Pyrrolidine-3-carboxylic Acids as Endothelin Antagonists. 3. Discovery of a Potent, 2-Nonaryl, Highly Selective ET_A Antagonist (A-216546)

Gang Liu,*,† Kenneth J. Henry, Jr.,*,‡,|| Bruce G. Szczepankiewicz, Martin Winn, Natasha S. Kozmina, Steven A. Boyd, James Wasicak,‡ Thomas W. von Geldern, Jinshyun R. Wu-Wong, William J. Chiou, Douglas B. Dixon, Bach Nguyen,§ Kennan C. Marsh,§ and Terry J. Opgenorth

Metabolic Disease Research, Cancer Research, and Drug Analysis Department, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois 60064-3500

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Previously we have reported the discovery of ABT-627 (**1**, A-147627, active enantiomer of A-127722), a 2,4-diaryl substituted pyrrolidine-3-carboxylic acid based endothelin receptor-A antagonist. This compound binds to the ET_A receptor with an affinity (K_i) of 0.034 nM and with a 2000-fold selectivity for the ET_A receptor versus the ET_B receptor. We have expanded our structure−activity studies in this series, in an attempt to further increase the ET_A selectivity. When the *p*-anisyl group of **1** was replaced by an *n*-pentyl group, the resultant antagonist **3** exhibited substantially increased ET_B/ET_A activity ratio, but a decreased ET_A affinity. Structure−activity studies revealed that substitution and geometry of this alkyl group, and substitution on the benzodioxolyl ring, are important in optimizing this series of highly ET_A selective antagonists. In particular, the combination of a (*E*)-2,2-dimethyl-3-pentenyl group and a 7-methoxy-1,3-benzodioxol-5-yl group provided hydrophobic compound **10b** with subnanomolar affinity for human ET_A receptor subtype and with an ET_B/ET_A activity ratio of over 130000. Meanwhile, synthetic efforts en route to olefinic compounds led to the discovery that 2-pyridylethyl (**9o**) and 2-(2-oxopyrrolidinyl)ethyl (**9u**) replacement of the *p*-anisyl group of **1** yielded very hydrophilic ET_A antagonists with potency and selectivity equal to those of **10b**. On the basis of overall superior affinity, high selectivity for the ET_A receptor (K_i, 0.46 nM for ET_A and 13000 nM for ET_B), and good oral bioavailability (48% in rats), A-216546 (**10a**) was selected as a potential clinical backup for **1**.

Introduction

The endothelins (ET-1, ET-2 and ET-3), 21-residue amino acid peptides discovered in 1988,[1,2] are potent, long-acting constrictors of vascular smooth muscle and are also potent mitogens.[3] ET-1, made primarily by endothelial cells, is thought to act as an autocrine/paracrine mediator in the regulation of vascular functions through modulation of tone, cell proliferation, vascular permeability, and hormone production.[4−7]

The biological effects of the ETs are mediated by G-protein-linked receptors. Two subtypes of human endothelin receptors (ET_A and ET_B) have been cloned and are approximately 55% homologous.[8,9] The ET_A receptor has greater affinity for ET-1 than ET-3 and is expressed in vascular smooth muscle cells. Binding of ET-1 to ET_A receptor mediates the vasoconstrictive and mitogenic effects of ET-1 both in vitro and in vivo.[3−7] The ET_B receptor has equal affinity for ET-1 and ET-3 and is expressed in endothelial cells. Binding of ET-1 or ET-3 to the ET_B receptor may attenuate the vasoconstrictive effects of local ET-1 by mediating production of nitric oxide[10] and by clearing ET-1 from the circulation.[11]

Endothelin-1 has been implicated as a contributing factor in many diseases.[3−7] In animal models and human studies, ET receptor antagonists have demonstrated clear benefit in acute myocardial infarction,[12] congestive heart failure,[13] pulmonary hypertension,[14] cerebral vasospasm,[15] renal failure,[16] and restenosis.[17] It is apparent from these studies that ET_A receptors mediate most of the actions of ET-1 associated with these pathological conditions, while the ET_B receptor may mediate some beneficial effects. These findings suggest that a selective ET_A receptor antagonist would be useful as a therapeutic agent for chronic treatment of the aforementioned pathological conditions.

Recently, our laboratories have disclosed the design and synthesis of a novel series of 1,2,4-trisubstituted pyrrolidine-3-carboxylic acid based ET_A receptor selective antagonists, exemplified by ABT-627 (**1**, A-147627, active enantiomer of A-127722).[18] This compound competitively inhibits [^{125}I]ET-1 binding to cloned human ET_A and ET_B receptors with K_i values of 0.034 and 63.3 nM, respectively. In our efforts to search for a follow-up compound possessing even greater selectivity for the ET_A receptor, the contributions to binding affinity or receptor subtype selectivity conferred by the *p*-anisyl group of **1** were investigated. When the *p*-anisyl substituent of A-127722 was changed to a *p*-ethylphenyl group (**2**), a decrease of ET_B affinity was observed with a slight increase of ET_A affinity (Scheme 1). Intrigued by this observation, we experimented with aliphatic groups and quickly discovered that the corresponding *n*-pentyl group (**3**) further improved upon the ET_B/ET_A activity ratio of **2**, though **3** exhibited decreased affinity

† Address correspondence to D-47V, AP-10, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-3500. E-mail: Gang.Liu@abbott.com.

‡ Cancer Research.

|| Current address: Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.

§ Drug Analysis Department.

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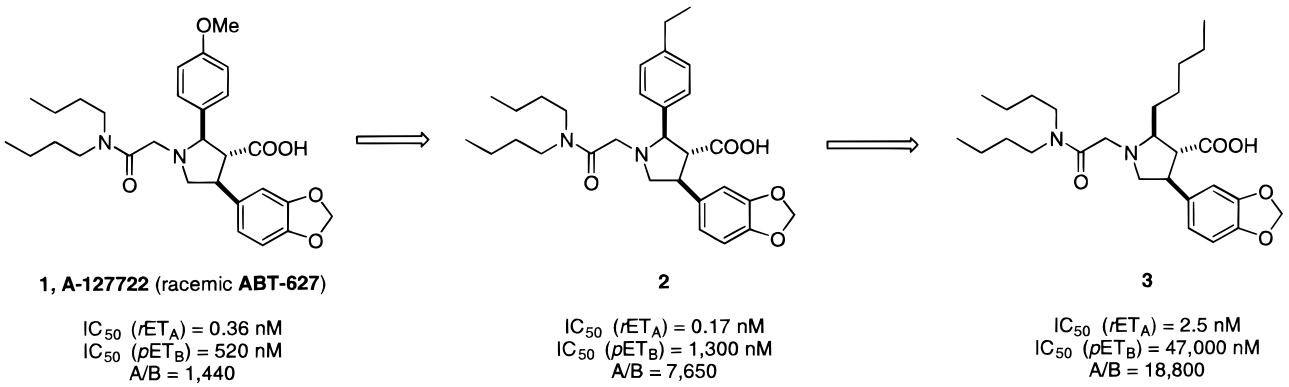
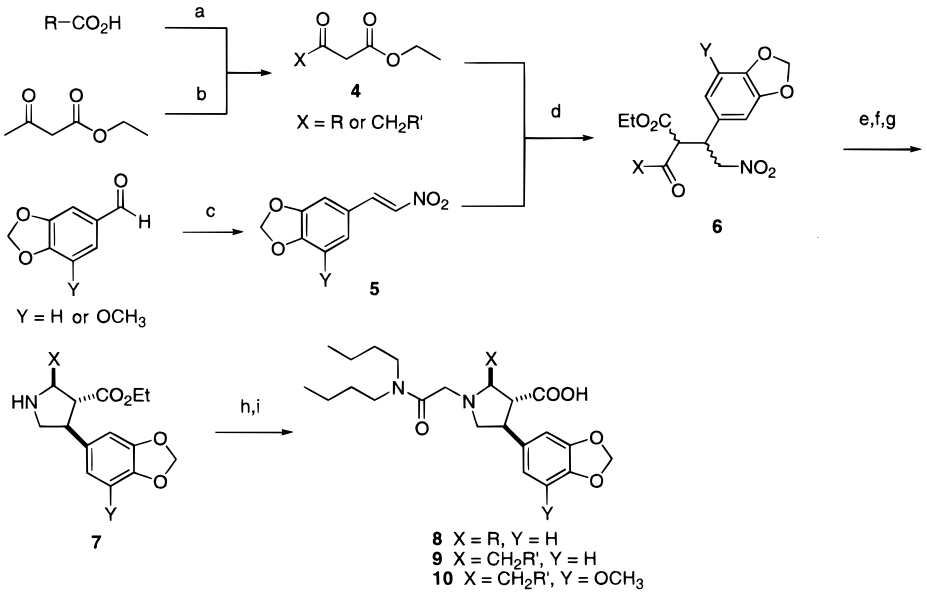
Scheme 1. Rationale for Developing the Alkyl-Based Highly ET_A Selective Antagonists



Scheme 2[a]



[a] (a) CDI, THF, then magnesium monoethylmalonate; (b) NaH, then *n*-BuLi, then R′-Br, THF; (c) $MeNO_2$, NH_4OAc, AcOH−EtOH, reflux; (d) cat. *t*-BuOK, THF; (e) H_2, Raney Ni, EtOAc; (f) $NaBH_3CN$, concentrated HCl, THF−EtOH, pH 5; (g) DBU, CH_3CN, reflux; (h) *n*-Bu_2NCOCH_2Br, *i*-Pr_2NEt, CH_3CN; (i) 6 N aqueous NaOH, EtOH.

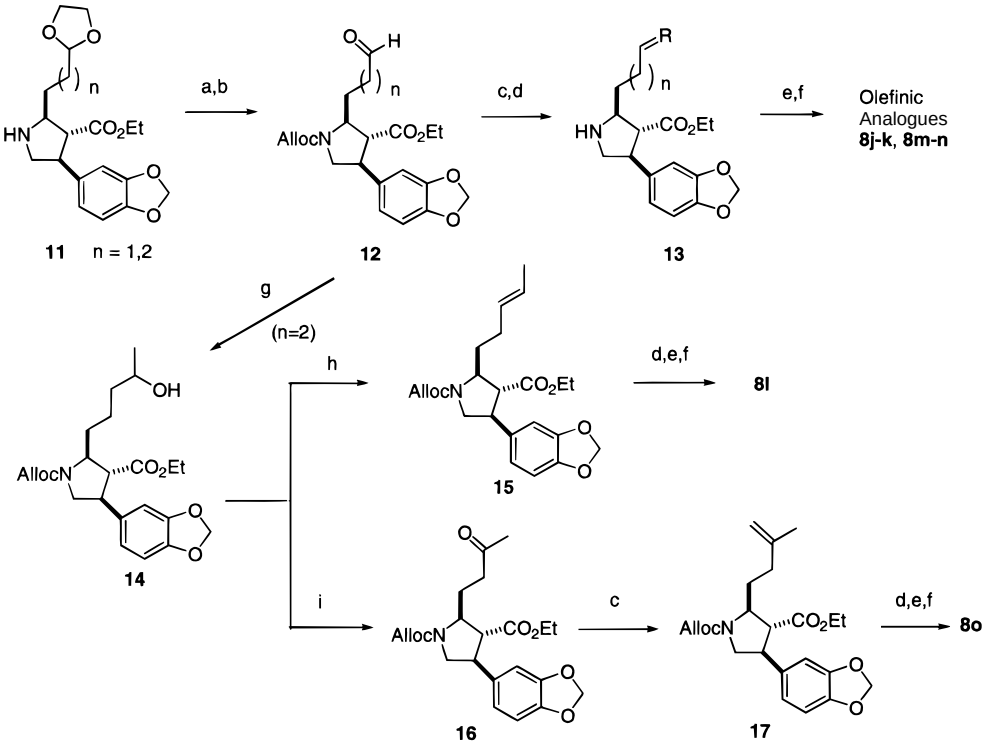
for ET_A receptor.[19] The net result is a 13-fold improvement in ET_A selectivity. The distinctive structure and selectivity feature of **3** served as a valuable lead for further exploration of this 2-alkylpyrrolidine-based, highly selective ET_A receptor antagonists.

Chemistry

Most of the new compounds in this report were synthesized by direct analogy to our earlier work on **1**,[18] as shown in Scheme 2. Various β-ketoesters **4** were prepared according to the published procedures, through either reacting the imidazolide of the carboxylic acid with magnesium monoethylmalonate followed by decarboxylation[20] (ca. 70% yield) or alkylating an acetoacetate dianion with an alkyl bromide[21] (20−70% yield). Condensation of nitromethane with piperonals provided the corresponding β-nitrostyrenes **5**.[22] Potassium *tert*-butoxide catalyzed Michael addition of β-ketoesters **4** to nitrostyrenes **5** yielded two isomers of the adducts **6** in ca. 80% yield. Raney nickel mediated hydrogenation of the adduct **6** and reduction of the resultant cyclic imine with sodium cyanoborohydride provided a mixture of three diastereomeric pyrrolidines in which the desired trans,trans isomer **7** predominated. The cis,cis isomer could be converted to trans,trans isomer by epimerization (DBU, CH_3CN). Chromatographic separation on silica gel afforded the essentially pure trans,trans isomer of the amino ester **7** in ca. 40% yield from the Michael adduct **6**. Alkylation of pyrrolidine **7** with *N*,*N*-dibutylbromoacetamide and subsequent hydrolysis of the ethyl ester furnished final compounds **8**−**10** in 80% yield for in vitro assays.

Compounds not suitable for the Raney nickel hydrogenation conditions (mainly olefin-containing substrates) were synthesized from dioxolanes **11** through a four-step sequence (Scheme 3). Protection of the pyrrolidine nitrogen as an allyl carbamate and hydrolysis of the acetal provided aldehyde **12**. Wittig reaction of the resulting aldehyde **12** and removal of the urethane yielded the corresponding olefin substituted pyrrolidine **13**, which was converted to the final antagonist as described in Scheme 2. Addition of methylmagnesium bromide to the aldehyde **12** and dehydration of the resulting alcohol **14** with Burgess reagent yielded the trans olefin **15**. The alcohol **14** was also oxidized to ketone **16**, which was converted to the terminal olefin **17** with an appropriate Wittig ylide.

Scheme 3[a]



[a] (a) Allyl chloroformate, py, DMAP, CH_2Cl_2; (b) 1 N aqueous HCl, THF, reflux; (c) $Ph_3P{=}R$, THF; (d) $Pd(Ph_3P)_4$, Ph_3P, pyrrolidine, CH_2Cl_2; (e) *n*-Bu_2NCOCH_2Br, *i*-Pr_2NEt, CH_3CN; (f) 6 N aqueous NaOH, EtOH; (g) MeMgBr, THF; (h) $CH_3O_2CNSO_2N(C_2H_5)_3$, CH_3CN, heat; (i) PCC, $MgSO_4$, CH_2Cl_2.

Intermediates **15** and **17** were then converted to the desired antagonists via steps described previously in Scheme 2.

