Pyrrolidine-3-carboxylic Acids as Endothelin Antagonists. 4. Side Chain Conformational Restriction Leads to ET_B Selectivity

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When the dialkylacetamide side chain of the ET_A-selective antagonist ABT-627 is replaced with a 2,6-dialkylacetanilide, the resultant analogues show a complete reversal of receptor selectivity, preferring ET_B over ET_A. By optimizing the aniline substitution pattern, as well as the alkoxy group on the 2-aryl substituent, it is possible to prepare antagonists with subnanomolar affinity for ET_B and with selectivities in excess of 4000-fold. A number of these compounds also show promising pharmacokinetic profiles; a useful balance of properties is found in A-192621 (**38**). Pharmacology studies with A-192621 serve to reveal the role of the ET_B receptor in modulating blood pressure; the observed hypertensive response to persistent ET_B blockade is consistent with previous postulates and indicates that ET_B-selective antagonists may not be suitable as agents for long-term systemic therapy.

Introduction

Endothelin (ET),[1] a 21-amino acid bicyclic peptide, is the most powerful peptidic constrictor of vascular smooth muscle reported to date, as well as a potent mitogen. Produced predominantly by endothelial cells, it acts in both an autocrine and a paracrine fashion as a mediator of vascular function. It has been implicated as a pathogenic factor for a variety of disease states,[2a] including asthma,[2b] coronary vasospasm and myocardial infarction,[2c] pulmonary hypertension,[2d] restenosis,[2e] and atherosclerosis,[2f] in which excessive vasoconstriction or smooth muscle proliferation plays a role.

Endothelin acts by binding to a family of membrane-associated G-protein coupled receptors (GPCRs).[3] Binding to the ET_A receptor subtype, which predominates in vascular smooth muscle cells, triggers a cascade of events which leads, via the hydrolysis of inositol phosphates, to the observed vasoconstrictive and proliferative responses. The results of binding to ET_B, which is the major receptor on endothelial cells, are less clearly understood. While this receptor mediates constriction in some tissue beds,[4] it has also been linked to vasodilation through the production of nitric oxide[5] and to the clearance of endogenous ET.[6]

A number of groups, including our own, have reported the development of potent nonpeptidic endothelin antagonists which bind selectively to the ET_A receptor subtype or are nonselective. These agents have provided valuable insights into the role of the ET_A receptor in mediating a variety of disease states. There have been fewer reports,[7] however, describing high-quality ET_B antagonists, and correspondingly the role of ET_B remains more obscure. In the course of a series of studies probing the conformation of the amide side chain of ABT-627 (**1**), we made an unexpected observation which led to a complete reversal of receptor selectivity and led to the first ET_B-selective agent of this class. This observation has been pursued and has led to the discovery of A-192621, a potent and orally deliverable ET_B-selective antagonist. As one example of how such a selective agent can improve our understanding of endothelin receptor biology, pharmacology studies employing A-192621 have helped to elucidate the role of ET_B receptors in the maintenance of vascular tone.

Key Observation

Several previous studies by our group have indicated the critical role that the N-linked side chain of **1** plays in determining receptor binding and selectivity (Scheme 1).[8,9] Structure−activity studies during the development of ABT-627 (IC_{50} = 0.08 nM against ET_A; 1800 times selective for ET_A versus ET_B) indicated to us the importance of the side chain amide carbonyl. We suspect that this carbonyl oxygen is involved in a hydrogen-bonding interaction with the ET_A receptor which serves to orient the butyl groups into appropriate hydrophobic binding pockets. Our work also indicated that the presence of *both* alkyl groups are required to maintain high ET_A selectivity. We later learned that, by modifying the orientation of these two alkyl groups, we can produce analogues which retain high affinity for ET_A but simultaneously bind to ET_B receptors as well. The result of these studies was A-182086 (**2**), the most potent and "balanced" antagonist reported to date (IC_{50} = 0.08 nM against ET_A; A/B = 3).

On the basis of the above results, we continued to explore conformational restriction as a route to improving the selectivity of compounds of this class. One way to restrict the motions of these side chain alkyl groups would be to tie them back as substituents on an aromatic ring (e.g. compound **3**). Fortunately this strat-

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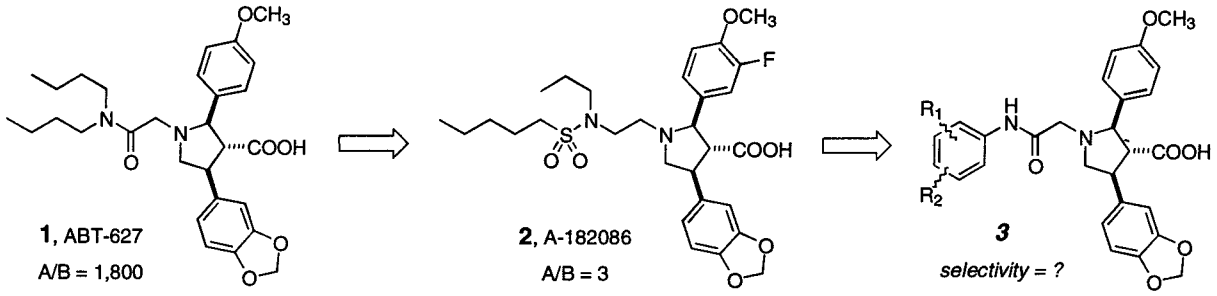
Scheme 1. Conceptual Framework for Side Chain Conformational Restriction Strategy



egy is an easy one to test and requires no knowledge of the preferred substituent orientation, since all of the required dimethylanilines are commercially available. We thus prepared a series of compounds (Table 1, **4**−**9**) which covers all of the possibilities. In general these compounds are relatively inactive and exhibit modest selectivity for the ET_A receptor subtype. A notable exception is analogue **7**, which carries methyl groups at the 2- and 6-positions. Surprisingly, this compound is slightly selective for the ET_B over ET_A receptor and has a relatively high affinity for ET_B as well (IC_{50} < 20 nM).

