased selectivity over the ET_B receptor (A/B = 24 400). Our summary of the functionality responsible for ET_A versus ET_B selectivity is shown in Figure 2.

Table 2. SAR of Non-Benzodioxole Analogues: Radioligand Binding



			IC_{50}(nM)			
Compd	R1	R2	ET_A binding	ET_B binding	(n)	A/B ratio
2a			0.6 (0.12-2.60)[b]	2670 (1380-5400)[c]	(4)	4,450
2b			1.20 (0.79-1.75)[b]	960 (593-1500)[c]	(3)	800
2c			1.50 (0.94-2.1)[b]	8000 (7250-11000)[c]	(2)	5,300
2d			0.48 (0.27-1.28)[b]	5080 (2490-8090)[c]	(3)	10,580
2e			0.85 (0.81-0.9)[b]	984 (860-1100)[c]	(2)	1,160
2f			0.78 (0.57-1.1)[b]	12000 (1110-1290)[c]	(2)	15,300
2g			0.92 (0.8-1.05)[b]	8130 (8130-8140)[c]	(2)	9,030
2h			5.2 (4.3-6.3)[b]	7700 (5940-9970)[c]	(2)	1,480
8			0.18(0.086-0.25)[a]	4400 (2500-5200)[a]	(2)	24,400
[**8a**]			[0.16(0.06-0.2)][a]	[6200(2900-6200)][a]	(2)	[38.700]
9			0.76[a]	3050[a]	(1)*	4,000
9a			0.87[a]	950[a]	(1)*	1,090

[a] Binding to human endothelin receptors in CHO cells. [b] Binding to MMQ cells, *r*ET_A(rat). [c] Expressed as *p*ET_B(porcine). *n*: Number of determinations in parentheses. *The variation of IC_{50}s from experiment to experiment is normally within 15%.

Table 3. Pharmacokinetic Profiles of the Leading Compounds

	pharmacokinetic profiles (rats)[a]					
	log *D* (calc)	$T_{1/2}$ iv (h)	$T_{1/2}$ oral (h)	C_{max} (μg/mL)	AUC (μg h/mL)	*F* (%)
1	3.26	3.5 (± 1.93)[b]	6.2 (± 3.62)[b]	3.44 (± 1.77)[b]	7.36 (± 1.72)[b]	60.2 (± 14.0)[b]
2	5.21	1.6 (± 0.86)[b]	2.5 (± 0.67)[b]	1.69 ± 0.41[c]	3.27 ± 0.45[c]	47.7 ± 6.56[c]
8a	4.32	2.1 (± 1.03)[b]	4.7 (± 2.10)[b]	1.84 ± 0.20[c]	12.5 ± 2.2[c]	71.7 ± 12.5[c]
8 (racemic)	4.32	4.7 (± 3.13)[b]	5.8 (± 2.13)[b]	0.93 ± 0.24[c]	9.4 ± 1.3[c]	65.6 ± 9.0[c]

[a] $T_{1/2}$ iv, half-life after intravenous dosing (5 mg/kg). $T_{1/2}$ oral, C_{max}, AUC, and *F* are half-life, maximum drug concentration, total drug exposure (area under the curve), and oral bioavailability after oral dosing (10 mg/kg) in three rats. [b] Standard deviation. [c] Standard error of the mean.

Compound Evaluations

After synthesizing a series of very potent and highly selective highly selective ET_A antagonists with varying hydrophobicity and hydrophilicity, a small set of compounds were prepared in enantiomerically pure form to evaluate the impact of this polarity swing on the pharmacokinetic profiles of the compounds. These enantiomers were first evaluated in our standard screening assay, which employed a rodent ET_A receptor derived from MMQ cells ($r$$ET_A$) and ET_B receptor ($p$$ET_B$) derived from porcine cerebellar tissue. IC_{50} data are recorded by measuring the displacement of [^{125}I]ET-1 from ET_A, or of [^{125}I]ET-3 from ET_B, as well as in a second set of binding assays employing human ET_A and ET_B receptors expressed in CHO cells. Pharmacokinetic properties were examined for the enantiomers of three leading ET_A-selective antagonists using a standard protocol which compares the time course of plasma drug levels after dosing in rats by intraveno injection and oral gavage (Table 3).