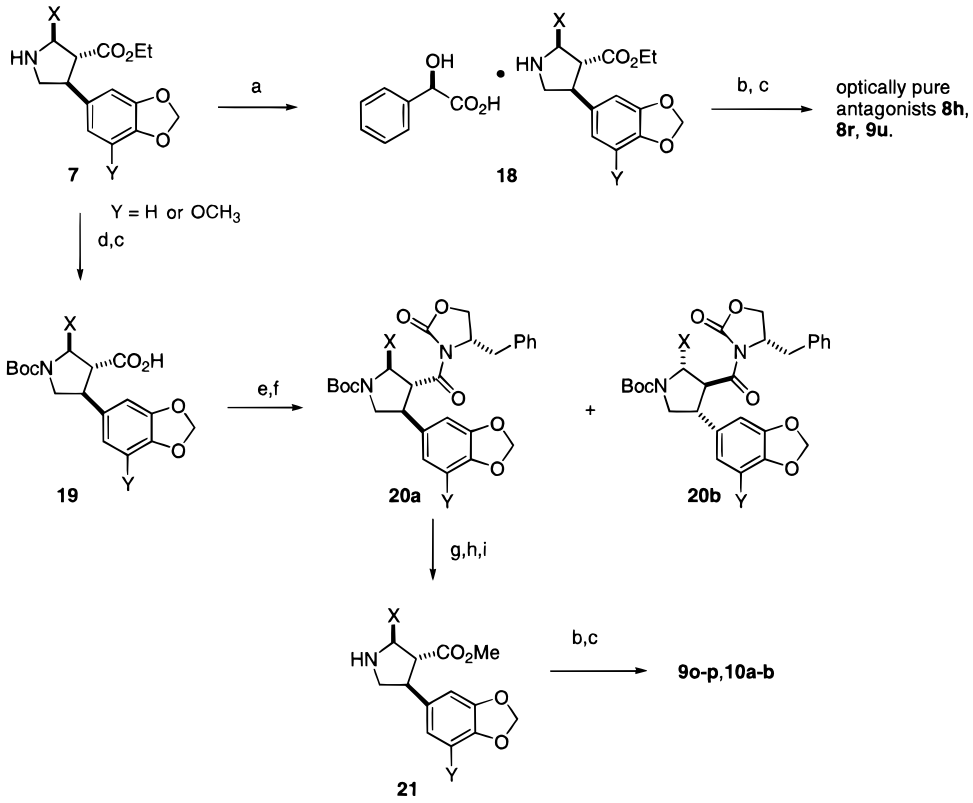
For detailed evaluation, a number of compounds have been prepared in optically pure form. The preparation of final products **8**−**10** as single enantiomers was generally accomplished through resolution of the pyrrolidine **7** prior to N-alkylation (Scheme 4). Certain racemic pyrrolidine esters **7** could be resolved via formation of chiral salts **18** with (*S*)-(+)-mandelic acid. Recrystallization produced material of >90% ee, as evaluated by chiral HPLC. The diastereomerically pure salt **18** was then converted to the optically pure final products as shown in Scheme 2. Alternatively, racemic pyrrolidine **7** could be converted to *N*-Boc carboxylic acid **19** in two steps. Acid **19** was then coupled with (*S*)-(−)-4-benzyl-2-oxazolidinone through a pentafluorophenylester to give diastereomers of **20a** and **20b**, which may be separated by silica flash chromatography. The desired acyl oxazolidinone **20a** was then cleaved with sodium methoxide. Treatment of the resulting *N*-Boc methyl ester with trifluoroacetic acid and neutralizing the salt with aqueous base provided the optically pure pyrrolidine **21**, which was converted to the final compounds.

Structure−Activity Relationships

The initial screening for the compounds described in this study was a measurement of their ability to displace endothelin from its receptors. We employed a rodent ET_A receptor derived from MMQ cells (rET_A); ET_B receptor (pET_B) was derived from porcine cerebellar tissue. IC_{50} data were recorded by measuring the displacement of [^{125}I]ET-1 from the ET_A receptor or [^{125}I]-ET-3 from the ET_B receptor. Confirmatory binding studies were performed in a similar fashion, using human ET_A and ET_B receptor (hET_A, hET_B) permanently expressed in CHO cells.

The decreased ET_B affinity of *n*-pentyl compound **3** suggested that the alkyl group might be finding a new hydrophobic binding site only present on the ET_A receptor or the increased hydrophobicity of **3** was less tolerated at the ET_B receptor than at the ET_A receptor. In a separate study, the length of the linear alkyl groups was investigated.[19] The optimal chain length appears to be five atoms. Further shortening (propyl and butyl) or lengthening (hexyl and heptyl) of the alkyl chain was detrimental to both the ET_A binding affinity and the ET_B/ET_A activity ratio. Only *n*-pentyl compound **3** provided reasonable affinity for ET_A receptor and the largest separation of ET_A and ET_B activity. With the optimal chain length established, the substitution pattern of the aliphatic chain was then explored (Table 1). Only the smallest alkyl substituent, a methyl group, was tolerated at position 4 and 2 of the pentyl chain (**8a** and **8c**), but not at position 3 or 1 (**8b** and **8d**). For the larger substituents, 3-ethylpentyl group (**8e**) was 4 times less active and selective than 2-propylpentyl group (**8f**), and neither improved upon the parent *n*-pentyl compound **3**. Fluorination at the terminal position of the pentyl chain has a slight negative effect on the affinity and selectivity of the antagonist **8g** for the ET_A receptor. Since a methyl group was tolerated at the 2-position of the alkyl chain, a *gem*-dimethyl group at the position 2 was introduced to eliminate the extra stereocenter. This modification (**8h**) slightly boosted the affinity for ET_A receptor and decreased the ET_B receptor

Scheme 4[a]



[a] (a) (*S*)-(+)-mandelic acid, Et_2O−hexanes; (b) *n*-Bu_2NCOCH_2Br, *i*-Pr_2NEt, CH_3CN; (c) 6 N aqueous NaOH, EtOH; (d) Boc_2O, Et_3N, CH_2Cl_2; (e) pentafluorophenol, EDCl, DMF; (f) lithium salt of (*S*)-(−)-4-benzyl-2-oxazolidinone, THF, then silica gel chromatography; (g) NaOMe, MeOH; (h) TFA (neat); (i) aqueous $NaHCO_3$, Et_2O extraction.

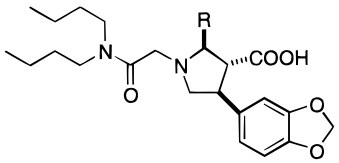
affinity, thus tripling the receptor selectivity observed with antagonist **3**.

Double bonds were then introduced to the different positions of the pentyl chain to increase the rigidity of the aliphatic chain and to better position the alkyl group in the binding site (Table 1). Generally, double bonds between the position 3 and 4 were well tolerated. Compound **8i**, which includes a 4-methyl-3-pentenyl group, was slightly more potent and selective than *n*-pentyl analogue **3**. One carbon extension of the chain (**8j**) increased the binding affinity, but not the receptor selectivity. A more rigid derivative of **8i**, cyclopropylidenepropyl group (**8k**), exhibited a subnanomolar affinity for ET_A, but without much improvement in selectivity. For the unsymmetric olefins, trans geometry seemed to have positive effects on increasing the affinity for ET_A receptor, exemplified by (*E*)-3-pentenyl replacement **8l**. Unfortunately, compromised by the slightly increased ET_B affinity, the subnanomolar ET_A affinity of **8l** only doubled the receptor selectivity over lead compound **3**. On the other hand, transition from trans to cis geometry (**8m**) resulted in over 6-fold loss of ET_A affinity and 3-fold loss of selectivity. Terminal olefins, both monosubstituted **8n** and disubstituted **8o**, have negative effects on the affinity and selectivity comparing to the aliphatic chain. Methyl substitution at the 3-position of trans olefin **8p** was tolerated. Compound **8q**, *gem*-dimethyl substituted **8i** at position 2, seemed to increase the selectivity for ET_A receptor slightly. The optimal antagonist was realized through combining the two structural features of **8h** and **8l**, 2,2-dimethyl substitution and 3,4-trans olefin, to give **8r**, the first antagonist with subnanomolar affinity and over 100000-fold selectivity for the ET_A receptor versus the ET_B receptor.

Because of the incompatibility of Raney nickel hydrogenation conditions with most of the olefinic compounds, 2-dioxolylalkyl substituted pyrrolidines **11** were prepared as the common intermediates for the olefin analogues (see Scheme 3). Surprisingly, alkylation of pyrrolidine **11** ($n = 1$) with the side chain of **1** and subsequent hydrolysis of the ester furnished potent (IC_{50} = 2.3 nM) and highly selective (> 43500×) antagonist **9a** (Table 2). The structural difference between dioxolylethyl and *n*-pentyl group intrigued us most, suggesting that we might have reached an alternative, hydrophilic binding site only present on the ET_A receptor. The structure−activity relationship of this serendipitously discovered lead was then explored.

Initial derivatization of the 1,3-dioxolane ring offered disappointing results. Methyl substituents on the dioxolane ring (**9b**) were not tolerated. The 1,3-dioxane **9c** was less potent and selective than the 1,3-dioxolane **9a**. Compared to propylene linker (**9d**), ethylene appeared to be the optimal link between the heterocycles. Thus this chain length was kept constant for the ensuing analogues. Interestingly, transition from 1,3-dioxolane to 1,3-dithioxolane ring structure (**9e**) resulted in a 2-fold loss of the potency and over 6-fold decrease in selectivity for the ET_A receptor, which strongly implicates hydrogen bonding as a factor contributing to the high selectivity. In contrast to the case of pentenyl compound **8r**, the introduction of a *gem*-dimethyl group at the α-carbon of the dioxolane (**9f**) only marginally

Table 1. SAR of 2-Acyclic Analogues: Radioligand Binding



		IC_{50} (nM)[a]			
compd	R	rET$_A$ binding mean (range)	pET$_B$ binding mean (range)	B/A ratio[b]	formula
3	$C_4H_9CH_2$	2.5 (3.3−1.8)	47000 (85000−26000)	18800	$C_{27}H_{42}N_2O_5 \cdot 0.5H_2O$
8a	$(CH_3)_2CHC_2H_4CH_2$	2.5 (5.5−1.1)	35800 (38500−33000)	14300	$C_{28}H_{44}N_2O_5 \cdot 0.65TFA$
8b	$C_2H_5CH(CH_3)CH_2CH_2$	16 (31−8.1)	80000 (80000)	5000	$C_{28}H_{44}N_2O_5 \cdot 0.60TFA \cdot 0.25Et_2O$
8c	$C_3H_7CH(CH_3)CH_2$	3.0 (3.3−2.8)	71000 (73300−68500)	23670	$C_{28}H_{44}N_2O_5 \cdot 1.0TFA \cdot 0.5H_2O$
8d	$C_4H_9C(CH_3)_2$	1300 (1800−950)	36000 (45800−28900)	28	$C_{29}H_{46}N_2O_5 \cdot 1.60HCl$
8e	$(C_2H_5)_2CHCH_2CH_2$	22 (*88−9.5*)	72000 (*83950−55270*)	3270	$C_{29}H_{46}N_2O_5 \cdot 0.3TFA$
8f	$(C_3H_7)_2CHCH_2$	6.0 (28−2.3)	95000 (95000)	15800	$C_{30}H_{48}N_2O_5 \cdot 0.35TFA$
8g	$F_3CC_3H_7CH_2$	4.0 (4.2−3.8)	34000 (48300−24400)	8500	$C_{27}H_{39}N_2O_5F_3 \cdot 1.05TFA$
8h	$C_3H_7C(CH_3)_2CH_2$	1.3 (1.3−1.2)	62000 (72000−54000)	47690	$C_{29}H_{46}N_2O_5 \cdot 1.05TFA$
8i	$(CH_3)_2C{=}CHCH_2CH_2$	1.3 (1.6−1.2)	33200 (38600−28500)	25500	$C_{28}H_{42}N_2O_5 \cdot 0.05H_2O \cdot 1.15TFA$
8j	$(C_2H_5)(CH_3)C{=}CHCH_2CH_2$	0.86 (1.5−0.4)	7000 (15800−3900)	8100	$C_{29}H_{44}N_2O_5 \cdot 3.80TFA$
8k	$(CH_2)_2C{=}CHCH_2CH_2$	0.64 (0.83−0.49)	10000 (12500−8070)	15600	$C_{28}H_{40}N_2O_5 \cdot 1.80TFA$
8l	(*E*)-$CH_3CH{=}CHCH_2CH_2$	0.78 (0.97−0.70)	26200 (52300−19000)	33600	$C_{27}H_{40}N_2O_5 \cdot 0.85TFA$
8m	(*Z*)-$CH_3CH{=}CHCH_2CH_2$	4.8 (10.9−2.3)	67000 (79900−56700)	14000	$C_{27}H_{40}N_2O_5 \cdot 1.30TFA$
8n	$CH_2{=}CC_2H_4CH_2$	9.9 (39−4.3)	60000 (73200−51600)	6060	$C_{27}H_{40}N_2O_5 \cdot 1.10TFA \cdot 1.05AcOH$
8o	$CH_2{=}C(CH_3)C_2H_4CH_2$	5.2 (5.7−4.8)	36000 (36000)	6900	$C_{28}H_{42}N_2O_5 \cdot 1.30TFA$
8p	(*E*)-$CH_3CH{=}C(CH_3)CH_2CH_2$	3.3 (6.8−1.7)	48000 (48000)	14500	$C_{28}H_{42}N_2O_5 \cdot 0.70TFA$
8q	$(CH_3)_2C{=}CHC(CH_3)_2CH_2$	2.7 (3.2−2.3)	64000 (64000)	23700	$C_{30}H_{46}N_2O_5 \cdot 1.05TFA$
8r (2*S*,3*R*,4*S*)	(*E*)-$CH_3CH{=}CHC(CH_3)_2CH_2$	0.78 (1.1−0.57)	>100000	>128000	$C_{29}H_{44}N_2O_5 \cdot 0.70TFA$

[a] IC_{50} calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} unless otherwise noted. [b] Expressed as $IC_{50}(ET_B)/IC_{50}(ET_A)$.

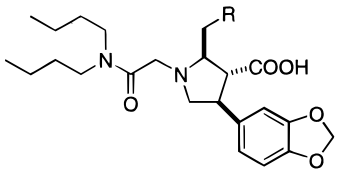
augmented the affinity and the selectivity. This result is consistent with our hypothesis that the hydrophobic and hydrophilic substituents bind to different sites of the ET_A receptor.

A pharmacokinetic study of the compound **9a** (in rats) indicated some hydrolysis of the dioxolane to the aldehyde in vivo. To eliminate the acid labile dioxolane moiety, heterocycles mimicking the dioxolane ring structure were sought. By replacing one of the oxygens of the dioxolane ring with carbon, furan and pyran derivatives were synthesized. Among this group, the best compound in terms of affinity and specificity for the ET_A receptor was the 2-tetrahydropyranyl compound **9g**, with slightly improved ET_A affinity and decreased receptor selectivity. Moving the oxygen to the position 4 of the ring resulted in antagonist **9h** with much lower affinity for the ET_A receptor. For the five-membered ring system, 2-furan **9i** was equally active and selective as 3-furan **9j**. The fully reduced 2-tetrahydrofuran **9k** was 6 times less active than the tetrahydropyan **9g**.