Chemistry

The core pyrrolidines employed in this study have been assembled (Scheme 2) by direct analogy to our earlier work.[8] Briefly, the Michael reaction between β-ketoesters **13** and nitrostyrene **11** provides a mixture of diastereomeric nitroketones. The ketoesters themselves are prepared from the corresponding ketones (by carbethoxylation) or benzoic acids (through activation and malonate homologation). Nitrostyrene **11** derives from Henry reaction of piperonal (**10**). The diastereomeric mixture of nitroketones is reductively cyclized in two steps (Raney nickel reduction to provide a cyclic imine, which is further reduced using cyanoborohydride) to give pyrrolidines **14**, generally as a mixture of three diastereomers in which the desired *trans,trans*-isomer predominates. An additional portion of *trans,trans*-material may be derived by epimerization (DBU, toluene) of the *cis,cis*-isomer, which is conveniently separated by chromatography.

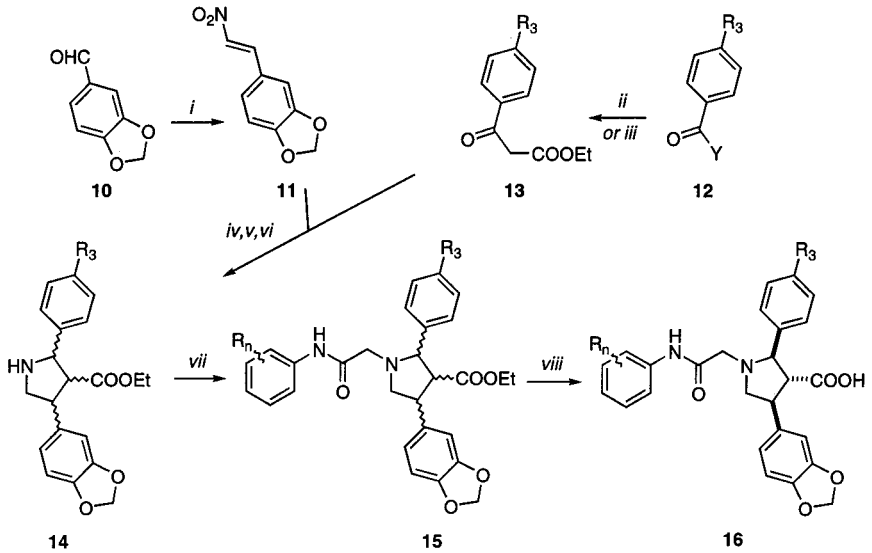
Appropriately substituted anilines are acylated with bromoacetyl bromide to provide the corresponding bromoacetanilides, which are used to alkylate the above pyrrolidines **14**. Under standard saponification conditions (NaOH, MeOH, room temperature, overnight) the hydrolysis of the *trans,trans*-ester is dramatically faster than that of any other isomer, providing the pure *trans,trans*-acid **16**. This valuable selective hydrolysis was first observed during the preparation of our ET_A-selective analogues.[5] When necessary, optically active analogues may be prepared through application of pyrrolidine core resolution strategies we have described previously.[8,9]

Compound Evaluation

The first line of biochemical analysis for the compounds described in this study is a measurement of their ability to displace endothelin from its receptor. For the purpose of screening we employ human ET_A and ET_B receptors (hET_A, hET_B) permanently expressed in CHO cells. 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.

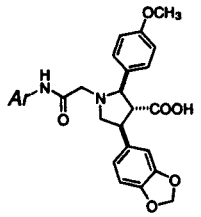
Analogues of particular interest are examined for their ability to block the ET-1-induced hydrolysis of

Scheme 2. Synthesis of Arylacetanilide Analogues[a]



[a] CH_3NO_2, NH_4OAc, HOAc, heat; (ii) NaH, $(EtO)_2CO$, THF; (iii) CDI, THF; MgOOCCHCOOEt, DMF; (iv) DBU, THF−iPrOH; (v) H_2, Ra−Ni, THF−HOAc; $NaBH_3CN$, THF−EtOH, pH 5; (vi) DBU, THF, heat; (vii) $BrCH_2CONHAr$, iPR_2NEt, CH_3CN; (viii) NaOH, H_2O−EtOH.