Results and Discussion

Structure−activity studies reveal that the compound **8a** is very potent and highly selective for ET_A vs ET_B receptors, with a half-life of ~5 h, AUC of 12.5 μg h/mL, and oral bioavailability of >70% in rats. We note that the half-life of the compound **8a** is similar to that of **1**. This suggests that the *o*-demethylation is not a rate-limiting metabolic event. However, replacement of the 4-OCH_3 group with 4-OH is well tolerated and has a little less binding affinity than **1**. Our clinical studies of **1** (Atrasentan) indicate a long half-life (28−32 h) in humans, suggesting that these second-generation agents should also have desirable profiles.

Conclusions

We have developed highly selective and very potent ET_A receptor antagonists to meet our back-up goal, based on the pyrrolidine-3-carboxylic acid template. Placement of a *p*-tolyl group at the 2-position and a 5-substituted dihydrobenzofuran at the 4-position of the pyrrolidine was required for this high level of selectivity for ET_A. Introducing a fluorine at 3-aryl further increases the binding affinity and improves the bioavailablity. In addition, these analogues no longer contain a benzodioxole moiety, eliminating any potential for inhibition cytochrome P450-enzymes (CYP 3A4). This type of metabolism may lead to nonlinear pharmacokinetics. The optimized analogue, A-306552 (**8a**), is a promising compound to meet our back-up goal as a ET_A-selective antagonist.

Experimental Section

General. Compounds were prepared in a previous fashion analogous to substituting the appropriate aldehyde.[11] Compounds **1a**−**1d**[15b] and **2a**−**2e**[11] were reported. Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. All reactions were performed under nitrogen atmosphere unless specifically noted. Flash column chromatography was done using silica gel (230−400 mesh) from E. M. Science. ^{1}H NMR spectra were recorded at 300 MHz; all values are referenced to tetramethylsilane as internal standard and are reported as shift (multiplicity, coupling constants, proton count). Mass spectral analysis is accomplished using fast atom bombardment (FAB-MS) or direct chemical ionization (DCI-MS) techniques. All elemental analyses are consistent with theoretical values to within ±0.4% unless indicated. Melting points were measured on a Thomas-Hoover apparatus and are uncorrected.

Abbreviations: DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; EtOAc, ethyl acetate; TFA, trifluoroacetic acid.

Ethyl 3-(3-Fluoro-4-methylphenyl)-3-oxo-propionate (3a). A solution: Ethyl malonate potassium salt (3.27 g, 0.0219 mol) and $MgCl_2$ (1.57 g, 0.0165 mol) was mixed in 32 mL of THF stirred and heated at 65 °C for 4 h. B solution: To the solution of 3-fluoro-4-methylbenzoic acid (2.5 g, 0.0165 mol) in 18 mL of THF was added 1,1′-carbonyldiimidazole (3.15 g, 0.0194 mol) in portion and heated for 30 min. The B solution added to A. The mixture was stirred overnight at room temperature to allow a white solid to suspend. The flask was cooled in an ice bath, and a dilute HCl solution (5 mL of concentrated HCl and 10 mL of H_2O) was added. EtOAc was added to extract the product, and the organic layer was washed with H_2O and brine and dried over Na_2SO_4. Yield: 3.45 g (95%). This crude product was used for the next step without further purification.