Dioxolane replacements oxazoles and pyrazole were also examined. Oxazole **9l** showed a decreased ET_B affinity while maintaining the ET_A affinity, which led to an antagonist with over 41700-fold selectivity for the ET_A receptor. Despite an overall increase in potency, dimethyl substituted oxazole **9m** showed a greater increase of ET_B affinity than ET_A affinity. Compound **9n**, a N-methylated pyrrole, did not offer any improvement over the 1,3-dioxolane. A more dramatic effect was seen in 2-pyridyl compound **9o**, which has subnanomolar affinity and almost 40000-fold selectivity for the ET_A receptor over the ET_B receptor.

On the basis of the active pyridine compound **9o**, a strategy of heteroatom frame shift was conceived. The ethylene linker was moved to the nitrogen of the heterocycle and the original connecting carbon center was changed to nitrogen or a carbonyl group. Compound **9p**, a pyrazole replacement, offered subnanomolar affinity and improved selectivity compared to 1,3-dioxolane **9a**. Succinimide **9q** was equally potent and

Table 2. SAR of 2-Heterocyclic Analogues: Radioligand Binding



		IC_{50} (nM)[a]			
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3	$C_3H_7CH_2$	2.5 (3.3−1.8)	47000 (85000−26000)	19000	$C_{27}H_{42}N_2O_5\cdot0.5H_2O$
9a	(1,3-dioxol-2-yl)CH_2	2.3 (3.1−1.7)	>100000	>43500	$C_{27}H_{40}N_2O_7\cdot1.2$TFA
9b	(4,5-dimethyl-1,3-dioxol-2-yl)CH_2	13 (28−5.9)	48000 (77500−29800)	3700	$C_{29}H_{44}N_2O_7\cdot1.35$TFA
9c	(1,3-dioxan-2-yl)CH_2	3.3 (7.6−1.2)	62000 (62000)	18800	$C_{28}H_{42}N_2O_7\cdot1.50$TFA
9d	(1,3-dioxol-2-yl)CH_2CH_2	7.0 (25−3.0)	50000 (66000−37900)	7140	$C_{28}H_{42}N_2O_7\cdot1.50$TFA
9e	(1,3-dithia)CH_2	4.5 (6.8−3.0)	29300 (33400−25700)	6500	$C_{27}H_{40}N_2O_5S_2\cdot1.15$TFA
9f	(1,3-dioxol-2-yl)$C(CH_3)_2$	1.4 (3.0−0.65)	>100000	>71400	$C_{29}H_{44}N_2O_7\cdot1.1TFA\cdot0.2H_2O$
9g	(2-tetrahydropyranyl)CH_2	1.8 (2.0−1.6)	47800 (60000−38000)	26600	$C_{29}H_{44}N_2O_6\cdot1.4$TFA
9h	(4-tetrahydropyranyl)CH_2	10 (17.6−5.9)	>100000	>10000	$C_{29}H_{44}N_2O_6$
9i	(2-furfuryl)CH_2	2.7 (2.8−2.7)	47000 (86000−18000)	17400	$C_{28}H_{38}N_2O_6\cdot0.8TFA\cdot0.15H_2O$
9j	(3-furfuryl)CH_2	2.2 (5.1−0.94)	26000 (69400−9980)	11800	$C_{28}H_{38}N_2O_6\cdot0.2$TFA
9k	(2-tetrahydrofuryl)CH_2	12 (35−2.6)	>100000	>9090	$C_{28}H_{42}N_2O_6\cdot0.45$TFA
9l	(1,3-oxazol-2-yl)CH_2	2.4 (2.7−2.1)	>100000	>41700	$C_{27}H_{37}N_3O_6\cdot1.70$TFA
9m	(4,5-dimethyl-1,3-oxazol-2-yl)CH_2	1.05 (1.11−0.99)	29700 (33000−26500)	28300	$C_{29}H_{41}N_3O_6\cdot1.05$TFA
9n	(*N*-methylpyrrol-2-yl)CH_2	2.5 (3.7−1.9)	43600 (77800−24400)	17400	$C_{29}H_{41}N_3O_5\cdot1.25$TFA
9o	(2-pyridyl)CH_2	0.38 (0.39−0.37)	14100 (16200−12200)	37000	$C_{29}H_{39}N_3O_5\cdot1.75$TFA
9p	(1-pyrazolyl)CH_2	0.51 (0.90−0.29)	28600 (28900−28200)	56000	$C_{27}H_{38}N_4O_5\cdot0.75$TFA
9q	(*N*-succinimido)CH_2	5.0 (7.1−3.5)	>100000	>20000	$C_{28}H_{39}N_3O_7\cdot1.05$TFA
9r	(*N*-propylsultamyl)CH_2	41 (71−25)	>100000	>2440	$C_{27}H_{41}N_3O_7S_1\cdot0.60$TFA
9s	(2-oxopiperidin-1-yl)CH_2	2.0 (3.1−1.3)	85000 (85000)	42500	$C_{29}H_{43}N_3O_6\cdot0.70$TFA
9t	(2-oxopyrid-1-yl)CH_2	2.2 (3.0−1.6)	41000 (41000)	18600	$C_{29}H_{39}N_3O_6\cdot0.70$TFA
9u	(2-oxopyrrolidin-1-yl)CH_2	0.42 (1.1−0.16)	65400 (65400)	156000	$C_{28}H_{41}N_3O_6\cdot1.4$TFA

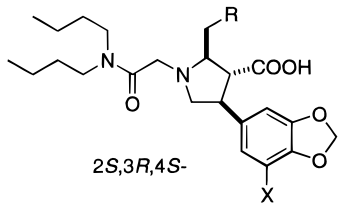
[a] IC_{50} calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} unless otherwise noted. [b] Expressed as $IC_{50}(ET_B)/IC_{50}(ET_A)$.

as selective as dioxolane **9a**, while sultam **9r** yielded a much weaker and less selective antagonist. Piperidinone **9s** was equally potent as pyridone **9t**, but twice as selective. The most potent and selective dioxolane mimic was five-membered 2-pyrrolidinone **9u**, which binds 10 times better than dioxolane **9a** to the ET_A receptor without much increase in the ET_B affinity. In general, any five- or six-membered heterocycles with hydrogen-bonding acceptors at the 2-position seem to be well tolerated without impairing the ET_A receptor selectivity. These efforts led to the optimal antagonist **9u** with subnanomolar affinity for the ET_A receptor and an ET_B/ET_A activity ratio of over 100000-fold.

Secondary Evaluations

After synthesizing a series of very potent and 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 compounds were first evaluated in our standard screening assay, as well as in a second set of binding assays employing human ET_A and ET_B receptors expressed in CHO cells (Table 3). As we have observed in previous studies,[18,23] the rET$_A$ and pET$_B$ receptors used in the screening assays reliably predict the relative and absolute affinities observed

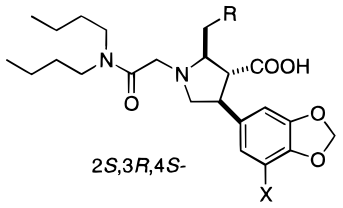
Table 3. Summary Data for Single Enantiomers



			binding IC_{50} (nM)[a,b]			binding IC_{50} (nM)[a,c]			
compd	R	X	$r\mathrm{ET_A}$ mean (range)	$p\mathrm{ET_B}$ mean (range)	B/A ratio[d]	$h\mathrm{ET_A}$ mean (range)	$h\mathrm{ET_B}$ mean (range)	B/A ratio[d]	formula
8h	$C_3H_7C(CH_3)_2$	H	0.67 (0.96−0.45)	2900 (5900−1600)	4300	0.29 (0.37−0.23)	2800 (4300−1900)	9700	nd
8r	(*E*)-$CH_3CH{=}CHC(CH_3)_2$	H	0.78 (1.1−0.57)	>100000	>128000	1.0 (1.3−0.77)	92700 (92700)	92700	$C_{29}H_{44}N_2O_5{\cdot}0.70$TFA
9o	(2-pyridyl)CH_2	H	0.72 (1.4−0.35)	26000 (36000−15000)	36000	0.29 (0.68−0.12)	32000 (41000−25000)	110000	$C_{29}H_{39}N_3O_5{\cdot}2.25TFA\cdot$ $1.10H_2O$
9p	(1-pyrazolyl)CH_2	H	0.68 (0.71−0.66)	26200 (33000−20600)	38500	1.06 (1.67−0.68)	42000 (83800−21100)	39600	$C_{27}H_{38}N_4O_5{\cdot}0.90$TFA
9u	(2-oxopyrrolidinyl)CH_2	H	0.22 (0.25−0.20)	24000 (27700−20600)	110000	0.17 (0.18−0.17)	18000 (23000−13800)	106000	$C_{28}H_{41}N_3O_6{\cdot}0.85$TFA
10a	$C_3H_7C(CH_3)_2$	OCH_3	0.56 (0.86−0.33)	16700 (24900−9100)	29800	0.49 (0.61−0.41)	15400 (20000−12400)	31400	$C_{30}H_{48}N_2O_6{\cdot}0.7$TFA
10b	(*E*)-$CH_3CH{=}CHC(CH_3)_2$	OCH_3	0.41 (0.41−0.40)	45300 (81000−23000)	110000	0.29 (0.68−0.13)	39700 (73300−27000)	137000	$C_{30}H_{46}N_2O_6{\cdot}0.80$TFA

[a] IC_{50} calculated using a mean of at least two measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} unless otherwise noted. [b] Binding assays recorded as described in Experimental Section, using MMQ cells ($r\mathrm{ET_A}$), porcine cerebellar tissue ($p\mathrm{ET_B}$). [c] Binding assays recorded as described in Experimental Section, using clonal CHO cell lines ($h\mathrm{ET_A}$ and $h\mathrm{ET_B}$). [d] Expressed as $IC_{50}(\mathrm{ET_B})/IC_{50}(\mathrm{ET_A})$.

Table 4. Summary of Pharmacokinetic Profiles for Single Enantiomers



			pharmacokinetic profiles (rats)[a]				
compd	R	X	$T_{1/2}$ iv (h)	$T_{1/2}$ oral (h)	C_{max} (μg/mL)	AUC (μg h/mL)	F (%)
8h	$C_3H_7C(CH_3)_2$	H	2.45	3.85	1.48	7.06	76.8
8r	(*E*)-$CH_3CH{=}CHC(CH_3)_2$	H	1.6	3.58	1.49	2.62	32.2
9o (racemic)	(2-pyridyl)CH_2	H	2.8	8.8	0.58	0.78	21.1
9u	(2-oxopyrrolidin-1-yl)CH_2	H	1.4	nd	0.016	0.009	0.3
10a	$C_3H_7C(CH_3)_2$	OCH_3	1.6	2.5	1.69	3.27	47.7
10b	(*E*)-$CH_3CH{=}CHC(CH_3)_2$	OCH_3	1.5	3.94	0.66	2.09	27.8

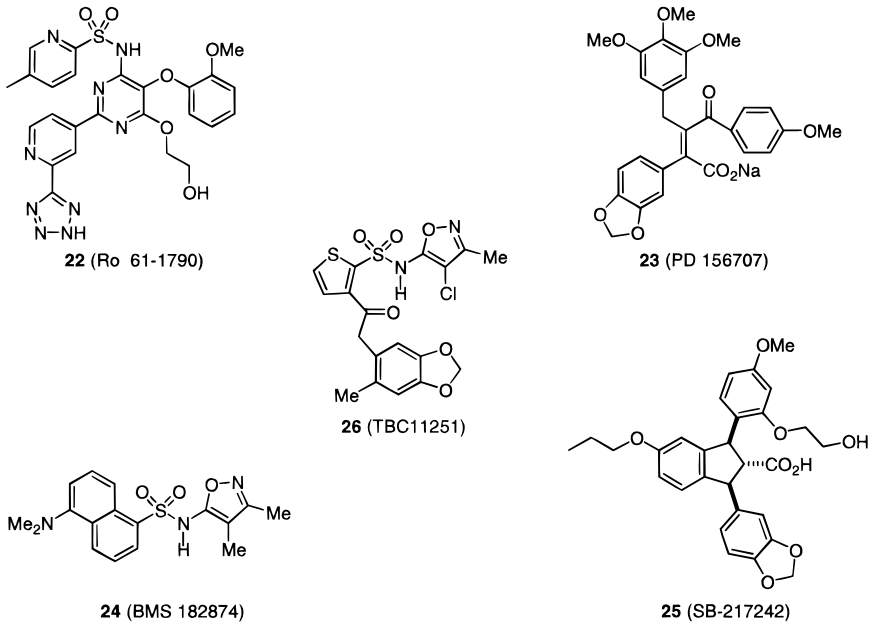
[a] $T_{1/2}$ iv, half-life after intravenous dosing. $T_{1/2}$ oral, C_{max}, AUC, and F are half-life, maximum drug concentration, total drug exposure (area under the curve) and oral bioavailability after oral dosing in rats. Calculated from raw pharmacokinetic data as described in the Experimental Section.

against human receptors, with some tendency for this class of compounds to exhibit a higher B/A ratio for the human genotypes. Most of the compounds in this group have subnanomolar potencies against $h\mathrm{ET_A}$ receptors; B/A ratios vary from 9700 to 137000.

Pharmacokinetic studies (in rats) of these enantiomers revealed that the vastly different pharmacokinetic profiles of these two series were associated with their distinctively different physical properties (Table 4). Oral bioavailability of **8h** is very good (77%) but with a very slow absorption rate, which could be explained by the extremely high hydrophobicity of the compound, compared to **1**. Compound **8r** has much lower bioavailability and a much smaller AUC values. On the other hand, the very hydrophilic series of compounds, exemplified by **9o** and **9u**, exhibit very poor pharmacokinetic profiles: low oral bioavailabilities and small C_{max} and AUC values. Rapid elimination and a tendency toward oxidation might contribute to the poor showing of oral bioavailability of a number of these compounds.

A separate unpublished SAR study by our group has indicated that the placement of a methoxy group at the position 7 of the benzodioxolane ring was well tolerated for the $\mathrm{ET_A}$ selective antagonists. To improve the absorption rate of compounds **8h** and **8r**, slightly more hydrophilicity was added to the alkyl series by introducing this modification. Initial biochemical assays indicated improved binding affinity and receptor selectivity for new analogs **10a** (A-216546) and **10b** over the parent compounds (Table 3). In fact, compound **10b** is arguably the most $\mathrm{ET_A}$ selective antagonist known to date against human receptors.