Table 1.



compound	Ar	*h*ET$_A$ binding IC$_{50}$, nM (range, N)[a]	*h*ET$_B$ binding IC$_{50}$, nM (range, N)[a]	A/B ratio	formula	characterization
4		16 (13-20, 2)	81 (72-91, 2)	0.2	$C_{29}H_{30}N_2O_6 \cdot 0.5\ H_2O$	NMR,MS,CHN
5		25 (18-34, 2)	74 (66-82, 2)	0.3	$C_{29}H_{30}N_2O_6 \cdot 0.8\ H_2O$	NMR,MS,CHN
6		33 (30-36, 2)	82 (79-86, 2)	0.4	$C_{29}H_{30}N_2O_6 \cdot 0.8\ H_2O$	NMR,MS,CHN
7		33 (27-40, 2)	16 (14-18, 2)	2	$C_{29}H_{30}N_2O_6 \cdot 0.5\ H_2O$	NMR,MS,CHN
8		50 (42-59, 2)	830 (810-840, 2)	0.06	$C_{29}H_{30}N_2O_6 \cdot 0.8\ H_2O$	NMR,MS,CHN
9		160 (96-300, 3)	2,700 (1,250-5,200, 3)	0.06	$C_{29}H_{30}N_2O_6 \cdot 0.5\ H_2O$	NMR,MS,CHN
17		15 (8-31, 3)	4.0 (2.1-6.7, 3)	4	$C_{30}H_{32}N_2O_6 \cdot 0.5\ H_2O$	NMR,MS,CHN
18		100 (78-160, 3)	20 (15-30, 3)	5	$C_{31}H_{34}N_2O_6 \cdot 0.5\ H_2O$	NMR,MS,CHN
19		45 (40-51, 2)	1.4 (1.1-1.7, 2)	32	$C_{31}H_{34}N_2O_6$	NMR,MS,CHN
20		15 (8.2-41, 4)	82 (41-140, 2)	0.2	$C_{32}H_{36}N_2O_6 \cdot 1.0$ TFA	NMR,MS,CHN
21		12 (4.1-30, 3)	460 (210-760, 3)	0.03	$C_{33}H_{38}N_2O_6 \cdot 0.5\ H_2O$	NMR,MS,CHN
22		920 (1)	26 (1)	35	$C_{33}H_{38}N_2O_6 \cdot 0.5\ H_2O$	NMR,MS,CHN
23		190 (100-660, 5)	600 (190-1,000, 4)	0.3	$C_{32}H_{36}N_2O_6$	NMR,MS,CHN
24		200 (160-270, 3)	5.8 (2.8-13, 3)	34	$C_{30}H_{32}N_2O_7 \cdot 0.7$ TFA	NMR,MS,CHN
25		16 (4.6-49, 4)	57 (17-170, 4)	0.3	$C_{29}H_{30}N_2O_8 \cdot 0.75$ HOAc	NMR,MS,CHN
26		61 (51-83, 3)	10 (6.1-17, 3)	6	$C_{27}H_{36}N_2O_8S \cdot 0.3\ H_2O$	NMR,MS,CHN
27		22 (4.5-65, 4)	0.8 (0.45-2.4, 3)	24	$C_{31}H_{33}N_2O_6F \cdot 0.3$ EtOAc	NMR,MS,CHN
28		18 (5.4-60, 3)	1.2 (0.5-1.8, 3)	15	$C_{32}H_{36}N_2O_6 \cdot 0.5\ H_2O$	NMR,MS,CHN
29		10 (2.7-33, 4)	1.5 (0.7-4.7, 4)	7	$C_{33}H_{38}N_2O_6 \cdot 0.25\ H_2O$	NMR,MS,CHN
30		840 (420-1,700, 3)	44 (28-71, 3)	19	$C_{32}H_{34}N_2O_8 \cdot 0.5\ H_2O$	NMR,MS,CHN

[a] IC$_{50}$ values calculated using a mean of at least 2 measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} M unless otherwise noted.

inositol phosphates in ET_B-CHO. In these experiments the compounds are simultaneously tested for the ability to function as agonists. This same subset of compounds of particular interest has also been examined for their pharmacokinetic properties using a standard protocol which compares the time course of plasma drug levels after dosing in rats by intravenous injection and oral gavage.

Results and Discussion

We initially chose to probe the question of substitution pattern on the aniline ring using two methyl groups, largely due to the ready availability of all possible dimethylanilines. Having determined that 2,6-substitution led to an intriguing result, we immediately followed up with a study of alkyl chain length. Our previous studies leading to ABT-627 had indicated that this parameter could be of critical importance in determining not only receptor affinity but also selectivity as well. In the event, one-carbon extension of the 2-substituent to give **17** led to slight improvements in both potency and ET_B selectivity (IC_{50} = 4.0 nM; A/B = 4). While branching (to give **18**) did not provide further benefit, a similar one-carbon homologation of the 6-methyl group was accompanied by additional improvements in potency and selectivity; the resultant 2,6-diethyl analogue **19** exhibits 1.4 nM affinity for ET_B and is >30-fold selective. Further homologation of one (**20**) or both (**21**) alkyl groups leads to a reversion to ET_A selectivity. Thus, it appears that a four-atom spacing between the amide nitrogen and the alkyl terminus is preferred for both alkyl groups. Interestingly, a similar four-atom spacing (albeit with a dramatically different geometry) provides the di-*n*-butylamide which defines the ET_A selectivity of ABT-627. Placement of a branched alkyl chain at both 2- and 6-positions (**22**) leads to a 20-fold decrease in activity, though selectivity remains high. Methylation of the amide nitrogen has a dramatic impact; the resultant analogue **23** is modestly ET_A-selective and has lost affinity for *both* ET_A and ET_B.