Ethyl 2-(3-Fluoro-4-methylbenzoyl)-4-nitro-3-(2,3-dihydrobenzofuran-5-yl)butyrate (5a). To a stirred solution of the nitrostyrene (2.94 g, 0.0154 mol) in 26 mL of THF were added the ketoester (3.45 g, 0.0154 mol) of **3a** and 30 mg of potassium *tert*-butoxide. The resulting mixture was warmed to dissolve the nitro compound, and stirring was continued for 3.5 h at room temperature. To the mixture were added 5 drops of concentrated HCl, water, and EtOAc to extract the product. The organic layer was washed with water and brine and dried over Na_2SO_4. This crude product (7.2 g) was used for next step without further purification.

Ethyl *trans,trans*-2-(3-Fluoro-4-methylphenyl)-4-(2,3-dihydrobenzofuran-5-yl)pyrrolidine-3-carboxylate (7). The product of **5a** (7.0 g, 16.87 mmol) in EtOAc (35 mL) solution was added to a 250 mL pressure bottle containing RaNi (16 g) which was washed with THF (4×) and with EtOAc (3×), and the mixture was shaken on a Parr hydrogenator for 4 h at room temperature under 60 psi of H_2. Then 2.6 mL of TFA was added, and the mixture was shaken for 24 h under H_2. The mixture was filtered, and most of the solvent was evaporated. Saturated $NaHCO_3$ solution was added and extracted with EtOAc (200 × 2), and the organic layers were combined, washed with H_2O and brine, and dried over Na_2SO_4 to give mostly *cis,cis*-isomer. This crude *cis,cis*-isomer (6.0 g, 16.26 mmol) was dissolved in toluene (50 mL), and 5.25 g of DBU was added. The mixture was refluxed for 12 h for epimerization. Solvent was evaporated and purified by silica column chromatography to elute with hexane and EtOAc (3:2) to give 4.2 g of *trans,trans*-isomer.

***trans,trans*-2-(3-Fluoro-4-methylphenyl)-4-(2,3-dihydrobenzofuran-5-yl)-1-(*N,N*-dibutylaminocarbonylme-**

thyl)-pyrrolidine-3-carboxylic Acid (8). This final compound was prepared by hydrolysis of the product from a reaction of **7** and *N*,*N*-di-*n*-butyl bromoacetamide with diisopropylethylamine in CH_3CN:[10a] white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 0.82 (t, J = 7.5 Hz, 3H), 0.88 (t, J = 7.5 Hz, 3H), 1.09 (sextet, J = 7.5 Hz, 2H), 1.20−1.35 (m, 3H), 1.37−1.52 (m, 3H), 2.25 (s, 3H), 2.86−2.96 (m, 2H), 3.03−3.12 (m, 2H), 3.20 (t, J = 9 Hz, 3H), 3.32−3.50 (m, 4H), 3.62 (sextet, J = 4.5 Hz, 1H), 3.83 (d, J = 9 Hz, 1H), 4.53 (t, J = 9 Hz, 2H), 6.67 (d, J = 7.5 Hz, 1H), 7.08−7.15 (m, 3H), 7.21 (t, J = 7.5 Hz, 1H), 7.30 (s, 1H); MS (ESI) *m/e* 511 $(M + H)^+$. Anal. ($C_{30}H_{39}N_2FO_4 \cdot 0.25H_2O$) C, H, N.

Resolution of Racemic Compound 7a. To the racemic compound (3.69 g, 10 mmol), dissolved in 25 mL of dichloromethane and cooled in an ice bath, was added di-*tertert*-butyl dicarbonate (2.40 g, 11 mmol). After being stirred for 2 h at room temperature, the solution was concentrated in vacuo; the residue was dissolved in ethanol (20 mL) and treated with a solution of 400 mg of NaOH in 5 mL of water. The solution was stirred for 2 h at room temperature, concentrated, and redissolved in 40 mL of water. The resultant mixture was extracted with 25 mL of diethyl ether; the ether layer was extracted with 10 mL of water. The combined aqueous phases were acidified with acetic acid; the mixture was stirred until a solid formed. The solid was filtered, washed with water, and dried in vacuo. The product was recrystallized from 1:1 ether−hexane to get 3.97 g (90% yield). The crude acid (1.76 g, 3.99 mmol) was dissolved in 60 mL of dry ether and treated with 484 mg (3.99 mmol) of (*R*)-(+)-α-methylbenzylamine. The solution was kept in a freezer overnight. A white crystal was collected and washed with ether to give 1.55 g (69%), mp 163−165 °C. Mother liquid was left at room temperature to give more crystal (590 mg, 26%), mp 125−126 °C. The first crop was recrystallized from EtOAc. After one recrystallization, chiral HPLC analysis, using a Regis Whelk-O column, indicated >98.0% ee. All mother liquids were combined, and the amine was washed out, treated with 0.5 equiv of (*R*)-(+)-α-methylbenzylamine, crystallized from EtOAc (89% ee), and recrystallized from CH_3CN (99% ee).