Chart 1



The results of pharmacokinetic profile change of these two compounds were mixed. The hydrophilicity modification improved the pharmacokinetic profiles of **10a**, but not of **10b**. The absorption rate of **10a** increased with improved hydrophilicity, comparing to compound **8h**. Compound **10a**, is also well absorbed (oral bioavailability $F = 48\%$), despite its relatively short iv half-life (1.6 h). In the phase I single dose human study, ABT-627 (**1**) exhibits a much longer elimination half-life (24 h) than that expected from the rat study (3.5 h). Both in vitro and in vivo metabolism studies have suggested that one of the major metabolic routes for **1** appears to be the glucuronidation of the carboxylic acid moiety. The in vivo studies with [^{14}C]A-127722 indicates that the drug is recovered through enterohepatic recycling of the glucuronidate of **1**, which explains its prolonged half-life in human. Apparently, this recycling process is much more profound in human than in rat. If the same trend holds, a longer half-life for **10a** in human than in rat is expected. The ability of **10a** to inhibit ET-1 stimulated phosphoinositol (PI) hydrolysis was also determined. Compound **10a** effectively inhibits ET-1 evoked PI hydrolysis in rat MMQ cells with an IC_{50} value of 0.59 nM, showing the compound indeed is a very potent functional antagonist of endothelin.

Since 1993, a number of non-peptide ET_A selective antagonists have been reported (Chart 1), including Ro 61-1790[24] (**22**), PD 156707[25] (**23**), BMS 182874[26] (**24**), SB 217242[27] (**25**), and TBC11251[28] (**26**). Except for **24**, all of these compounds exhibit subnanomolar affinities for the ET_A receptor (Table 5). The selectivity of these antagonists for human ET_A receptor is about 1000-fold, with the exception of **25** and **26**. Compound **26** is the most selective agent (>10000-fold for ET_A vs ET_B) reported to date and is well-absorbed orally (60% in rats). By comparison, compound **10a** appears to be as potent against the ET_A receptor as any of the above antagonists; it also shows the least affinity for ET_B receptor, which translates into the largest ET_B/ET_A ratio (over 30000).

Table 5. Comparison Data for Representative ET_A Selective Antagonists

	K_i (nM)[a]				
compd	*h*ET_A binding	*h*ET_B binding	B/A ratio[b]	iv half-life[c] (h)	F[c] (%)
10a	0.46	13000	28260	1.6	48
22	0.13	175	1346	0.8	N/A
23	0.17	139	818	1.0	41
24	48	>50000	>1040	N/A	N/A
25	1.1	111	100	3.3	66
26	0.43	>4300	>10000	6.7	60

[a] Human receptor data acquired in a variety of systems, collected from a variety of sources. [b] Expressed as $K_i(ET_B)/K_i(ET_A)$. [c] $T_{1/2}$ and oral bioavailability (F) data, measured in rats, collected from a variety of sources.

Conclusions

Two novel series of pyrrolidine-3-carboxylic acid based highly ET_A selective antagonists have been identified through modification of the *p*-anisyl group of the ET_A-selective endothelin antagonist **1**. In the first series, structure−activity studies revealed that the *p*-anisyl group of **1** could be replaced with hydrophobic 2,2-dimethylpentyl (**8h**) and 2,2-dimethylpentenyl (**8r**) groups. The resultant analogues retain the high ET_A affinity of **1**, but exhibit substantially reduced ET_B activity. In particular, the combination of a 2,2-dimethylpentenyl group, *N*,*N*-dibutylacetamide side chain, and a 7-methoxy-1,3-benzodioxol-5-yl group provides antagonist **10b** with subnanomolar affinity for the ET_A receptor and over 100000-fold selectivity. A number of these compounds also exhibit oral bioavailabilities (in rats) in the 28−77% range and have good plasma half-lives. Of these, compound **10a**, exhibits the best combination of biochemical and pharmacological properties, and has been identified as a potential backup to **1**.

For the alkyl heterocycle series, it was found that the size of the heterocycles and positioning of the hydrogen bonding acceptors are important in optimizing this series of highly selective antagonists. In particular, 2-pyridylethyl (**9o**) and 2-(2-oxopyrrolidinyl)ethyl (**9u**) groups have the best combination of potency and

selectivity. Unfortunately, poor pharmacokinetic profiles are generally exhibited by these very hydrophilic compounds, which prevent them from being orally dosed.

The highly selective receptor-binding profile of these potent and orally bioavailable compounds further improved the ET_A selectivity observed with **1**. It remains to be seen whether reduced ET_B antagonism will prove to be advantageous in treating diseases in which endothelin-1 plays a pathogenic role.

Experimental Section

General. 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 chromatography was performed using silica gel (230−400 mesh) from E. M. Science. Proton NMR spectra were recorded on a General Electric QE300 instrument with Me_4Si as an internal standard and are reported as shift (multiplicity, coupling constants, proton counts). Mass spectral analyses were accomplished using different techniques, including direct chemical ionization (DCI), atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI), and fast atom bombardment (FAB), as specified for individual compounds. Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ, and are consistent with theoretical values to within 0.4% unless indicated.

Ethyl 2-(*n*-Hexanoyl)-3-(nitromethyl)-1,3-benzodioxol-5-propionate (6, X = $C_4H_9CH_2$, Y = H). To a solution of ethyl 3-oxo-6-octanoate (5.0 g, 26.9 mmol) in 100 mL of THF was added 3,4-methylenedioxy-β-nitrostyrene (5.1 g, 26.4 mmol), followed by the addition of potassium *tert*-butoxide (30 mg, 0.27 mmol). The mixture was stirred at room temperature for 2 h, during which the solid dissolved and the solution became reddish. After 2 h, TLC (ethyl acetate−hexanes, 1:5) indicated complete consumption of the ketoester. The solution was then concentrated in vacuo, and the residue was flash chromatographed on silica gel eluting with 20% ethyl acetate in hexane to produce 8.7 g (23.0 mmol, 85%) of the title compound **6** as a mixture of diastereomers in a 1:1 ratio.

***trans,trans*-Ethyl 2-(*n*-Pentyl)-4-(1,3-benzodioxol-5-yl)-pyrrolidine-3-carboxylate (7, X = $C_4H_9CH_2$, Y = H).** The nitro ketone **6** (8.7 g, 23.0 mmol) in 150 mL of ethyl acetate was hydrogenated under 4 atm of hydrogen pressure using a Raney nickel 2800 catalyst (8.7 g). The Raney nickel was washed sequentially with methanol, THF, and EtOAc before use. The catalyst was then removed by filtration, and the solution was concentrated under reduced pressure. The resulting crude imine (7.7 g, 23.3 mmol) was dissolved in 25 mL of tetrahydrofuran and 50 mL of ethanol. Sodium cyanoborohydride (1.6 g, 25.5 mmol) and 2 mg of bromocresol green were added. To this blue solution was added dropwise a solution of 1:2 concentrated HCl in ethanol at such a rate that the color was kept at light yellow-green. After the yellow color persisted without additional HCl, the solution was stirred for an additional 40 min. The solution was then concentrated in vacuo and partitioned between chloroform and an aqueous saturated sodium bicarbonate solution. The organic phase was separated, dried over sodium sulfate, and concentrated under reduced pressure. The residue was dissolved in 35 mL of acetonitrile and DBU (4.3 g, 28.2 mmol) was added. The solution was refluxed overnight. TLC (EtOAc) showed no more cis,cis isomer. The solvent was removed in vacuo and the crude product was flash chromatographed on silica gel eluting with 75:25 ethyl acetate-hexane to give 3.5 g of pure trans,-trans compound **7** (10.5 mmol, 46% from **6**) as a light yellow oil.

Resolution of Compounds. Method A. *trans,trans*-Ethyl [2*S*,3*R*,4*S*]-2-(2,2-Dimethylpentyl)-4-(1,3-benzodioxol-5-yl)pyrrolidine-3-carboxylate (18, X = $C_3H_7C(CH_3)_2CH_2$, Y = H). The amino ester **7** (6.8 g, 18.8 mmol) was dissolved in 100 mL of ether; a solution of 1.6 g (10.5 mmol) of (*S*)-(+)-mandelic acid in 60 mL of ether was added, the total volume was made up to ∼200 mL, and the solution was seeded. The mixture was stirred slowly overnight. The resultant crystals were collected by filtration and recrystallized from ether/EtOAc to give 1.8 g (3.5 mmol, 37%) of a white solid. This material was partitioned between sodium bicarbonate and ether; the ether layer was washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give the enantiomerically pure product (>98% ee), as indicated by chiral HPLC analysis, using a Regis Whelk-O column.

Method B. *trans,trans*-Methyl [2*S*,3*R*,4*S*]-2-(2,2-Dimethylpentyl)-4-(7-methoxy-1,3-benzodioxol-5-yl)pyrrolidine-3-carboxylate (21, X = $C_3H_7C(CH_3)_2CH_2$, Y = OCH_3). To a solution of crude amino ester **7** (2.0 g, 5.1 mmol) in 40 mL of dichloromethane with 4 mL (28.7 mmol) of triethylamine was added 2.0 g (9.2 mmol) of di-*tert*-butyl dicarbonate, and the mixture was stirred at ambient temperature for 5 h. Solvents were then removed in vacuo, and the residue was taken up in 60 mL of ethanol. Aqueous sodium hydroxide (10 mL of 2.5 N solution, 25 mmol) was added, and the resultant solution was stirred overnight. Solvents were removed in vacuo; the residue was taken up in water and extracted with ether. The aqueous phase was acidified with aqueous 1 N phosphoric acid and extracted with EtOAc. The organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated to give 1.0 g of a colorless oil. A sample of this material (0.734 g, 1.58 mmol) was combined with 0.35 g (1.9 mmol) of pentafluorophenol and 0.364 g (1.9 mmol) of EDAC in 5 mL of DMF. The resultant solution was stirred at ambient temperature for 1 h, then was poured onto 50 mL of 0.6 M sodium bicarbonate solution and extracted (3 15 mL) with ether. The combined ether extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to give a foam, which was dissolved in 5 mL of THF and cooled to 0 °C. Simultaneously, 0.418 g (2.37 mmol) of (*S*)-(−)-4-benzyl-2-oxazolidinone was combined with ∼0.1 mg of pyreneacetic acid in 5 mL of THF and cooled to 0 C. *N*-Butyllithium (1.6 M in hexanes) was added to a red endpoint (persists ∼10 s), and the solution was stirred for 10 min. The solution was transferred into the THF solution of the pentafluorophenyl ester, and the resultant solution was stirred at 0 °C for 40 min. Solvents were removed in vacuo; the residue was taken up in sodium bicarbonate and extracted with ether (3 × 10 mL). The combined ether extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude mixture of diastereomeric products was separated by flash chromatography on silica gel, eluting with a gradient from 4:1 → 3:1 → 2:1 hexanes/EtOAc, giving 423 mg (0.68 mmol) of the faster moving and 389 mg (0.63 mmol) of the slower moving diastereomer, respectively. The faster moving diastereomer was dissolved in 2 mL of a 2.0 M solution of sodium methoxide (4.0 mmol) in methanol (freshly prepared, containing 5% methyl formate by volume) and stirred at ambient temperature for 16 h. Solvents were removed in vacuo, and the residue was partitioned between ether and aqueous 1 N sodium hydroxide. The ether layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with 4:1 hexanes/EtOAc. The resultant material was dissolved in 5 mL of TFA and stirred at ambient temperature for 1 h. Solvents were removed in vacuo; the residue was suspended in sodium bicarbonate solution and extracted with EtOAc. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo to give 98 mg (0.25 mmol, 33% from the *N*-Boc acid) of the resolved amino ester.

***N,N*-Dibutylbromoacetamide.** The bromoacetamide employed in this study was prepared using the method of Weaver, as described in our earlier work.

***trans,trans*-2-(*n*-Pentyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (3).** The pyrrolidine **7** (48 mg, 0.14 mmol) was combined with 35 mg (0.14 mmol) of the *N,N*-dibutylbromoac-

etamide in 3 mL of acetonitrile; 0.5 mL (2.9 mmol) of Hünig's base was added, and the solution was allowed to stir overnight at ambient temperature. Solvents were removed in vacuo; the residue was partitioned between EtOAc and aqueous 1 N phosphoric acid. The organic layer was washed with sodium bicarbonate solution and brine, then dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography on silica gel, eluting with 2:1 hexanes/EtOAc. The product was dissolved in 4 mL of ethanol; 1 mL of 2.5 N aqueous sodium hydroxide (2.5 mmol) was added, and the resultant solution was stirred overnight at ambient temperature. Solvents were removed in vacuo; the residue was taken up in water and extracted with ether. The aqueous phase was acidified to pH 5 with aqueous 1 N phosphoric acid and extracted with EtOAc. The organic extracts were washed with brine, dried over sodium sulfate, and filtered. The solvents were removed in vacuo to give 56 mg (0.12 mmol, 86%) of the title compound as white foam: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87 (t, J = 7.5 Hz, 3H), 0.89 (t, J = 7.5 Hz, 3H), 0.97 (t, J = 7.5 Hz, 3H), 1.21−1.42 (brm, 10H), 1.43−1.78 (brm, 6H), 2.76 (t, J = 7.0 Hz, 1H), 3.02−3.30 (brm, 6H), 3.40−3.60 (m, 3H), 3.73 (d, J = 14.0 Hz, 1H), 5.98 (AB, 2H), 6.70 (d, J = 8.1 Hz, 1H), 6.77 (dd, J = 1.8, 8.1 Hz, 1H), 6.89 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 475. Anal. Calcd for $C_{27}H_{42}N_2O_5 \cdot 0.5H_2O$: C, 67.05; H, 8.96; N, 5.79. Found: C, 67.30; H, 8.77; N, 5.68.

The following compounds were prepared using the procedures described above for compound **3**.

***trans,trans*-2-(4-Methylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8a):** an amorphous solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.83 (d, J = 6.9 Hz, 3H), 0.84 (d, J = 6.9 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H), 0.96 (t, J = 7.5 Hz, 3H), 1.13−1.90 (m, 15H), 3.00−4.20 (m, 11H), 5.93 (s, 2H), 6.74 (d, J = 8.1 Hz, 1H), 6.78 (dd, J = 1.8, 8.1 Hz, 1H), 6.87 (d, J = 1.8 Hz, 1H); MS (APCI) $(M + H)^+$ at m/z 489. Anal. Calcd for $C_{28}H_{44}N_2O_5 \cdot 0.65$TFA: C, 62.53; H, 8.00; N, 4.98. Found: C, 62.50; H, 8.02; N, 4.96.

***trans,trans*-2-(3-Methylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8b):** an amorphous solid; ^{1}H NMR ($CDCl_3$, 300 MHz, 1:1 mixture of diastereomers) δ 0.83 (t, J = 7.5 Hz, 3H), 0.85 (d, J = 7.5 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H), 0.97 (t, J = 7.5 Hz, 3H), 1.05−1.22 (m, 2H), 1.22−1.41 (m, 7H), 1.43−1.68 (m, 5H), 1.89 (m, 1H), 2.94 (t, J = 6.0 Hz, 1H), 3.15−3.27 (m, 3H), 3.29−3.60 (m, 5H), 3.72 (brd, J = 6.0 Hz 1H), 3.92 (brd, J = 13.5 Hz, 1H), 5.93 (dd, J = 2.0, 4.0 Hz, 2H), 6.73 (d, J = 8.1 Hz, 1H), 6.78 (dd, J = 1.8, 8.1 Hz, 1H), 6.88 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 489. Anal. Calcd for $C_{28}H_{44}N_2O_5 \cdot 0.60TFA\cdot 0.25Et_2O$: C, 66.08; H, 9.02; N, 4.99. Found: C, 65.93; H, 8.81; N, 4.84.