Other substituents are tolerated at the 2- and 6-positions as well, though none is the equal of ethyl. Sequential isosteric replacement of each ethyl group with methoxy (**24**, **25**) causes a loss of 4−10-fold in ET_B binding affinity in each case; while the first of these replacements has little effect on selectivity, this is eroded as well in the 2,6-dimethoxy analogue. In similar fashion, 2,6-dibromo analogue **26** is a poorer ligand for ET_B as well (IC_{50} = 10 nM). Substitution at the 4-position is well-tolerated in some circumstances. Addition of a 4-fluorine atom provides a compound (**27**) which retains the activity/selectivity profile of analogue **19**; a small 4-alkyl group (**28**, **29**) decreases ET_B selectivity 2-fold with little effect on ET_B binding, while 4-carboxy substitution (**30**) leads to a substantial decrease in activity. In the end, none of these modifications provides a significant improvement over the 2,6-diethylacetanilide. It is clear from the above results that there is a very limited hydrophobic space on the ET_B receptor which is efficiently occupied by this conformationally restricted side chain.

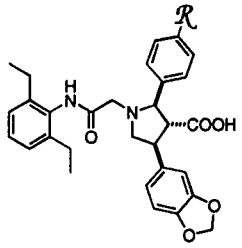
Our previous studies had highlighted the importance of the 2-aryl ring in "tuning" the activity and selectivity profile for this family of pyrrolidine-based ET antagonist molecules, once the essential nature of the profile (e.g. ET_A-selective, balanced) had been established through modifications of the side chain group. Having now identified a new class of antagonist, ET_B-selective by virtue of a 2,6-dialkylanilide moiety, we began a short series of studies to optimize this profile through a similar strategy (Table 2). As in past studies, this exercise proved fruitful. Sequential homologation of the *p*-methoxy substituent to ethoxy (**31**, IC_{50} = 3.6 nM) and propoxy (**32**, IC_{50} = 12 nM) led to small but significant losses of ET_B affinity (~10-fold total decrease) over the series, with a substantially larger decrease (>200-fold total) at ET_A. As a result, the extended versions were increasingly less active, but more selective for ET_B. Replacement of the linear alkyl group with isopropyl (**33**) caused a loss of activity; however, the *p*-methoxyethoxy group of **34** was well-tolerated and led for the first time to an antagonist with nanomolar ET_B affinity and >1000-fold selectivity. This latter result led us to examine the roles of the two oxygen atoms on this *para*-substituent; indeed, when the proximal ether is removed (to give **35**), potency and selectivity remain high.

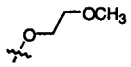
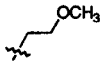
Preliminary pharmacokinetic analysis of a number of the compounds described above, as racemates (data not shown), suggested a smaller subset (**19**, **31**, **32**, **34**) which warranted further study as single enantiomers. In general, the relevant pyrrolidine cores may be resolved through one of several general strategies we have previously described. The active enantiomers were identified and characterized in some detail (Table 3). Because our primary goal for this effort is to identify an orally deliverable agent which can help to address the role of ET_B receptor blockade in disease therapy, we felt that it was important to emphasize selectivity over potency in choosing a candidate for further evaluation in disease models. This analysis led us to pick compound **38** (A-192621) for scaleup and additional studies.

To confirm that these compounds are antagonists, several of the compounds in Table 3 have been evaluated in a functional assay measuring ET receptor-mediated hydrolysis of inositol phosphates. The compounds show a high level of antagonist activity in this assay and exhibit no agonist effect even at high doses (data not shown), confirming that as a class they are pure, functional antagonists of the ET_B receptor.

Pharmacology of ET_B Blockade. To determine whether the in vitro profile described above for A-192621 was manifest in vivo, we began by examining the effects of acute dosing of the compound in an ET_A/ET_B pseudoefficacy model (Figure 1). Briefly, a number of groups have demonstrated that a bolus injection of ET-1 produces a biphasic response on blood pressure. A rapid, sharp, and transient period of hypotension, which has been demonstrated to be mediated through ET_B-induced release of nitric oxide, is followed by a long period of elevated pressure which has a slower onset and which is mediated through the ET_A receptor. A single oral dose of A-192621 (30 mg/kg, by gavage), given 1 h prior to repeating this study, completely blocks the ET-1-induced depressor effect, a result which is expected with a potent ET_B antagonist. As would be predicted for a selective agent, we observe no decrease in the pressor response. In fact, this pressor effect appears to have a