***trans,trans*-2-(3,4-Dimethoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*,*N*-dibutylaminocarbonylmethyl)-pyrrolidine-3-carboxylic acid (1e):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (t, J = 7 Hz, 3H), 0.88 (t, J = 7 Hz, 3H), 1.05 (q, J = 7 Hz, 2H), 1.26 (m, 4H), 1.44 (q, J = 7 Hz, 2H), 2.83 (d, J = 13.5 Hz, 1H), 2.98 (m, 3H), 3.12(t, J = 9 Hz, 1H), 3.25 (m, 1H), 3.45 (m, 3H), 3.62 (m, 1H), 3.82 (s, 3H), 3.86 (s, 4H), 5.94 (s, 2H), 6.73 (d, J = 7.5 Hz, 1H), 6.80 (d, J = 7.5 Hz, 1H), 6.92 (m, 3H), 7.06 (s, 1H); MS (DCI/NH_3) $(M + H)^+$ at *m/z* 541. Anal. ($C_{30}H_{40}N_2O_7 \cdot 0.5H_2O$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*,*N*-dibutylaminocarbonylmethyl)-pyrrolidine-3-carboxylic acid (1f):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7 Hz, 3H), 0.88 (t, J = 7 Hz, 3H), 1.10 (q, J = 7 Hz, 2H), 1.25 (m, 3H), 1.43 (m, 3H), 2.82 (d, J = 13.5 Hz, 1H), 2.93 (dd, J = 7.5 Hz, 1H), 3.06 (m, 3H), 3.28 (m, 1H), 3.40 (d, J = 13.5 Hz, 1H), 3.94 (m, 2H), 3.61 (m, 1H), 3.82 (d, J = 7.5 Hz, 1H), 3.88 (s, 3H), 5.93 (s, 2H), 6.72 (d, J = 8 Hz, 1H), 6.88 (m, 2H), 7.00 (s, 1H), 7.12 (m, 2H); MS (DCI/NH_3) $(M + H)^+$ at *m/z* 529. Anal. ($C_{29}H_{37}N_2FO_6$) C, H, N.