***trans,trans*-2-(2-Methylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butyl)aminocarbonylmethyl]pyrrolidine-3-carboxylic Acid (8c):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz, 1:1 mixture of diastereomers) δ 0.8−1.0 (m, 12H), 1.20−1.40 (m, 7H), 1.45−1.60 (m, 6H), 1.60−1.74 (m, 1H), 1.80−2.0 (m, 1H), 3.10−3.40 (m, 5H), 3.67−3.78 (m, 1H), 3.80−3.91 (m, 1H), 4.0−4.20 (m, 2H), 4.30−4.50 (m, 2H), 5.93 (d, J = 1.5 Hz, 2H), 6.73 (dd, J = 1.8, 8.1 Hz, 1H), 6.79 (ddd, J = 1.8, 1.8, 8.1 Hz, 1H), 6.86 (dd, J = 1.8, 3.9 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 489. Anal. Calcd for $C_{28}H_{44}N_2O_5 \cdot 1.0TFA\cdot 0.5H_2O$: C, 58.91; H, 7.58; N, 4.58. Found: C, 58.91; H, 7.58; N, 4.45.

***trans,trans*-2-(1,1-Dimethylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8d):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, J = 7.5 Hz, 3H), 0.94 (t, J = 7.5 Hz, 3H), 0.95 (t, J = 7.5 Hz, 3H), 1.06 (s, 3H), 1.12 (s, 3H), 1.20−1.42 (m, 10H), 1.44−1.68 (m, 4H), 3.16−3.60 (m, 6H), 3.91 (brdd, J = 6.0, 11.4 Hz, 1H), 4.08 (d, J = 16.8 Hz, 1H), 4.27 (m, 1H), 4.33 (d, J = 9.0 Hz, 1H), 5.87 (brd, J = 18.0 Hz, 1H), 5.94 (s, 2H), 6.70 (d, J = 8.1 Hz, 1H), 6.82 (dd, J = 1.8, 8.1 Hz, 1H), 6.85 (d, J = 1.8 Hz, 1H); MS (APCI/NH_3) $(M + H)^+$ at m/z 503. Anal. Calcd for $C_{29}H_{46}N_2O_5 \cdot 1.60$HCl: C, 62.09; H, 8.55; N, 4.99. Found: C, 62.09; H, 8.21; N, 4.65.

***trans,trans*-2-(3-Ethylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8e):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81 (t, J = 7.5 Hz, 6H), 0.90 (t, J = 7.5 Hz, 3H), 0.96 (t, J = 7.5 Hz, 3H), 1.10−1.42 (m, 12H), 1.43−1.62 (m, 4H), 1.68−1.72 (brm, 1H), 2.91 (brt, J = 7.5 Hz, 1H), 3.14−3.28 (m, 2H), 3.28−3.52 (m, 6H), 3.70 (brd, J = 6.6 Hz, 1H), 3.92 (brd, J = 16.5 Hz, 1H), 5.92 (dd, J = 2.0, 4.0 Hz, 2H), 6.71 (d, J = 8.1 Hz, 1H), 6.79 (dd, J = 1.8, 8.1 Hz, 1H), 6.88 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 503. Anal. Calcd for $C_{29}H_{46}N_2O_5 \cdot 0.3$TFA: C, 66.22; H, 8.69; N, 5.22. Found: C, 66.40; H, 9.08; N, 5.10.

***trans,trans*-2-(2-Propylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8f):** an amorphous solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.85 (m, 6H), 0.92 (t, J = 7.5 Hz, 3H), 0.97 (t, J = 7.5 Hz, 3H), 1.12−1.40 (m, 13H), 1.42−1.68 (m, 6H), 2.90 (m, 1H), 3.14−3.30 (m, 3H), 3.33 (m, 5H), 3.72 (brm, 1H), 3.90 (brm, 1H), 5.93 (dd, J = 2.0, 4.0 Hz, 2H), 6.73 (d, J = 8.1 Hz, 1H), 6.78 (dd, J = 1.8, 8.1 Hz, 1H), 6.88 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 517. Anal. Calcd for $C_{30}H_{48}N_2O_5 \cdot 0.35$TFA: C, 66.24; H, 8.76; N, 5.03. Found: C, 66.26; H, 8.82; N, 4.98.

***trans,trans*-2-(5,5,5-Trifluoropentyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8g):** an amorphous solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, J = 7.5 Hz, 3H), 0.95 (t, J = 7.5 Hz, 3H), 1.31 (septet, J = 7.5 Hz, 4H), 1.41−1.66 (m, 8H), 1.76−2.15 (m, 4H), 3.10−3.25 (m, 3H), 3.30 (dd, J = 6.0, 12.6 Hz, 1H), 3.40 (dd, J = 6.0, 12.6 Hz, 1H), 3.67 (t, J = 10.8 Hz, 1H), 3.77 (t, J = 10.8 Hz, 1H), 3.99 (dd, J = 9.9, 19.2 Hz, 1H), 4.03 (d, J = 16.5 Hz, 1H), 4.23−4.33 (m, 1H), 4.35 (d, J = 16.5 Hz, 1H), 5.94 (s, 2H), 6.73 (d, J = 8.1 Hz, 1H), 6.79 (dd, J = 1.8, 8.1 Hz, 1H), 6.82 (d, J = 1.8 Hz, 1H); MS (ESI) $(M + H)^+$ at m/z 529. Anal. Calcd for $C_{27}H_{39}N_2O_5F_3 \cdot 1.05$TFA: C, 53.91; H, 6.23; N, 4.32. Found: C, 53.99; H, 6.08; N, 4.09.

***trans,trans*-2-(2,2-Dimethylpentyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8h):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80−0.99 (m, 15H), 1.10−1.37 (m, 8H), 1.43−1.58 (m, 4H), 1.77−1.97 (m, 2H), 3.48−3.12 (m, 5H), 3.60−3.69 (m, 1H), 3.75−3.86 (m, 1H), 3.95−4.16 (m, 2H), 4.28−4.4 (m, 2H), 5.94 (s, 2H), 6.74 (d, J = 8.1 Hz, 1H), 6.8 (dd, J = 1.8, 8.1 Hz, 1H), 6.87 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 503. Anal. Calcd for $C_{29}H_{46}N_2O_5 \cdot 1.05$TFA: C, 60.01; H, 7.62; N, 4.50. Found: C, 60.21; H, 7.37; N, 4.33.

***trans,trans*-2-(4-Methyl-3-pentenyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8i):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, J = 7.5 Hz, 3H), 0.96 (t, J = 7.5 Hz, 3H), 1.24−1.38 (m, 4H), 1.35−1.57 (m, 4H), 1.57 (s, 3H), 1.64 (s, 3H), 1.83−2.16 (m, 4H), 3.10−3.40 (m, 5H), 3.69−3.88 (m, 2H), 3.95−4.10 (m, 1H), 4.19 (d, J = 15.9 Hz, 1H), 4.26−4.34 (m, 1H), 4.39 (d, J = 16.2 Hz, 1H), 5.00−5.07 (m, 1H), 5.94 (s, 2H), 6.73 (d, J = 8.1 Hz, 1H), 6.78 (dd, J = 1.8, 8.1 Hz, 1H), 6.85 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 487. Anal. Calcd for $C_{28}H_{42}N_2O_5 \cdot 0.05H_2O \cdot 1.15$TFA: C, 58.79; H, 6.90; N, 4.34. Found: C, 58.82; H, 7.05; N, 4.53.

***trans,trans*-2-(4-Methyl-4-hexenyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8j):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz, 1:1 mixture of geometric isomers) δ 0.87−1.01 (m, 6H), 1.23−1.39 (m, 4H), 1.45−1.60 (m, 4H), 1.54 (brs, 3H), 1.66 (brs, 1H), 1.83−2.20 (m, 6H), 1.96 (q, J = 7.5 Hz, 2H), 3.16 (brt, J = 7.8 Hz, 2H), 3.22−3.32 (m, 1H), 3.36 (brtm, J = 7.5 Hz, 2H), 3.69−3.83 (brm, 2H), 4.05 (brdd, J = 9.6, 18.0 Hz, 1H), 4.23−4.42 (m, 3H), 4.97−5.06 (brm, 1H), 5.96 (s, 2H), 6.77 (brs, 2H), 6.84 (brs, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 501. Anal. Calcd for $C_{29}H_{44}N_2O_5 \cdot 3.80$TFA: C, 47.07; H, 5.16; N, 3.00. Found: C, 47.14; H, 4.96; N, 2.97.

***trans,trans*-2-(3-Cyclopropylidenepropyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]-pyrrolidine-3-carboxylic Acid (8k):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, J = 7.5 Hz, 3H), 0.95 (t, J = 7.5

Hz, 3H), 1.01 (s, 4H), 1.31 (septet, $J = 7.5$ Hz, 4H), 1.51 (sextet, $J = 7.5$ Hz, 4H), 1.95−2.41 (m, 4H), 3.16 (t, $J = 7.5$ Hz, 2H), 3.24−3.43 (m, 3H), 3.73−3.89 (brm, 2H), 4.03 (dd, $J = 10.5$, 19.5 Hz, 1H), 4.21 (d, $J = 16.5$ Hz, 1H), 4.34 (m, 1H), 4.36 (d, $J = 18.0$ Hz, 1H), 5.70 (brm, 1H), 5.94 (s, 2H), 6.74 (d, $J = 8.1$ Hz, 1H), 6.79 (dd, $J = 1.8$, 8.1 Hz, 1H), 6.86 (d, $J = 1.8$ Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 485. Anal. Calcd for C28H40N2O5·1.80TFA: C, 55.02; H, 6.11; N, 4.06. Found: C, 55.12; H, 6.18; N, 4.13.

***trans,trans*-2-[3(*E*)-Pentenyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8l):** white solid; ^{1}H NMR $CDCl_3$, 300 MHz) δ 0.93 (t, $J = 7.5$ Hz, 3H), 0.96 (t, $J = 7.5$ Hz, 3H), 1.32 (septet, $J = 7.5$ Hz, 4H), 1.51 (sextet, $J = 7.5$ Hz, 4H), 1.60 (dd, $J = 6.0$, 14.7 Hz, 3H), 1.88−2.21 (m, 4H), 3.10−4.43 (m, 5H), 3.72-(dd, $J = 10.2$, 22.8 Hz, 1H), 3.77 (dd, $J = 12.0$, 21.0 Hz, 1H), 3.95−4.10 (m, 2H), 4.26 (m, 1H), 4.29 (d, $J = 16.5$ Hz, 1H), 5.23−5.38 (m, 1H), 5.48 (qd, $J = 6.0$, 21.0 Hz, 1H), 5.94 (s, 2H), 6.83 (d, $J = 8.1$ Hz, 1H), 6.89 (dd, $J = 1.8$, 8.1 Hz, 1H), 6.98 (d, $J = 1.8$ Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 473. Anal. Calcd for $C_{27}H_{40}N_2O_5$·0.85TFA: C, 60.52; H, 7.23; N, 4.92. Found: C, 60.63; H, 7.27; N, 4.88.

***trans,trans*-2-[3(*Z*)-Pentenyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8m):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, $J = 7.5$ Hz, 3H), 0.95 (t, $J = 7.5$ Hz, 3H), 1.20−1.40 (m, 4H), 1.45−1.63 (m, 4H), 1.57 (dd, $J = 1.5$, 6.9 Hz, 3H), 1.88−2.24 (m, 4H), 3.15 (td, $J = 1.8$, 7.8 Hz, 2H), 3.25−3.42 (m, 3H), 3.68−3.88 (m, 2H), 4.03 (dd, $J = 9.0$, 11.1 Hz, 1H), 4.12 (d, $J = 16.8$ Hz, 1H), 4.32 (brm, 1H), 4.35 (d, $J = 16.8$ Hz, 1H), 5.29 (brm, 1H), 5.49 (dd, $J = 6.0$, 11.1 Hz, 1H), 5.94 (s, 2H), 6.74 (d, $J = 8.1$ Hz, 1H), 6.81 (dd, $J = 1.8$, 8.1 Hz, 1H), 6.88 (d, $J = 1.8$ Hz, 1H); MS (APCI) $(M + H)^+$ at m/z 473. Anal. Calcd for $C_{27}H_{40}N_2O_5$·1.10TFA·1.05AcOH: C, 57.10; H, 6.96; N, 4.25. Found: C, 57.17; H, 6.70; N, 3.97.

***trans,trans*-2-(4-Pentenyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8n):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, $J = 7.5$ Hz, 3H), 0.96 (t, $J = 7.5$ Hz, 3H), 1.24−1.38 (m, 4H), 1.42−1.65 (m, 6H), 1.80−2.03 (m, 2H), 2.09 (q, $J = 7.0$ Hz, 2H), 3.12−3.44 (m, 5H), 3.72 (t, $J = 11.3$ Hz, 1H), 3.84 (t, $J = 10.5$ Hz, 1H), 3.98 (d, $J = 12.0$ Hz, 1H), 4.03 (d, $J = 16.8$ Hz, 1H), 4.30−4.40 (m, 1H), 4.37 (d, $J = 16.8$ Hz, 1H), 4.96 (m, 1H), 5.01 (dd, $J = 2.4$, 11.7 Hz, 1H), 5.71 (tdd, $J = 3.3$, 6.9, 18.0 Hz, 1H), 5.95 (s, 2H), 6.74 (d, $J = 8.1$ Hz, 1H), 6.81 (dd, $J = 1.8$, 8.1 Hz, 1H), 6.87 (d, $J = 1.8$ Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 473. Anal. Calcd for $C_{27}H_{40}N_2O_5$·1.05TFA: C, 59.01; H, 6.99; N, 4.73. Found: C, 58.91; H, 6.72; N, 4.50.

***trans,trans*-2-(4-Methyl-4-pentenyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8o):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, $J = 7.5$ Hz, 3H), 0.96 (t, $J = 7.5$ Hz, 3H), 1.31 (septet, $J = 7.5$ Hz, 4H), 1.53 (sextet, $J = 7.5$ Hz, 4H), 1.67 (s, 3H), 1.87−2.0 (m, 2H), 2.04 (t, $J = 7.2$ Hz, 2H), 3.17 (brtd, $J = 3.0$, 9.0 Hz, 2H), 3.37 (dd, $J = 8.4$, 18.0 Hz, 2H), 3.38 (dd, $J = 6.0$, 13.8 Hz, 2H), 3.75 (t, $J = 11.4$ Hz, 1H), 3.86 (t, $J = 9.0$ Hz, 1H), 4.04 (dd, $J = 11.1$, 21.0 Hz, 1H), 4.11 (d, $J = 16.8$ Hz, 1H), 4.37 (d, $J = 16.8$ Hz, 1H), 4.40 (brm, 1H), 4.64 (s, 1H), 4.72 (s, 1H), 5.95 (s, 2H), 6.75 (d, $J = 8.1$ Hz, 1H), 6.82 (d, $J = 1.8$, 8.1 Hz, 1H), 6.89 (d, $J = 1.8$ Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 487. Anal. Calcd for $C_{28}H_{42}N_2O_5$·1.30TFA: C, 57.89; H, 6.87; N, 4.41. Found: C, 57.97; H, 6.84; N, 4.48.