Table 2. Effects of Modifying the Phenoxyalkyl Substituent



compound	$\mathcal{R}$	*h*ET$_A$ IC_{50}, nM (range, N)[a]	*h*ET$_B$ IC_{50}, nM (range, N)[a]	A/B ratio	formula	characterization
19		45	1.4	32	---	---
31		910 (290-1700, 4)	3.6 (2.5-5.7, 4)	250	$C_{32}H_{36}N_2O_6 \cdot 0.5\ H_2O$	NMR,MS,CHN
32		9,900 (3,500-16,000, 4)	12 (9-19, 4)	820	$C_{33}H_{38}N_2O_6 \cdot 0.25\ H_2O$	NMR,MS,CHN
33		1,500 (1,000-2,000, 3)	6.9 (4.9-10, 3)	220	$C_{33}H_{38}N_2O_6 \cdot 0.7$ TFA	NMR,MS,CHN
34		4,200 (2,700-5,000, 3)	1.1 (0.82-1.2, 3)	4,000	$C_{33}H_{38}N_2O_7$	NMR,MS,CHN
35		11,000 (5,000-19,000, 3)	3.8 (1.9-7.3, 3)	3,000	$C_{33}H_{38}N_2O_6 \cdot 0.2\ H_3PO_4$	NMR,MS,CHN

[a] IC_{50} values calculated using a mean of at least 2 measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} M unless otherwise noted.

more rapid onset, as well as a substantially longer duration of action. The apparently more rapid onset may be an unmasking effect which results from removal of the early depressor component. The increased half-life of this response probably reflects the postulated role of ET_B as a clearance receptor. With a primary clearance pathway removed through ET_B blockade, exogenously administered ET-1 is expected (and has been demonstrated[10]) to have an increased circulating half-life.

The above results suggest the possibility that long-term treatment with an effective ET_B-selective antagonist like A-192621 might produce hypertension in a normal animal as well. Such a hypertensive liability would substantially limit the utility of these agents in chronic therapy. To test this possibility, we performed a study in which normal rats received A-192621 (30 mg/kg/day in food) for 3 days. The results (Table 4) confirm that, while no significant effects on food or water intake were observed, a substantial increase in mean arterial pressure (23 mmHg) results, along with a significant decrease in heart rate (12%). Importantly, the addition of an ET_A-selective antagonist rapidly returns the elevated blood pressure to normal values.[11] ET-1 levels increase 5-fold in the drug-treated group, again confirming the role of ET_B as a clearance receptor and strongly suggesting that the hypertensive effect is related to an increase in the amount of circulating endothelin.

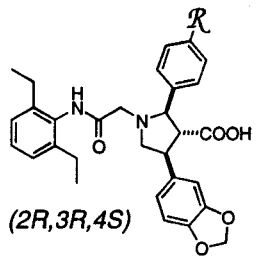
Conclusions

An unexpected observation during the course of a series of conformational studies has led us to the observation that a 2,6-dialkylacetanilide side chain imparts ET_B selectivity to this family of pyrrolidine-based endothelin antagonists. Optimization of substituents on the aniline ring and of the alkoxy moiety on the 2-aryl group led to compounds with subnanomolar affinities for ET_B and selectivities of up to 4000-fold for ET_B versus ET_A. Some of the antagonists in this series are orally deliverable. A series of analogues were prepared in optically active form for detailed evaluation, and compound **38**, A-192621, was chosen for further study based on a combination of biochemical and pharmacokinetic properties. Pharmacology studies of A-192621 in rats confirm that this compound does indeed function as an ET_B-selective agent in vivo. Three-day oral dosing in food leads to a substantial increase in blood pressure, indicating that the ET_B receptor plays a role in the maintenance of basal tone, presumably by acting as a scavenger for circulating endothelins. This latter result seems to suggest that long-term ET_B antagonism may only be acceptable as a therapeutic modality in the context of simultaneous ET_A blockade.

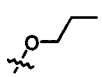
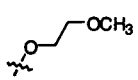
Experimental Section

Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. THF was dried over sodium and purified by distillation. All reactions were performed under nitrogen atmosphere unless specifically noted. Flash 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

Table 3. Summary Data for Selected Enantiomerically Pure Analogues



(2R,3R,4S)

compound	R	hET_A IC_{50}, nM (range, N)[a]	hET_B IC_{50}, nM (range, N)[a]	A/B ratio	PI hydrolysis IC_{50} (nM)[a]	i.v. $T_{1/2}$ (hr)	oral C_{max} / T_{max} (μg/mL ; hr)	AUC_{oral} (μg-hr/mL)	F (%)	formula	characterization
36		89 (78-95, 3)	0.27 (0.24-0.31, 2)	330	---	6.4	0.93 / 1.0	3.9	49%	$C_{31}H_{34}N_2O_6$	NMR,MS,HRMS
37		340 (200-740, 6)	1.2 (0.34-3.8, 6)	280	7.9	3.1	1.1 / 1.3	3.4	37%	$C_{32}H_{36}N_2O_6$	NMR,MS,HRMS
38 *(A-192621)*		8,200 (4,500-14,000, 6)	6.4 (3.0-16, 6)	1,300	0.65	5.0	0.81 / 1.6	2.5	35%	$C_{33}H_{38}N_2O_6$.HCl	NMR,MS,CHN
39		3,700 (1,400-7,800, 3)	0.85 (0.76-0.95, 2)	4,400	---	2.6	0.34 / 1.0	0.65	9%	$C_{33}H_{38}N_2O_7$	NMR,MS,HRMS

[a] IC_{50} values calculated using a mean of at least 2 measurements (all duplicates) for 11 concentrations from 10^{-10} to 10^{-5} M unless otherwise noted.