***trans,trans*-2-(3,4-Difluorophenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*,*N*-dibutylaminocarbonylmethyl)-pyrrolidine-3-carboxylic acid (1g):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7 Hz, 3H), 0.90 (t, J = 7 Hz, 3H), 1.15 (q, J = 7 Hz, 2H), (m, 3H), 1.44 (m, 3H), 2.90 (m, 2H), 3.03−3.18 (m, 4H), 3.42 (m, 3H), 3.63 (m, 1H), 4.96 (d, J = 9 Hz, 1H), 5.93(s, 2H), 6.75 (d, J = 7.5 Hz, 1H), 6.87 (d, J = 7.5 Hz, 1H), 6.98 (s, 1H), 7.12 (m, 2H), 7.26 (m, 1H); MS (APCI) $(M + H)^+$ at *m/z* 517. Anal. ($C_{28}H_{34}N_2F_2O_5$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-ethylphenyl)-4-(2,3-dihydrobenzofuran-5-yl)-1-(*N*,*N*-dibutylaminocarbonylmethyl)-pyrrolidine-3-carboxylic acid (9):** white solid; ^{1}H NMR (300 MHz,CD_3OD) δ 0.82 (t, J = 7.5 Hz, 3H), 0.88 (t, J = 7.5 Hz, 3H), 1.07 (sextet, J = 7.5 Hz, 2H), 1.20 (t, J = 7.5 Hz, 3H), 1.28 (m, 3H), 1.45 (m, 3H), 2.65 (q, J = 7.5 Hz, 2H), 2.92 (m, 2H), 3.08 (m, 2H), 3.20 (t, J = 9 Hz, 2H), 3.32−3.49 (m, 5H), 3.60 (m, 1H), 3.83 (d, J = 9 Hz, 1H), 4.53 (t, J = 9 Hz, 2H), 6.67 (d, J = 9 Hz, 1H), 7.13 (m, 3H), 7.25 (t, J = 7.5 Hz, 1H), 7.32 (s, 1H). MS (ESI) *m/z* 525 $(M + H)^+$. Anal. ($C_{31}H_{41}N_2FO_4$) C, H, N.

***trans,trans*-2-(3-Fluoro-4-ethylphenyl)-4-(1,4-bezodioxan-6-yl)-1-(*N*,*N*-dibutylaminocarbonylmethyl)-pyrrolidine-3-carboxylic acid (9a):** white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 0.82 (t, J = 7.5 Hz, 3H), 0.88 (t, J = 7.5 Hz, 3H), 1.07 (sextet, J = 7.5 Hz, 2H), 1.20 (t, J = 7.5 Hz, 3H), 1.28 (m, 3H), 1.45 (m, 3H), 2.65 (q, J = 7.5 Hz, 2H), 2.83 (d, J = 12 Hz, 1H), 2.92 (dd, J = 9 Hz, 9 Hz, 1H), 3.06 (m, 3H), 3.34 (m, 1H), 3.49 (m, 4H), 3.77 (d, J = 9 Hz, 1H), 4.22 (s, 4H), 6.77 (d, J = 7.5 Hz, 1H), 6.85 (dd, J = 7.5 Hz, 2 Hz, 1H), 6.96 (d, J = 2 Hz, 1H), 7.11 (m, 2H), 7.34 (t, J = 7.5 Hz, 1H). MS (ESI) *m/z* 541 $(M + H)^+$. Anal. ($C_{31}H_{41}N_2FO_5$) C, H, N.

***trans,trans*-2-(4-Methylphenyl)-4-(2,3-dihydrobenzofuran-5-yl)-1-(*N*,*N*-dibutylaminocarbonylmethyl)-pyrrolidine-3-carboxylic Acid (2f):** white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, J = 9 Hz, 3H), 0.95 (t, J = 9 Hz, 3H), 1.14 (m, 2H), 1.32 (m, 4H), 1.53 (m, 2H), 2.37 (s, 3H), 2.98 (m, 2H), 3.22 (m, 2H), 3.38 (m, 2H), 3.59 (m, 1H), 3.70−4.30 (m, 4H), 4.56 (t, J = 12 Hz, 2H), 5.45 (m, 1H), 6.72 (d, J = 8 Hz, 2H), 7.22 (m, 3H), 7.60 (m, 3H); MS (DCI/NH_3) *m/z* 493 $(M + H)^+$. Anal. ($C_{30}H_{40}N_2O_4 \cdot 1.25TFA$) C, H, N.