***trans,trans*-2-[3-Methyl-3(*E*)-pentenyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8p):** white amorphous solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, $J = 7.5$ Hz, 3H), 0.97 (t, $J = 7.5$ Hz, 3H), 1.22−1.40 (m, 5H), 1.44−1.61 (m, 8H), 1.82 (brm, 1H), 2.02 (m, 3H), 3.05−3.30 (m, 5H), 3.38 (m, 1H), 3.55 (brm, 2H), 3.85 (m, 3H), 4.12 (brd, $J = 15.0$ Hz, 1H), 5.21 (dd, $J = 6.0$, 12.0 Hz, 1H), 5.93 (s, 2H), 6.73 (d, $J = 8.1$ Hz, 1H), 6.78 (dd, $J = 1.8$, 8.1 Hz, 1H), 6.88 (d, $J = 1.8$ Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 487. Anal. Calcd for $C_{28}H_{42}N_2O_5$·0.70TFA: C, 62.34; H, 7.60; N, 4.95. Found: C, 62.49; H, 7.43; N, 4.73.

***trans,trans*-2-(2,2,4-Trimethyl-3-pentenyl)-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8q):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, $J = 7.5$ Hz, 3H), 0.94 (t, $J = 7.5$ Hz, 3H), 1.11 (s, 3H), 1.13 (s, 3H), 1.24−1.37 (m, 4H), 1.46−1.59 (m, 4H), 1.61 (d, $J = 1.2$ Hz, 3H), 1.69 (d, $J = 1.2$ Hz, 3H), 2.04−2.11 (m, 2H), 3.10−3.20 (m, 2H), 3.30−3.39 (m, 3H), 3.67−3.82 (m, 2H), 3.95−4.08 (m, 1H), 4.32 (m, 2H), 4.37−4.47 (m, 1H), 4.99 (s, 1H), 5.95 (s, 2H), 6.73 (d, $J = 8.1$ Hz, 1H), 6.78 (dd, $J = 1.8$, 8.1 Hz, 1H), 6.84 (d, $J = 1.8$ Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 515. Anal. Calcd for $C_{30}H_{46}N_2O_5$·1.05TFA: C, 60.77; H, 7.48; N, 4.42. Found: C, 60.83; H, 7.20; N, 4.43.

***trans,trans*-[2*S*,3*R*,4*S*]-2-[2,2-Dimethyl-3(*E*)-pentenyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (8r):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, $J = 7.5$ Hz, 3H), 0.96 (t, $J = 7.5$ Hz, 3H), 0.98 (s, 3H), 1.00 (s, 3H), 1.32 (septet, $J = 7.5$ Hz, 4H), 1.54 (sextet, $J = 7.5$ Hz, 4H), 1.60 (d, $J = 5.7$ Hz, 3H), 1.80−1.94 (m, 1H), 1.96−2.06 (m, 1H), 3.12−3.32 (m, 3H), 3.35 (td, $J = 3.0$, 9.6 Hz, 2H), 3.60−3.69 (m, 2H), 3.87−4.20 (m, 3H), 4.14 (brd, $J = 15.0$ Hz, 1H), 5.30 (d, $J = 15.6$ Hz, 1H), 5.39 (qd, $J = 6.0$, 15.6 Hz, 1H), 5.94 (s, 2H), 6.74 (d, $J = 8.1$ Hz, 1H), 6.82 (dd, $J = 1.8$, 8.1 Hz, 1H), 6.88 (d, $J = 1.8$ Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 501. Anal. Calcd for $C_{29}H_{44}N_2O_5$·0.70TFA: C, 62.90; H, 7.76; N, 4.83. Found: C, 62.72; H, 7.83; N, 4.82.

***trans,trans*-2-[2-(1,3-Dioxol-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9a):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, $J = 7.5$ Hz, 3H), 0.95 (t, $J = 7.5$ Hz, 3H), 1.23−1.38 (m, 4H), 1.52 (sextet, $J = 7.5$ Hz, 4H), 1.85−1.95 (m, 2H), 2.02−2.17 (m, 2H), 3.18 (dd, $J = 6.0$, 9.0 Hz, 2H), 3.30 (dd, $J = 9.0$, 18.0 Hz, 2H), 3.35 (m, 1H), 3.79 (dd, $J = 3.6$, 6.9 Hz, 1H), 3.83−3.88 (m, 3H), 3.97 (dd, $J = 4.8$, 6.0 Hz, 1H), 4.05 (q, $J = 9.6$ Hz, 2H), 4.30−4.40 (m, 1H), 4.37 (s, 2H), 4.87 (t, $J = 4.1$ Hz, 1H), 5.94 (s, 2H), 6.73 (d, $J = 8.1$ Hz, 1H), 6.79 (dd, $J = 1.8$, 8.1 Hz, 1H), 6.87 (d, $J = 1.8$ Hz, 1H); MS (APCI) $(M + H)^+$ at m/z 505. Anal. Calcd for $C_{27}H_{40}N_2O_7$·1.20TFA: C, 55.05; H, 6.47; N, 4.37. Found: C, 55.12; H, 6.44; N, 4.27.

***trans,trans*-2-[2-(4,5-Dimethyl-1,3-dioxol-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9b):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz, mixture of diastereomers) δ 0.89−0.99 (m, 6H), 1.15 (t, $J = 7.5$ Hz, 3H), 1.20−1.38 (m, 7H), 1.44−1.60 (m, 4H), 1.75−1.95 (m, 2H), 1.97−2.26 (m, 2H), 3.13−3.40 (m, 4H), 3.62−3.70 (m, 1H), 3.70−3.88 (m, 2H), 4.05 (m, 1H), 4.16 (m, 1H), 4.25−4.45 (m, 4H), 4.88 (t, $J = 4.4$ Hz) and 5.06 (td, $J = 1.2$, 4.4 Hz, 1H in total), 5.96 (s, 2H), 6.77 (brm, 2H), 6.83 (brs, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 533. Anal. Calcd for $C_{29}H_{44}N_2O_7$·1.35TFA: C, 55.45; H, 6.66; N, 4.08. Found: C, 55.47; H, 6.47; N, 4.03.

***trans,trans*-2-[2-(1,3-Dioxan-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9c):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (t, $J = 7.5$ Hz, 3H), 0.96 (t, $J = 7.5$ Hz, 3H), 1.23−1.41 (m, 6H), 1.42−1.62 (m, 6H), 1.62−1.75 (m, 2H), 2.66 (dd, $J = 6.9$, 8.4 Hz, 1H), 2.83−2.94 (m, 3H), 3.07 (td, $J = 7.8$, 15.0 Hz, 1H), 3.16 (dd, $J = 3.9$, 9.6 Hz, 2H), 3.38−3.69 (m, 3H), 3.72(d, $J = 8.4$ Hz, 1H), 3.76 (dd, $J = 3.0$, 10.2 Hz, 2H), 4.07 (dd, $J = 4.2$, 11.1 Hz, 2H), 4.52 (t, $J = 4.5$ Hz, 1H), 5.91 (dd, $J = 2.0$, 4.0 Hz, 2H), 6.68 (d, $J = 8.1$ Hz, 1H), 6.73 (dd, $J = 1.8$, 8.1 Hz, 1H), 6.86 (d, $J = 1.8$ Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 519. Anal. Calcd for $C_{28}H_{42}N_2O_7$·1.50TFA: C, 53.99; H, 6.36; N, 4.06. Found: C, 54.06; H, 6.50; N, 3.99.

***trans,trans*-2-[3-(1,3-Dioxol-2-yl)propyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9d):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, $J = 7.5$ Hz, 3H), 0.95 (t, $J = 7.5$

Hz, 3H), 1.32 (septet, J = 7.5 Hz, 4H), 1.52 (sextet, J = 7.5 Hz, 6H), 1.65−1.75 (m, 2H), 1.85−2.12 (m, 2H), 3.17 (dd, J = 6.3, 9.3 Hz, 2H), 3.21−3.45 (m, 3H), 3.78 (dd, J = 3.6, 6.9 Hz, 1H), 3.80−3.97 (m, 5H), 4.03 (q, J = 9.7 Hz, 1H), 4.16 (d, J = 16.5 Hz, 1H), 4.33 (m, 1H), 4.37 (d, J = 16.5 Hz, 1H), 4.82 (t, J = 4.5 Hz, 1H), 5.95 (s, 2H), 6.75 (d, J = 8.1 Hz, 1H), 6.81 (dd, J = 1.8, 8.1 Hz, 1H), 6.88 (d, J = 1.8 Hz, 1H); MS (APCI) $(M + H)^+$ at m/z 519. Anal. Calcd for $C_{28}H_{42}N_2O_7$·1.50TFA: C, 53.99; H, 6.36; N, 4.06. Found: C, 54.06; H, 6.26; N, 4.05.

***trans,trans*-2-[2-(1,3-Dithia)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9e):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, J = 7.5 Hz, 3H), 0.95 (t, J = 7.5 Hz, 3H), 1.24−1.40 (m, 4H), 1.45−1.61 (m, 4H), 1.89 (q, J = 7.4 Hz, 2H), 1.97−2.10 (m, 1H), 2.15−2.25 (m, 1H), 3.13−3.45 (m, 9H), 3.75−3.93 (m, 2H), 4.03 (dd, J = 9.6, 18.9 Hz, 1H), 4.25 (d, J = 15.9 Hz, 1H), 4.28−4.39 (m, 1H), 4.44 (d, J = 15.9 Hz, 1H), 4.50 (t, J = 6.5 Hz, 1H), 5.96 (s, 2H), 6.76 (d, J = 8.1 Hz, 1H), 6.79 (dd, J = 1.8, 8.1 Hz, 1H), 6.87 (d, J = 1.8 Hz, 1H); MS (APCI) $(M + H)^+$ at m/z 537. Anal. Calcd for $C_{27}H_{40}N_2O_5S_2$·1.15TFA: C, 52.69; H, 6.21; N, 4.19. Found: C, 52.68; H, 5.97; N, 4.00.

***trans,trans*-2-[2,2-Dimethyl-2-(1,3-dioxol-1-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9f):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82−1.00 (m, 12H), 1.24−1.40 (m, 4H), 1.43−1.64 (m, 5H), 1.76−1.84 (m, 1H), 2.93−3.00 (m, 1H), 3.15−3.47 (m, 6H), 3.60−3.70 (m, 3H), 3.74−3.95 (m, 5H), 4.48 (s, 1H), 5.94 (m, 2H), 6.72 (d, J = 8.1 Hz, 1H), 6.83 (dd, J = 1.8, 8.0 Hz, 1H), 6.94 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 533 Anal. Calcd for $C_{29}H_{44}N_2O_7$·1.10TFA·0.2H_2O: C, 56.63; H, 6.93; N, 4.23. Found: C, 56.60; H, 6.96; N, 4.25.

***trans,trans*-2-[2-(2-Tetrahydropyranyl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9g):** an amorphous white solid; ^{1}H NMR ($CDCl_3$, 300 MHz, 1:1 mixture of two diastereomers) δ 0.89 (t, J = 7.5 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H), 1.30 (septet, 5H), 1.42−1.66 (m, 8H), 1.71 (brm, 1H), 1.85 (brm, 1H), 1.96−2.23 (brm, 2H), 3.10−3.29 (m, 4H), 3.29−3.52 (m, 3H), 3.54−3.81 (m, 3H), 4.01 (q, J = 9.0 Hz, 1H), 4.12−4.25 (m, 2H), 4.43 (d, J = 9.0 Hz, 1H), 4.50 (d, J = 2.7 Hz, 1H), 5.94 (s) and 5.95 (s, 2H in total), 6.76 (s, 2H), 6.81 (s, 1H); MS (APCI) $(M + H)^+$ at m/z 517. Anal. Calcd for $C_{29}H_{44}N_2O_6$·1.40TFA: C, 56.48; H, 6.77; N, 4.14. Found: C, 56.46; H, 6.99; N, 3.83.

***trans,trans*-2-[2-(4-Tetrahydro-2*H*-pyranyl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9h):** an amorphous white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (t, J = 7.5 Hz, 3H), 0.97 (t, J = 7.5 Hz, 3H), 1.17−1.42 (m, 8H), 1.42−1.66 (m, 8H), 1.66−1.81 (m, 1H), 2.73 (t, J = 7.2 Hz, 1H), 3.06 (dd, J = 9.6, 18.6 Hz, 2H), 3.11−3.60 (m, 9H), 3.68 (d, J = 13.5 Hz, 1H), 3.94 (dd, J = 2.7, 12.0 Hz, 2H), 5.91 (s, 2H), 6.71 (d, J = 8.1 Hz, 1H), 6.78 (dd, J = 1.8, 8.1 Hz, 1H), 6.87 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 517. Anal. Calcd for $C_{29}H_{44}N_2O_6$: C, 67.42; H, 8.58; N, 5.42. Found: C, 67.39; H, 8.72; N, 5.30.

***trans,trans*-2-[2-(2-Furfurylethyl)]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9i):** an amorphous white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.89−0.96 (m, 6H), 1.21−1.38 (m, 4H), 1.42−1.57 (m, 4H), 2.19 (m, 1H), 2.31 (m, 1H), 2.63−2.85 (m, 2H), 3.08−3.39 (m, 5H), 3.69 (d, J = 10 Hz, 2H), 3.90−4.01 (m, 2H), 4.12−4.21 (m, 2H), 5.92 (s, 2H), 6.03 (d, J = 3.0 Hz, 1H), 6.24 (dd, J = 2.0, 3.0 Hz, 1H), 6.75 (d, J = 8.1 Hz, 1H), 6.79 (dd, J = 1.8, 8.1 Hz, 1H), 6.88 (d, J = 1.8 Hz, 1H), 7.25 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 499. Anal. Calcd for $C_{28}H_{38}N_2O_6$·0.80TFA·0.15H_2O: C, 60.00; H, 6.65; N, 4.73. Found: C, 60.00; H, 6.69; N, 4.55.

***trans,trans*-2-[2-(3-Furfuryl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9j):** an amorphous white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87−0.96 (m, 6H), 1.21−1.39 (m, 4H), 1.42−1.60 (m, 4H), 1.90 (m, 1H), 2.03 (m, 1H), 2.39−2.60 (m, 2H), 2.96 (m, 1H), 3.15−3.50 (m, 8H), 3.65−3.85 (m, 2H), 5.91 (s, 2H), 6.24 (s, 1H), 6.70 (d, J = 8.1 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 6.90 (s, 1H), 7.20 (s, 1H), 7.32 (s, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 499 Anal. Calcd for $C_{28}H_{38}N_2O_6$·0.20TFA: C, 65.42; H, 7.38; N, 5.37. Found: C, 65.25; H, 7.41; N, 5.27.