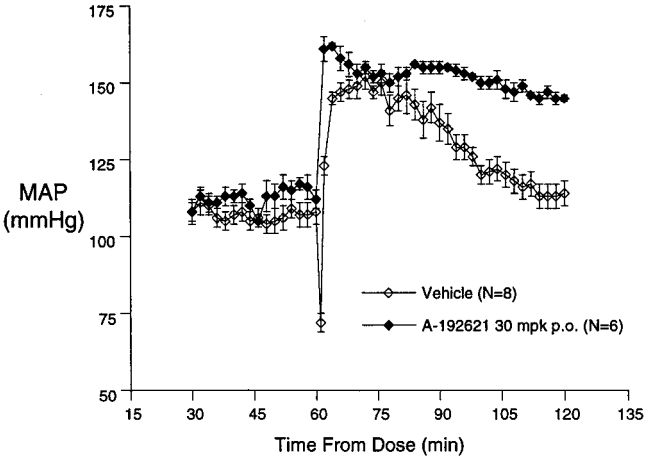


Figure 1. Pseudoefficacy data: effect on mean arterial pressure (MAP) of a bolus dose of ET-1 (0.3 nmol/kg, given at $T = 60$ min) to anesthetized rats in the absence (open diamonds) or presence (solid diamonds) of a prior dose of A-192621 (administered orally at $T = 0$). The ET_B antagonist completely blocks the transient hypotensive spike, unveiling an earlier phase of the hypertensive response. The hypertensive phase also persists longer, presumably due to reduced ET_B-mediated clearance of ET-1.

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.

General Synthesis of Core Compounds 14. In general, ketoesters **13** and core pyrrolidines **14** were prepared, and pyrrolidines **14** were resolved, according to the procedures we have described previously[5] and employing commercially available starting materials. An exception is described below.

4-(2-Methoxyethyl)benzoic Acid (12, $R_3 = CH_2CH_2OCH_3$, Y = OH). A three-necked 50-mL flask was charged with 800 mg (20 mmol) of 60% NaH in mineral oil. The suspension was washed and decanted with hexanes three times, while keeping a positive pressure of N_2 over the NaH. Next, 5 mL of THF was added, and the suspension was cooled with an ice bath. A solution of 2.01 g (10.0 mmol) of 4-bromophenethanol in 5 mL of THF was added via cannula, then the ice bath was removed, and the reaction was stirred for 10 min. After this time 700 mL (11.2 mmol) of methyl iodide was added, and the reaction was stirred at ambient temperature. After 1 h, the reaction was cooled with an ice bath, and the excess NaH was quenched by addition of 1 mL of H_2O. The mixture was poured into 60 mL of water and extracted with diethyl ether. The combined ether layers were back-extracted with brine (1 × 20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to 2.08 g (97%) of 1-bromo-4-(2-methoxyethyl)benzene as a colorless oil. To a solution of 1.03 g (4.78 mmol) of this compound in 10 mL of THF were added 400 mg of Mg and 1 crystal of I_2. The mixture was warmed to reflux for 5 min and then cooled to ambient temperature. The resultant Grignard reagent was transferred via syringe to a 50-mL three-necked flask under N_2 equipped with a CO_2 inlet. The CO_2 inlet was opened, and the red color faded to pale yellow. After 1 h, the reaction was concentrated in vacuo. The residue was taken up in 20 mL of H_2O, acidified with 12 M HCl, and extracted with diethyl ether (3 × 10 mL). The combined ether layers were extracted with 2 M NaOH (3 × 5 mL); then the combined basic layers were extracted with ether (2 × 5 mL). The basic layer was acidified with 12 M HCl and then extracted with ethyl acetate (2 × 5 mL). The combined ethyl acetate layers were back-extracted with brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated to 427 mg of a solid (50%).

General Synthesis of Bromoacetanilides. A solution of aniline (3 mmol) in 1.5 mL of dichloromethane was cooled on ice; 1.05 equiv of bromoacetyl bromide in 1 mL of dichloromethane was added dropwise, followed by 1.0 equiv of diisopropylethylamine. The solution turned dark and a precipate formed, as the reaction mixture was warmed to ambient temperature over 1.5 h. The mixture was taken up in heptane and extracted three times with water; the organic phase was dried and purified by silica gel chromatography. All anilines are commercially available or were prepared according to literature precedent, except as described below.

Table 4. Effects of Long-Term ET_B Blockade[a]

	MAP (mmHg)	ET-1 level (pg/mL)
control	127	4
A-192621-treated	150	19

[a] Normal (Sprague−Dawley) rats received ET_B-selective antagonist A-192621 in food (30 mpk/day) for 21 days. The resultant substantial rise in MAP occurs concommitantly with a 5 times increase in circulating levels of ET-1, suggesting that the ET_B receptor serves a clearance role.