***trans,trans*-2-(4-Ethylphenyl)-4-(2,3-dihydrobenzofuran-5-yl)-1-(*N*,*N*-dibutylaminocarbonylmethyl)-pyrrolidine-3-carboxylic acid (2g):** white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (m, 3H), 7.22 (d, J = 8 Hz, 2H), 7.13 (dd, J = 8 and 3 Hz, 1H), 6.72 (d, J = 9 Hz, 1H), 5.28 (d, J = 12 Hz, 1H), 4.55 (t, J = 9 Hz, 2H), 4.15 (d, J = 18 Hz, 1H), 4.03 (m, 2H), 3.75 (m, 2H), 3.40 (m, 2H), 3.20 (t, J = 9 Hz, 2H), 3.15 (m, 1H), 3.10−2.90 (m, 2H), 2.63 (q, J = 9 Hz, 2H), 1.47 (m, 2H), 1.31 (m, 4H), 1.12 (t, J = 8 Hz, 3H), 1.10 (m, 2H), 0.92 (t, J = 9 Hz, 3H), 0.80 (t, J = 9 Hz, 3H); MS (DCI/NH_3) *m/z* 507 $(M + H)^+$. Anal. ($C_{31}H_{42}N_2O_4 \cdot 1TFA$) C, H, N.

***trans,trans*-2-(4-Ethylphenyl)-4-(1,4-benzodioxan-6-yl)-1-(*N*,*N*-dibutylaminocarbonylmethyl)-pyrrolidine-3-carboxylic acid (2h):** white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 0.95 (t, J = 7 Hz, 3H), 1.03 (t, J = 7 Hz, 3H), 1.18 (q, J = 7 Hz, 2H), 1.37 (t, J = 7 Hz, 3H), 1.42 (m, 3H), 1.58 (m, 3H), 2.78 (q, J = 7 Hz, 2H), 3.18 (m, 4H), 3.30 (t, J = 8 Hz, 1H), 3.56 (m, 3H), 3.70 (d, J = 10 Hz, 1H), 3.73 (m, 1H), 4.08 (d, J = 8 Hz, 1H), 4.38 (s, 4H), 6.93 (d, J = 7 Hz, 1H), 7.03 (dd, J = 1.5 and 7 Hz, 1H), 7.13 (d, J = 1.5 Hz, 1H), 7.37 (d, J = 8 Hz, 2H), 7.50 (d, J = 8 Hz, 2H); MS (APCI) at *m/z* 523 $(M + H)^+$. Anal. ($C_{31}H_{42}N_2O_5 \cdot 1.1HOAc$) C, H, N.

References

(1) Yanagisawa, M.; Kurihara, H.; Kimura, S.; Tomobe, Y.; Kobayashi, Y.; Mitsui, Y.; Yazaki, Y.; Goto, K.; Masaki, T. Novel Potent Vasoconstrictor Peptide Produced by Vascular Endothelin Cells. *Nature (London)* **1988**, *332*, 411−415.

(2) (a) Inoue, A.; Yanagisawa, M.; Kimura, S.; Kasuya, Y.; Miyauchi, T.; Goto, K.; Masaki, T. The Human Endothelin Family: Three Structurally and Pharmacologically Distinct Isopeptides Predicted by Three Separate Genes. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2863−2867. (b) Ames, R. S.; Sarau, H. M.; Chambers, J. K.; Willette, R. N.; Aiyar, N. V.; Romanic, A. M.; Lo, C. S. Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. *Nature* **1999**, *401*, 282−286.

(3) (a) Opgenoth, T. J. Endothelin Receptor Antagonists. *Adv. Pharmacol.* **1995**, *33*, 1−65. (b) Levin, E. R. Mechanisms of Disease: Endothelins. *New Engl. J. Med.* **1995**, *333* (6), 356−363.

(4) Arai, H.; Hori, S.; Aramori, I.; Ohkubo, H.; Nakanishi, S. Cloning and Expression of a cDNA Encoding an Endothelin Receptor. *Nature* **1990**, *348*, 730−732.

(5) Warner, T.; Mitchell, J. A.; De Nucci, G.; Vane, J. R. Endothelin-1 and Endothelin-3 Release EDRF from Isolated Perfused Arterial Vessels of the Rat and Rabbit. *J. Cardiovasc. Pharmacol.* **1989**, *13* (Suppl. 5), S85−88.