***trans,trans*-2-[(2-Tetrahydrofuranyl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9k):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz, a 1:1 mixture of two diastereomers) δ 0.86−1.00 (m, 6H), 1.22−1.40 (m, 4H), 1.40−1.62 (m, 6H), 1.63−1.77 (m, 1H), 1.80−2.07 (m, 5H), 2.95−3.62 (m, 8H), 3.68−4.20 (m, 6H), 5.92 (s, 2H), 6.72 (dd, J = 1.8, 8.1 Hz, 1H), 6.80 (dm, J = 8.1 Hz, 1H), 6.86 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 503. Anal. Calcd for $C_{28}H_{42}N_2O_6$·0.45TFA: C, 62.66; H, 7.72; N, 5.06. Found: C, 62.62; H, 7.53; N, 4.93.

***trans,trans*-2-[2-(1,3-Oxazol-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9l):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (t, J = 7.5 Hz, 3H), 0.93 (t, J = 7.5 Hz, 3H), 1.22−1.36 (m, 4H), 1.43−1.60 (m, 4H), 2.35−2.60 (brm, 2H), 3.08−3.35 (m, 7H), 3.65 (dd, J = 9.0, 12.3 Hz, 1H), 3.88 (t, J = 11.3 Hz, 1H), 4.07 (dd, J = 9.9, 20.0 Hz, 1H), 4.40 (brm, 2H), 4.57 (d, J = 16.5 Hz, 1H), 5.92 (s, 2H), 6.76 (m, 3H), 7.08 (s, 1H), 7.64 (s, 1H); MS (ESI) $(M + H)^+$ at m/z 500. Anal. Calcd for $C_{27}H_{37}N_3O_6$·1.70TFA: C, 52.66; H, 5.63; N, 6.06. Found: C, 52.75; H, 5.80; N, 6.09.

***trans,trans*-2-[2-(4,5-Dimethyl-1,3-oxazol-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9m):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, J = 7.5 Hz, 3H), 0.92 (t, J = 7.5 Hz, 3H), 1.21−1.37 (m, 4H), 1.43−1.57 (m, 4H), 2.11 (s, 3H), 2.21 (s, 3H), 2.31−2.47 (m, 2H), 2.95−3.41 (m, 7H), 3.62 (dd, J = 9.0, 12.3 Hz, 1H), 3.83 (t, J = 12.3 Hz, 1H), 4.10 (dd, J = 10.2, 19.8 Hz, 1H), 4.30−4.40 (brm, 1H), 4.37 (d, J = 16.5 Hz, 1H), 4.57 (d, J = 16.5 Hz, 1H), 5.91 (s, 2H), 6.71 (dd, J = 1.8, 8.1 Hz, 2H), 6.76 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 528. Anal. Calcd for $C_{29}H_{41}N_3O_6$·1.05TFA: C, 57.70; H, 6.55; N, 6.49. Found: C, 57.89; H, 6.33; N, 6.41.

***trans,trans*-2-[2-(*N*-Methylpyrrol-2-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9n):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, J = 7.5 Hz, 3H), 0.96 (t, J = 7.5 Hz, 3H), 1.22−1.37 (m, 4H), 1.42−1.56 (m, 4H), 2.11−2.36 (m, 2H), 2.55−2.80 (m, 2H), 3.04−3.15 (m, 2H), 3.26−3.40 (m, 3H), 3.50 (s, 3H), 3.74 (d, J = 9.6 Hz, 2H), 3.88 (d, J = 16.5 Hz, 1H), 4.02 (dd, J = 9.0, 18.6 Hz, 1H), 4.21 (d, J = 16.5 Hz, 1H), 4.32−4.43 (m, 1H), 5.86−5.89 (m, 1H), 5.91 (s, 2H), 6.00 (t, J = 4.5 Hz, 1H), 6.52 (t, J = 3.0 Hz, 1H), 6.72 (d, J = 8.1 Hz, 1H), 6.80 (dd, J = 1.8, 8.0 Hz, 1H), 6.87 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 512. Anal. Calcd for $C_{29}H_{41}N_3O_5$·1.25TFA: C, 57.83; H, 6.51; N, 6.42. Found: C, 57.68; H, 6.69; N, 6.49.

***trans,trans*-2-[2-(2-Pyridyl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9o):** an amorphous white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (t, J = 7.5 Hz, 6H) 1.22−1.37 (m, 4H), 1.43−1.60 (m, 4H), 2.51 (brs, 2H), 3.13−3.46 (m, 7H), 3.62 (dd, J = 9.6, 13.8 Hz, 1H), 3.84 (t, J = 12.6 Hz, 1 H), 4.04 (dd, J = 10.5, 20.1 Hz, 1 H), 4.18−4.29 (m, 1H), 4.45 (s, 2H), 5.91 (s, 2H), 6.66 (d, J = 8.1 Hz, 1H), 6.75 (dd, J = 1.8, 8.1 Hz, 1H), 6.80 (d, J = 1.8 Hz, 1H), 7.51 (t, J = 6.9 Hz, 1H), 7.70 (d, J = 9.0 Hz, 1H), 8.06 (t, J = 6.9 Hz, 1H), 8.65 (d, J = 6.0 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 510. Anal. Calcd for $C_{29}H_{39}N_3O_5$·1.75TFA: C, 55.04; H, 5.79; N, 5.92. Found: C, 55.08; H, 5.64; N, 5.81.

***trans,trans*-2-[2-(1-Pyrazolyl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9p):** an amorphous solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (t, J = 7.5 Hz, 6H), 1.21−1.38 (m, 4H), 1.42−1.59 (m, 4H), 2.43−2.69 (m, 2H), 3.12−3.36 (m, 5H), 3.40−3.61 (t, J = 10.5 Hz, 1 H), 3.72−3.83 (t, J = 10.5 Hz, 1H), 3.98−4.55 (m, 6H), 5.91 (s, 2H), 6.28 (t, J = 3.0 Hz, 1H),

6.67 (d, J = 8.1 Hz, 1H), 6.75 (dd, J = 1.8, 8.1 Hz, 1H), 6.81 (d, J = 1.8 Hz, 1H), 7.50 (d, J = 3.0 Hz, 1H), 7.56 (d, J = 3.0 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 499. Anal. Calcd for $C_{27}H_{38}N_4O_5$·0.75TFA: C, 58.60; H, 6.69; N, 9.59. Found: C, 58.53; H, 6.45; N, 9.67.

***trans,trans*-2-[2-(Succinimido)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (9q):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, J = 7.5 Hz, 3H), 0.95 (t, J = 7.5 Hz, 3H), 1.23−1.41 (m, 4H), 1.44−1.63 (m, 4H), 2.10−2.25 (m, 1H), 2.37−2.48 (m, 1H), 2.69 (s, 4H), 3.20 (t, J = 7.8 Hz, 2H), 3.26−3.40 (m, 3H), 3.50−3.73 (m, 3H), 3.81 (t, J = 10.5 Hz, 1H), 3.94 (dd, J = 9.0, 19.2 Hz, 1H), 4.09−4.21 (m, 2H), 4.33 (d, J = 16.8 Hz, 1H), 5.92 (s, 2H), 6.72 (d, J = 8.1 Hz, 1H), 6.81 (dd, J = 1.8, 8.1 Hz, 1H), 6.87 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 530. Anal. Calcd for $C_{28}H_{39}N_3O_7$·1.05TFA: C, 55.68; H, 6.62; N, 6.47. Found: C, 55.77; H, 6.02; N, 6.19.

***trans,trans*-2-[2-(Propylsultam-1-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]-pyrrolidine-3-carboxylic Acid (9r):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, J = 7.5 Hz, 3H), 0.95 (t, J = 7.5 Hz, 3H), 1.23−1.38 (m, 4H), 1.44−1.61 (m, 4H), 2.10−2.42 (m, 4H), 3.02−3.26 (m, 7H), 3.26−3.44 (m, 5H), 3.73 (brt, J = 10.0 Hz, 1H), 3.90 (q, J = 8.0 Hz, 1H), 3.93−4.10 (m, 2H), 4.26 (d, J = 15.3 Hz, 1H), 5.94 (s, 2H), 6.73 (d, J = 8.1 Hz, 1H), 6.81 (dd, J = 1.8, 8.1 Hz, 1H), 6.92 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 552. Anal. Calcd for $C_{27}H_{41}N_3O_7S_1$·0.60TFA: C, 54.62; H, 6.76; N, 6.78. Found: C, 54.74; H, 6.74; N, 6.69.

***trans,trans*-2-[2-(2-Oxopiperidin-1-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]-pyrrolidine-3-carboxylic Acid (9s):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (t, J = 7.5 Hz, 3H), 0.93 (t, J = 7.5 Hz, 3H), 1.22−1.38 (m, 4H), 1.42−1.60 (m, 4H), 1.70−1.87 (m, 4H), 2.10−2.23 (brm, 1H), 2.23−2.50 (m, 3H), 3.05 (t, J = 9.0 Hz, 1H), 3.19−3.40 (m, 7H), 3.40−3.60 (m, 2H), 3.61−3.74 (m, 1H), 3.75−3.99 (m, 3H), 4.22 (brs, 1H), 5.92 (m, 2H), 6.72 (d, J = 8.1 Hz, 1H), 6.78 (dd, J = 1.8, 8.1 Hz, 1H), 6.87 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 530. Anal. Calcd for $C_{29}H_{43}N_3O_6$·0.70TFA: C, 59.91; H, 7.23; N, 6.89. Found: C, 59.92; H, 7.23; N, 6.80.

***trans,trans*-2-[2-(2-Oxopyrid-1-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]-pyrrolidine-3-carboxylic Acid (9t):** white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, J = 7.5 Hz, 3H), 0.94 (t, J = 7.5 Hz, 3H), 1.21−1.39 (m, 4H), 1.41−1.61 (m, 4H), 2.38 (brm, 2H), 3.09−3.24 (m, 3H), 3.32 (t, J = 8.4 Hz, 2H), 3.54 (brm, 1H), 3.73 (t, J = 11.4 Hz, 1H), 3.88 (dd, J = 8.4, 18.3 Hz, 1H), 3.94−4.10 (m, 2H), 4.15−4.30 (m, 3H), 5.96 (s, 2H), 6.31 (td, J = 1.5, 6.9 Hz, 1H), 6.58 (d, J = 9.0 Hz, 1H), 6.72 (d, J = 8.1 Hz, 1H), 6.77 (dd, J = 1.8, 8.1 Hz, 1H), 6.84 (d, J = 1.8 Hz, 1H), 7.40 (td, J = 1.8, 6.3 Hz, 1H), 7.60 (dd, J = 1.5, 6.0 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 526. Anal. Calcd for $C_{29}H_{39}N_3O_6$·0.70TFA: C, 60.31; H, 6.61; N, 6.94. Found: C, 60.17; H, 6.59; N, 6.91.

***trans,trans*-2-[2-(2-Oxopyrrolidin-1-yl)ethyl]-4-(1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]-pyrrolidine-3-carboxylic Acid (9u):** an amorphous white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (t, J = 7.5 Hz, 3H), 0.94 (t, J = 7.5 Hz, 3H), 1.23−1.38 (m, 4H), 1.44−1.60 (m, 4H), 2.05 (t, J = 6.9 Hz, 2H), 2.12−2.25 (m, 1H), 2.38 (td, J = 4.2, 8.4 Hz, 2H), 2.47−2.61 (m, 1H), 3.17 (dd, J = 6.0, 8.7 Hz, 2H), 3.24 (t, J = 9.0 Hz, 1H), 3.32 (t, J = 7.8 Hz, 2H), 3.38−3.48 (m, 3H), 3.52 (t, J = 9.0 Hz, 1H), 3.66 (t, J = 6.9 Hz, 1H), 3.96 (m, 2H), 4.14 (m, 1H), 4.38 (brs, 2H), 5.93 (s, 2H), 6.74 (d, J = 8.1 Hz, 1H), 6.89 (dd, J = 1.8, 8.1 Hz, 1H), 6.87 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 516. Anal. Calcd for $C_{28}H_{41}N_3O_6$·1.40TFA: C, 54.78; H, 6.33; N, 6.22. Found: C, 54.69; H, 6.33; N, 6.14.

***trans,trans*-[2*S*,3*R*,4*S*]-2-(2,2-Dimethylpentyl)-4-(7-methoxy-1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (10a):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81 (s, 3H), 0.84 (s, 3H), 0.86 (t, J = 7.5 Hz, 3H), 0.93 (t, J = 7.5 Hz, 3H), 0.96 (t, J = 7.5 Hz, 3H), 1.09−1.38 (m, 8H), 1.45−1.59 (m, 4H), 1.84−2.00 (m, 2H), 3.15 (dd, J = 6.9, 10.0 Hz, 2H), 3.30−3.42 (m, 3H), 3.72 (t, J = 10.5 Hz, 1H), 3.86 (t, J = 10.5 Hz, 1H), 3.88 (s, 3H), 4.02 (q, J = 10.0 Hz, 1H), 4.12 (d, J = 16.8 Hz, 1H), 4.29 (d, J = 16.8 Hz, 1H), 4.41 (brm, 1H), 5.94 (s, 2H), 6.52 (d, J = 1.8 Hz, 1H), 6.67 (d, J = 1.8 Hz, 1H); MS (ESI) $(M + H)^+$ at m/z 533. Anal. Calcd for $C_{30}H_{48}N_2O_6$·0.7TFA: C, 61.57; H, 8.01; N, 4.57. Found: C, 61.59; H, 8.20; N, 4.63.

***trans,trans*-[2*S*,3*R*,4*S*]-2-[2,2-Dimethyl-3(*E*)-pentenyl]-4-(7-methoxy-1,3-benzodioxol-5-yl)-1-[(*N,N*-di-*n*-butylamino)carbonylmethyl]pyrrolidine-3-carboxylic Acid (10b):** white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, J = 7.5 Hz, 3H), 0.95 (t, J = 7.5 Hz, 3H), 0.97 (s, 3H), 0.99 (s, 3H), 1.24−1.40 (m, 4H), 1.46−1.60 (m, 4H), 1.60 (d, J = 5.4 Hz, 3H), 1.92 (dd, J = 6.6, 15.0 Hz, 1H), 2.04 (d, J = 15.0 Hz, 1H), 3.17 (td, J = 3.0, 11.4 Hz, 2H), 3.25−3.40 (m, 3H), 3.55−3.75 (m, 2H), 3.87 (s, 3H), 3.99 (q, J = 9.0 Hz, 1H), 4.11−4.30 (m, 3H), 5.29 (d, J = 15.6 Hz, 1H), 5.38 (qd, J = 6.0, 15.6 Hz, 1H), 5.94 (s, 2H), 6.50 (d, J = 1.8 Hz, 1H), 6.63 (d, J = 1.8 Hz, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 531. Anal. Calcd for $C_{30}H_{46}N_2O_6$·0.80TFA: C, 61.03; H, 7.58; N, 4.50. Found: C, 61.04; H, 7.65; N, 4.46.