2,6-Diethyl-4-fluoroaniline. To an ice-cooled solution of 1.00 g (9.00 mmol) of 4-fluoroaniline in 20 mL of CH_2Cl_2 was added a solution of 5:1 CH_2Cl_2:Br_2 until an orange color persisted. During the reaction, a precipitate formed. The reaction was extracted with water (1 × 10 mL), saturated aqueous $NaHCO_3$ solution (1 × 10 mL), and then brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to 2.04 g of a solid. TLC (5% EtOAc/hexanes) showed only one spot, representing 2,6-dibromo-4-fluoroaniline. To a mixture of 122 mg (0.15 mmol) of (dppf)$PdCl_2$ and 3.48 g (10.7 mmol) of $CsCO_3$ was added a solution of 480 mg (1.78 mmol) of 2,6-dibromo-4-fluoroaniline in 10 mL of DMF, all under N_2. Next, 4 mL of 1.0 M triethylborane in hexanes was added. The reaction was heated at 50 °C for 22 h, then poured into 50 mL of water, and extracted with diethyl ether (3 × 10 mL). The combined ether layers were back-extracted with saturated aqueous $NaHCO_3$ solution (1 × 10 mL) and brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to an oil. The product was purified via silica gel chromatography, eluting with 20% EtOAc/hexanes to give 192 mg (64%) of a colorless oil.

2-Ethyl-6-methoxyaniline. Potassium ethylmalonate (3.68 g) was combined with 2.29 g of magnesium chloride in 12 mL of DMF; the reaction mixture was heated at 60 °C for 4 h. The resultant mixture was cooled to ambient temperature. Simultaneously, 3-methoxy-2-nitrobenzoic acid (3.4 g) was dissolved in 12 mL of DMF; 3.06 g of 1,1-carbonyldiimidazole was added (gas evolves), and the resultant solution (after stirring at ambient temperature for 4 h) was added to the malonate mixture. The resultant slurry was stirred at ambient temperature for 14 h. Solvents were removed in vacuo; the residue was taken up in EtOAc, washed sequentially with 1 N H_3PO_4, bicarbonate, and brine, and concentrated in vacuo. The crude product (3.2 g) was dissolved in 50 mL of concentrated sulfuric acid and stirred at ambient temperature for 48 h. The reaction mixture was poured onto 300 mL of ice and extracted twice with EtOAc. The organic extracts were washed sequentially with water, bicarbonate, and brine and concentrated in vacuo. The crude product was heated neat at 160 °C for 3 h. The resultant dark brown residue was extracted with EtOAc. The organic extracts were concentrated. The crude product was dissolved in 15 mL of ethanol, sodium borohydride (450 mg) was added, and the resultant solution was stirred at ambient temperature for for 2 h. The solvents were removed in vacuo; the residue was taken up in 10% aqueous HCl and stirred for 15 min. The mixture was extracted with EtOAc; the organic extracts were washed sequentially with bicarbonate and brine and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel, eluting with 1:1 EtOAc/hexanes, to give 1.08 g (32% overall) of the title compound as a colorless oil. A sample of this material (310 mg) was dissolved in 10 mL of THF; 1.5 mL of H_3PO_4 was added, followed by 50 mg of 10% palladium-on-charcoal. The resultant mixture was purged with nitrogen, then placed under a balloon of hydrogen, and stirred overnight. Bicarbonate was added carefully, and the mixture was filtered through a pad of Celite. The filtrate was extracted with EtOAc; the

organic extracts were washed with bicarbonate and brine and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel, eluting with 1:1 ether/hexanes, to give 102 mg (43% yield) of the title compound as a colorless oil.

2-Ethyl-6-propylaniline. To a stirred solution of 2-ethylaniline (5.00 g., 41.3 mmol) in tetrahydrofuran (50 mL) at ambient temperature were added benzyl bromide (4.91 mL, 41.3 mmol) and triethylamine (6.90 mL, 49.5 mmol). The reaction was heated at reflux overnight. Reaction was diluted with ethyl ether and washed with water and brine. Organic phase was dried with sodium sulfate, and solvents were removed in vacuo. Residue was purified on silica gel eluting with 98:2 hexanes−ethyl acetate to yield *N*-benzyl-2-ethylaniline as a yellow oil (2.20 g, 25% yield). This material was combined with allyl bromide (1.80 mL, 20.9 mmol) and sodium carbonate (663 mg, 6.26 mmol) in 4:1 ethanol/water (20 mL) at ambient temperature. Reaction was stirred overnight at ambient temperature. Reaction was diluted with ethyl ether and washed with water and brine. Organic phase was dried with sodium sulfate, and solvents were removed in vacuo. Residue was purified on silica gel eluting with 99:1 hexanes−ethyl ether to yield *N*-allyl-*N*-benzyl-2-ethylaniline as a colorless oil (1.90 g, 73% yield). To a stirred solution of this compound (1.00 g, 4.0 mmol) in anhydrous *o*-xylene (8 mL) at −78 °C under a nitrogen atmosphere was added zinc chloride (654 mg, 4.80 mmol). Reaction was allowed to warm to ambient temperature and then heated at reflux overnight. Reaction was cooled to 0 °C and quenched with 1 N sodium hydroxide. This was diluted with ethyl ether, and layers were separated. Organic phase was then washed with brine and dried with sodium sulfate. Solvents were removed in vacuo. Residue was purified on silica gel eluting with hexane to 99:1 hexanes−ethyl acetate to 97:3 hexanes−ethyl acetate to yield the desired product in 70% purity. This partially purified material, dissolved in 5 mL of acetic acid, was added to a flask under a nitrogen atmosphere containing 10% palladium-on-carbon catalyst (100 mg). Mixture was stirred vigorously for 3 h at ambient temperature under an atmosphere of hydrogen. Catalyst was filtered off through Celite and washed with ethyl acetate. Solvents were removed in vacuo. Residue was purified on silica gel eluting with 97:3 hexanes−ethyl acetate to 9:1 hexanes−ethyl acetate to yield the desired product (126 mg, 19% yield).