(6) Moreland, S.; McMullen, D. M.; Delaney, C. L.; Lee, V. G.; Hunt, J. T. Venous Smooth Muscle Contains Vasoconstrictor ET_B-Like Receptors. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 100−106.

(7) Hay, D. W. P.; Luttmann, M. A.; Hubbard, W. C.; Undem, B. J. Endothelin receptor subtypes in human and guinea-pig pulmonary tissues. *Br. J. Pharmacol.* **1993**, *110*, 1175−1183.

(8) (a) Wu-Wong, J. R. Endothelin receptor antagonists: Past, present and future. *Curr. Opin. Cardiovasc. Pulm. Renal Invest. Drugs* **1999**, *1*, 346−351. (b) Sasaki, T.; Noguchi, T.; Komamura, K.; Nishkimi, T.; Yoshikawa, H.; Miyatake, K. Differential Roles of Endothlin-1 in the Development of Secondary Pulmonary Hypertension in Patients with Left Heart Failure with or without Acute Exacerbation. *J. Card. Failure* **1999**, *5*, 38.

(9) Kumagai, Y.; Fukuto, J. M.; Cho, A. K. The Biochemical Disposition of Methylenedioxyphenyl Compounds. *Curr. Med. Chem*. **1994**, *4*, 254−261.

(10) (a) Winn, M.; von Geldern, T. W.; Opgenorth, T. J.; Jae, H.-S.; Tasker, A.; Boyd, S.; Kester, J.; Mantei, R.; Bal, R.; Sorensen, B.; Wu-Wong, J. R.; Choiu, W.; Dixon, D.; Novosad, E.; Hernandez, L.; Marsh, K. C. 2,4-Diarylpyrrolidine-3-carboxylic Acids-Potent ET_A Selective Endothlin Receptor Antagonists. 1. Discovery of A-127722. *J. Med. Chem*. **1996**, *39*, 1039−1048. (b) Jae, H.-S.; Winn, M.; Dixon, D.; Marsh, K.; Nguyen, B.; Opgenorth, T. J.; von Geldern, T. W. Pyrrolidine-3-carboxylic Acids as Endothelin Antagonists. 2. Sulfonamide-Based ET_A/ET_B Mixed Antagonists. *J. Med. Chem*. **1997**, *40*, 3217−3227.

(11) Tasker A.; Sorensen, B.; Jae, H.-S.; Winn, M.; von Geldern, T. W.; Dixon, D.; Chiou, W.; Dayton, B.; Calzadila, S.; Hernandez, L.; Marsh, K.; Wu-Wong, J. R.; Opgenorth, T. J. Potent and Selective Non-Benzodioxole-Containing Endothelin-A Receptor Antagonists. *J. Med. Chem*. **1997**, *40*, 322−330.

(12) Echavarren, A. M.; Stille, J. K. Palladium-Catalyzed Coupling of Aryl Triflates with Organostannanes. *J. Am. Chem. Soc*. **1987**, *109*, 5478−5486.

(13) von Geldern, T. W.; Tasker, A. S.; Sorensen, B. K.; Winn, M.; Szczepankiewicz, B. G.; Dixon, D. B.; Chiou, W. J.; Wang, L.; Wessale, J. L.; Alder, A.; Marsh, K. C.; Nguyen, B.; Opgenorth, T. J. Pyrrolidine-3-carboxylic Acids as Endothelin Antagonists. 4. Side Chain Conformational Restriction Leads to ET_B Selectivity. *J. Med. Chem*. **1999**, *42*, 3668−3678.

(14) Liu, G.; Kozmina, N. S.; Winn, M.; von Geldern, T. W.; Chiou, W. J.; Dixon, D. B.; Nguyen, B.; Marsh, K. C.; Opgenorth, T. J. Design, Synthesis, and Activity of a Series of Pyrrolidine-3-carboxylic Acid-Based, Highly Specific, Orally Active ET_B Antagonists Containing a Diphenylmethylamine Acetamide Side Chain. *J. Med. Chem*. **1999**, *42*, 3679−3689.