Receptor Binding Assays. All samples were kept at 4 °C throughout the process of membrane isolation. MMQ cells (prolactin secreting rat pituitary cells known to contain ET_A receptors), porcine cerebellar tissues (known to contain ET_B receptors), or Chinese hamster ovary cells (CHO) permanently transfected with the human ET_A or ET_B receptor are homogenized in 25 mL of 10 mM Hepes (pH 7.4) containing 0.25 M sucrose and a protease inhibitor cocktail [50 mM EDTA , 0.1 mM PMSF, and 5 μg/mL Pepstatin A, and 0.025% Bacitracin] using a micro ultrasonic cell disruptor (Kontes). The mixture was centrifuged at 1000*g* for 10 min. The supernatant was collected and centrifuged at 60000*g* for 60 min. The precipitate was resuspended in 20 mM Tris, pH 7.4, containing protease inhibitor cocktail and centrifuged again. The final membrane pellet was resuspended in 20 mM Tris, pH 7.4, containing protease inhibitors and stored at −80 °C until used. Protein content was determined by the Bio-Rad dye-binding protein assay.

Binding assays were performed in 96-well microtiter plates pretreated with 0.1% BSA. Membranes were diluted ∼100-fold in buffer B (20 mM Tris, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.4, with 0.2% BSA, 0.1 mM PMSF, 5 μg/mL Pepstatin A, 0.025% bacitracin, and 50 mM EDTA) to a final concentration of 0.2 mg/mL of protein. In competition binding studies, membranes (0.02 mg) were incubated with 0.1 nM [^{125}I]ET-1 (for ET_A assay in MMQ or CHO cells) or [^{125}I]ET-3 (for ET_B assay in porcine cerebellum or CHO cells) in buffer B (final volume: 0.2 mL) in the presence of increasing concentrations of the test compound for 3 h at 25 °C. After incubation, unbound ligand was separated from bound ligand by a vacuum filtration method using glass-fiber filter strips in PHD cell harvesters (Cambridge Technology, Inc., MA), washing the filter strips three times with saline (1 mL). Nonspecific binding was determined in the presence of 1 μM unlabeled ET-1. IC_{50} values are calculated using an average of at least two separate determinations.

Phosphoinositol Hydrolysis Assays. ET_A. MMQ cells (0.4×10^6 cells/mL) were labeled with 10 μCi/mL of [^{3}H]-myoinositol in RPMI for 16 h. The cells were washed with PBS and then incubated with buffer A (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, 25 mM Hepes, pH 7.4) containing protease inhibitors and 10 mM LiCl for 60 min. The cells were incubated with test compounds for 5 min and then challenged with 1 nM ET-1. ET-1 challenge was terminated by the addition of 1.5 mL of 1:2 (v/v) chloroform−methanol. Total inositol phosphates were extracted after adding chloroform and water to give final proportions of 1:1:0.9 (v/v/v) chloroform−methanol−water of as described by Berridge.[29] The upper aqueous phase (1 mL) was retained, and a small portion (100 μL) was counted. The rest of the

aqueous sample was analyzed by batch chromatography using anion-exchange resin AG1-X8 (Bio-Rad).

ET_B. Chinese hamster ovary cells (CHO) permanently transfected with the human ET_B receptor were grown to confluence in 24-well tissue culture plates and labeled with 5 μCi/well of [^{3}H]myoinositol in F-12 media + 10% FBS + 1×P/S/F. The adherent cells were washed gently with PBS and then incubated in 200 μL of buffer A containing protease inhibitors and 10 mM LiCl for 60 min at 37 °C in a CO_2 incubator. Test compounds were then added followed by the addition of 1 nM ET-1, and the mixtures were incubated for 30 min at 37 °C. The cells were then solubilized by the addition of 50 μL of 1 N NaOH and then neutralized by the addition of 50 μL of 1 N HCl. The solubilized cell suspension was transferred to glass tubes and extracted by the addition of 1.5 mL of 1:2 (v/v) chloroform−methanol. Total inositol phosphates were extracted and analyzed by batch chromatography on anion-exchange resin as above. All IC_{50} values are calculated using an average of at least two separate determinations.

Pharmacokinetic Analysis. The pharmacokinetic behavior of compounds were evaluated in male Sprague−Dawley rats. Briefly, the test compound was prepared as a 10 mg/mL solution in an ethanol−propylene glycol−D5W (20:30:50, by volume) vehicle containing 1 molar equiv of sodium hydroxide. Groups of rats ($n = 4$ per group) received either a 10 mg/kg (1 mL/kg) intravenous dose administered as a slow bolus in the jugular vein or a 10 mg/kg (1 mL/kg) oral dose administered by gavage. Heparinized blood samples (~0.4 mL/sample) were obtained from a tail vein of each rat 0.1 (iv only), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, and 12 h after dosing. The samples were analyzed by reverse phase HPLC following liquid−liquid extraction from the plasma. Initial estimates of the pharmacokinetic parameters (e.g., the maximum concentration C_{max}) for NONLIN84[30] were obtained with the program CSTRIP.[31] Area under the curve (AUC) values were calculated by the trapezoidal rule over the time course of the study. The terminal-phase rate constant (β) was utilized in the extrapolation of the AUC from 12 h to infinity to provide an $AUC_{0-\infty}$ value and in the calculation of $T_{1/2}$ values. Assuming dose proportionality and correcting for the differences in dosing, a comparison of the AUC following oral dosing with that obtained following an intravenous dose provided an estimate of the bioavailability (F).

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References

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

(2) 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.

(3) For recent reviews on the endothelin system, see: (a) Doherty, A. M. Endothelin: A New Challenge. *J. Med. Chem.* **1992**, *35*, 1493−1508. (b) Shiosaki, K. Recent Advances in Endothelin Receptor Antagonists. *Exp. Opin. Ther. Pat.* **1994**, *4*, 1361−1365. (c) Opgenorth, T. J. Endothelin Receptor Antagonism. *Adv. Pharmacol.* **1995**, *33*, 1−65. (d) Williams, D. L.; Walsh, T. F. Endothelin Blockade and Hypertension. In *Antihypertensive Drugs*; van Zwieten, P. A., Greenlee, W. J., Eds.; Harwood Academic: Amsterdam, 1997; pp 213−280. (e) Lago, M. A.; Luengo, J. I. Endothelin Antagonists. *Annu. Rep. Med. Chem.* **1996**, *31*, 81−90.

(4) Rubanyi, G. M.; Polokoff, M. A. Endothelins: Molecular Biology, Biochemistry, Pharmacology, Physiology, and Pathophysiology. *Pharmacol. Rev.* **1994**, *46*, 325−415.

(5) Simonson, M. S. Endothelins: Multifunctional Renal Peptides. *Physiol. Rev.* **1993**, *73*, 375−411.

(6) Haynes, W. G.; Webb, D. J. The Endothelin Family of Peptides: Local Hormones with Diverse Roles in Health and Disease? *Clin. Sci.* **1993**, *84*, 485−500.

(7) Battistini, B.; Chailler, P.; D'Orléans-Juste, P.; Briére, N.; Sirois, P. Growth Regulatory Properties of Endothelins. *Peptides* **1993**, *14*, 385−399.

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

(9) Sakurai, T.; Yanagisawa, M.; Takuwa, Y.; Miyazaki, H.; Kimura, S.; Goto, K.; Masaki, T. Cloning of a *c*DNA Encoding a Non-isopeptide-selective Subtype of the Endothelin Receptor. *Nature* **1990**, *348*, 732−735.

(10) Warner, T. D.; 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.

(11) Ishikawa, K.; Ihara, M.; Noguchi, K.; Mase, T.; Mino, N.; Saeki, T.; Fukuroda, T.; Fukami, T.; Ozaki, S.; Nagase, T.; Nishikibe, M.; Yano, M. Biochemical and Pharmacological Profile of a Potent and Selective EndothelinB-receptor Antagonist, BQ-788. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4892−4896.

(12) Vitola, J. V.; Forman, M. B.; Holsinger, J. P.; Kawana, M.; Atkinson, J. B.; Quertermous, T.; Jackson, E. K.; Murray, J. J. Role of Endothelin in a Rabbit Model of Acute Myocardial Infarction: Effects of Receptor Antagonists. *J. Cardiovasc. Pharmacol.* **1996**, *28*, 774−783.

(13) 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.

(14) (a) Underwood, D. C.; Bochnowicz, S.; Osborn, R. R.; Luttmann, M. A.; Hay, D. W. Nonpeptide Endothelin Receptor Antagonists. X. Inhibition of Endothelin-1- and Hypoxia-induced Pulmonary Pressor Responses in the Guinea Pig by the Endothelin Receptor Antagonist, SB 217242. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 1130−1137. (b) 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.

(15) Zimmermann, M. Endothelin in Cerebral Vasospasm. Clinical and Experimental Results. *J. Neurosurg. Sci.* **1997**, *41*, 139−151.

(16) Pollock, D. M.; Polakowski, J. S.; Wegner, C. D.; Opgenorth, T. J. Beneficial Effect of ET_A Receptor Blockade in a Rat Model of Radiocontrast-induced Nephropathy. *Ren. Fail.* **1997**, *19*, 753−761.

(17) 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.

(18) Winn, M.; von Geldern, T. W.; Opgenorth, T. J.; Jae, H.-S.; Tasker, A. S.; Boyd, S. A.; Kester, J. A.; Bal, R.; Sorensen, B.; Wu-Wong, J. R.; Chiou, W.; Dixon, D. B.; Novosad, E. I.; Hernandez, L.; Marsh, K. C. 2,4-Diarylpyrrolidine-3-carboxylic Acids−Potent ET_A Selective Endothelin Receptor Antagonists. 1. Discovery of A-127722. *J. Med. Chem.* **1996**, *39*, 1039−1048.

(19) Boyd, S. A.; Mantei, R. A.; Tasker, A. S.; Liu, G.; Sorensen, B. K.; Henry, K. J., Jr.; von Geldern, T. W.; Winn, M.; Wu-Wong, J. R.; Chiou, W. J.; Dixon, D. B.; Hutchins, C. W.; Opgenorth, T. J. Discovery of a Series of Pyrrolidine-based Endothelin Receptor Antagonists with Enhanced ET_A Receptor Selectivity. *Bioorg. Med. Chem. Lett.*, submitted.

(20) Brooks, D. W.; Lu, L. D.-L.; Masamune, S. C-Acylation Under Virtually Neutral Conditions. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 72−74.

(21) Weiler, L. Alkylation of the Dianion of β-Keto Esters. *J. Am. Chem. Soc.* **1970**, *92*, 6702−6704.

(22) Krapcho, A. P.; Diamani, J.; Cayen, C.; Bingham, R. 2-Carbethoxy Cyclooctanone. *Organic Synthesis*; John Wiley; New York, 1967; Vol. 47, p 20.

(23) von Geldern, T. W.; Hoffman, D. J.; Kester, J. A.; Nellans, H. N.; Dayton, B. D.; Calzadilla, S. V.; Marsh, K. C.; Hernandez, L.; Chiou, W.; Dixon, D. B.; Wu-Wong, J. R.; Opgenorth, T. J. Azole Endothelin Antagonist. III. Using ΔlogP as a Tool to Improve Absorption. *J. Med. Chem.* **1996**, *39*, 982−991.

(24) Roux, S.; Breu, V.; Giller, T.; Neidhart, W.; Ramuz, H.; Coassolo, P.; Clozel, J. P.; Clozel, M. Ro 61-1790, a New Hydrosoluble Endothelin Antagonist: General Pharmacology and Effects on Experimental Cerebral Vasospasm. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 1110−1118.

(25) Doherty, A. M.; Patt, W. C.; Edmunds, J. J.; Berryman, K. A.; Reisdorph, B. R.; Plummer, M. S.; Shahripour, A.; Lee, C.; Cheng, X.-M.; Walker, D. M.; Haleen, S. J.; Keiser, J. A.; Flynn,

M. A.; Welch, K. M.; Hallak, H.; Taylor, D. G.; Reynolds, E. E. Discovery of a Novel Series of Orally Active Non-Peptide Endothelin-A (ET_A) Receptor-Selective Antagonists. *J. Med. Chem.* **1995**, *38*, 1259−1263.

(26) (a) Stein, P. D.; Hunt, J. T.; Floyd, D. M.; Moreland, S.; Dickinson, K. E. J.; Mitchell, C.; Liu, E. C.-K.; Webb, M. L.; Murugesan, N.; Dickey, J.; McMullen, D.; Zhang, R.; Lee, V. G.; Serafino, R.; Delaney, C.; Schaeffer, T. R.; Kozlowski, M. The Discovery of Sulfonamide Endothelin Antagonists and the Development of the Orally Active ET_A Antagonist 5-(Dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulfonamide. *J. Med. Chem.* **1994**, *37*, 329−331. (b) Webb, M. L.; Bird, J. E.; Liu, E.C.-K.; Rose, P. M.; Serafino, R.; Stein, P. D.; Moreland, S. BMS-182874 Is a Selective, Nonpeptide Endothelin ET_A Receptor Antagonist. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 1124−1134.

(27) (a) Ohlstein, E. H.; Nambi, P.; Douglas, S. A.; Edward, R. M.; Gellai, M.; Lago, M. A.; Leber, J .D.; Cousins, R. D.; Gao, A.; Frazee, J. S.; Peishoff, C. E.; Bean, J. W.; Eggleston, D. S.; Elshourbagy, N. A.; Kumar, C.; Lee, J. A.; Yu, T.-L.; Louden, C.; Brooks, D. P.; Weinstock, J.; Feuerstein, G.; Poste, G.; Ruffolo, R. R., Jr.; Gleason, J. G.; Elliott, J. D. SB 209670, a Rationally Designed Potent Nonpeptide Endothelin Receptor Antagonist. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8052−8056. (b) Ohlstein, E. H.; Nambi, P.; Lago, A.; Hay, D. W. P.; Beck, G.; Fong, K.-L.; Eddy, E. P.; Smith, P.; Ellens, H.; Elliott, J. D. Nonpeptide Endothelin Receptor Antagonists. VI: Pharmacological Characterization of SB 217242, A Potent and Highly Bioavailable Endothelin Receptor Antagonist. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 609−615.

(28) Wu, C.; Chan, M. F.; Stavros, F.; Raju, B.; Okun, I.; Mong, S.; Keller, K. M.; Brock, T.; Kogan, T. P.; Dixon, R. A. F. Discovery of TBC11251, a Potent, Long Acting, Orally Active Endothelin Receptor-A Selective Antagonist. *J. Med. Chem.* **1997**, *40*, 1690−1697.

(29) Berridge, M. J. ; Dowens, C. P.; Hanley, M. R. Lithium Amplifies Agonist Dependent Phosphatidylinositol Responses in Brain and Salivary Glands. *Biochem. J.* **1982**, *206*, 587−595.

(30) Statistical Consultants, Inc. PCNONLiN and NONLIN84: Software for the Statistical Analysis of Nonlinear Models. *Am. Stat.* **1986**, *40*, 52.

(31) Sedman, A. J.; Wagner, J. G. CSTRIP-A FORTRAN Computer Program for Obtaining Initial Polyexponential Estimates. *J. Pharm. Sci.* **1976**, *65*, 1006−1010.

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