(15) (a) Liu, G.; Henry, K. J.; Szczepankiewicz, B. G.; Winn, M.; Kozmina, N. S.; Boyd, S. A.; Wasicak, J.; von Geldern, T. W.; Wu-Wong, J. R.; Choiu, W. J.; Dixon, D. B.; Nguyen, B.; Marsh, K. C.; Opgenorth, T. J. Pyrrolidine-3-carboxylic Acids as Endothelin Antagonists. 3. Discovery of a Potent, 2-Nonaryl, Highly Selective ET_A Antagonist (A-216546). *J. Med. Chem*. **1998**, *41*, 3261−3275. (b) Boyd, S. A.; Mantei, R. A.; Tasker, A. S.; Liu, G.; Sorensen, B. K.; Henry, K. J.; von Geldern, T. W.; Winn, M.; Wu-Wong, J. R.; Chiou, W. J.; Dixon, D. B.; Hutchins, C. W.; Marsh, K. C.; Nguyen, B.; Opgenoth, T. J. Discovery of a Series of Pyrrolidine-based Endothelin Receptor Antagonists with Enhanced ET_A Receptor Selectivity. *Bioorg. Med. Chem*. **1999**, *7*, 991−1002.

(16) Kiowski, W.; Sutsch, G.; Hunziker, P.; Muller, P.; Kim, J.; Oechslin, E.; Schmitt, R.; Jones, R.; Bertel, O. Evidence for Endothelin-1-mediated Vasoconstriction in Severe Chronic Heart Failure. *Lancet* **1995**, *346*, 732−736.

(17) Golfman, L.; Hata, T.; Beamish, R.; Dhall, N. Role of Endothelin in Heart Function and Disease. *Can. J. Cardiol*. **1993**, *9*, 635−653.

(18) Chen, S. J.; Chen, Y. F.; Opgenorth, T. J.; Wessale, J. L.; Meng, Q. C.; Durand, J.; DiCarlo, V. S.; Oparil, S. The Orally Active Nonpeptide Endothelin A-receptor Antagonist A-127722 Prevents and Reverses Hypoxia-induced Pulmonary Hypertension and Pulmonary Vascular Remodeling in Sprague−Dawley Rats. *J. Cardiovasc. Pharmacol*. **1997**, *29*, 713−725.

(19) Pollock, D. M.; Polakowski, J. S.; Wegner, C. D.; Opgenorth, T. J. Beneficial Effect of ETA Receptor Blockade in a Rat Model of Radiocontrast-induced Nephropathy. *Renal Failure* **1977**, *19*, 753−761.

(20) Mattoli, S.; Soloperto, M.; Marini, M.; Fasoli, A. Levels of Endothelin in the Bronchoalveolar Lavage Fluid of Patients with Symptomatic Asthma and Reversible Airflow Obstruction. *J. Allergy Clin. Immunol*. **1991**, *88*, 376−384.

(21) Burke, S. E.; Lubbers, N. L.; Gagne, G. D.; Wessale, J. L.; Dayton, B. D.; Wegner, C. D.; Opgenorth, T. J. Selective Antagonism of the ET(A) Receptor Reduces Neointimal Hyperplasia After Balloon-induced Vascular Injury in Pigs. *J. Cardiovasc. Pharmacol*. **1997**, *30*, 33−41.

(22) (a) Dawas, K.; Loizidou, M.; Shankar, A.; Ali. H.; Taylor, I. Angiogenesis in cancer: the role of endothelin-1. [Review] *Ann. R. Coll. Surg. Engl.* **1999**, *81*, 306−310. (b) Ali, H.; Dashwood, M.; Dawas, K.; Loizidou, M.; Savage, F.; Taylor, I. Endothelin receptor expression in colorectal cancer. *J. Cardiovasc. Pharmacol*. **2000**, *36*, S69−71

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