4-Bromo-2,6-diethylaniline. To a stirred solution of 2,6-diethylaniline (10.0 g, 67.0 mmol) in acetic acid (50 mL) at ambient temperature was added bromine (10.4 mL, 201 mmol). The reaction was stirred overnight at ambient temperature. The reaction mixture was diluted with diethyl ether (200 mL) and washed with 5% sodium bisulfite (4 × 50 mL) and brine. The organic phase was dried with sodium sulfate, and the solvents were removed in vacuo. The residue was chromatographed on silica gel, eluting with 9:1 hexanes−ethyl acetate to give the title compound (3.28 g, 21% yield).

General Synthesis of Antagonists. Preparation of final products was accomplished via alkylation of cores **14** with bromoacetanilides, followed by selective hydrolysis, as we have described previously.[8]

Compound 4, ***trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,3-dimethylphenyl]acetamido)-pyrrolidine-3-carboxylic acid:** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.16 (s, 3H), 2.30 (s, 3H), 2.97 (d, J = 17 Hz, 1H), 3.07 (t, J = 10 Hz, 1H), 3.18 (dd, J = 9, 10 Hz, 1H), 3.45 (d, J = 17 Hz, 1H), 3.50 (dd, J = 3, 10 Hz, 1H), 3.70 (m, 1H), 3.79 (s, 3H), 3.93 (d, J = 9 Hz, 1H), 5.95 (dd, J = 1, 2 Hz, 2H), 6.75 (d, J = 8 Hz, 1H), 6.83 (dd, J = 1, 8 Hz, 1H), 6.9 (m, 3H), 6.97 (d, J = 8 Hz, 1H), 7.08 (t, J = 7 Hz, 1H), 7.36 (brd d, J = 9 Hz, 2H), 7.64 (d, J = 7 Hz, 1H), 8.67 (brd s, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 503. Anal. Calcd for $C_{29}H_{30}N_2O_6 \cdot 0.5H_2O$: C, 68.09; H, 6.11; N, 5.48. Found: C, 68.13; H, 5.91; N, 5.29.

Compound 5, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,4-dimethylphenyl]acetamido)-pyrrolidine-3-carboxylic acid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.23 (s, 3H), 2.28 (s, 3H), 2.95 (d, J = 17 Hz, 1H), 3.05 (t, J = 10 Hz, 1H), 3.18 (dd, J = 9, 10 Hz, 1H), 3.45 (d, J = 17 Hz, 1H), 3.50 (dd, J = 3, 10 Hz, 1H), 3.70 (m, 1H), 3.79 (s, 3H), 3.93 (d, J = 9 Hz, 1H), 5.95 (dd, J = 1, 2 Hz, 2H), 6.75 (d, J = 8 Hz, 1H), 6.83 (dd, J = 1, 8 Hz, 1H), 6.9 (m, 3H), 7.0 (m, 2H), 7.36 (brd d, J = 9 Hz, 2H), 7.78 (d, J = 7 Hz, 1H), 8.60 (brd s, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 503. Anal. Calcd for $C_{29}H_{30}N_2O_6 \cdot 0.8H_2O$: C, 67.38; H, 6.16; N, 5.42. Found: C, 67.42; H, 5.95; N, 5.13.

Compound 6, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,5-dimethylphenyl]acetamido)-pyrrolidine-3-carboxylic acid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.24 (s, 3H), 2.29 (s, 3H), 2.97 (d, J = 17 Hz, 1H), 3.07 (t, J = 10 Hz, 1H), 3.19 (dd, J = 9, 10 Hz, 1H), 3.45 (d, J = 17 Hz, 1H), 3.50 (dd, J = 3, 10 Hz, 1H), 3.70 (m, 1H), 3.79 (s, 3H), 3.93 (d, J = 9 Hz, 1H), 5.95 (dd, J = 1, 2 Hz, 2H), 6.74 (d, J = 8 Hz, 1H), 6.83 (dd, J = 1, 8 Hz, 1H), 6.9 (m, 4H), 7.05 (d, J = 8 Hz, 1H), 7.36 (brd d, J = 9 Hz, 2H), 7.78 (s, 1H), 8.63 (brd s, 1H); MS (APCI) $(M + H)^+$ at m/z 503. Anal. Calcd for $C_{29}H_{30}N_2O_6 \cdot 0.8H_2O$: C, 67.38; H, 6.16; N, 5.42. Found: C, 67.72; H, 5.89; N, 5.25.

Compound 7, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-dimethylphenyl]acetamido)-pyrrolidine-3-carboxylic acid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.10 (s, 6H), 3.00 (d, J = 18 Hz, 1H), 3.13 (m, 2H), 3.46 (d, J = 18 Hz, 1H), 3.55 (dd, J = 5,10 Hz, 1H), 3.72 (m, 1H), 3.81 (s, 3H), 3.95 (d, J = 10 Hz, 1H), 5.93 (d, J = 2 Hz, 1H), 5.95 (d, J = 2 Hz, 1H), 6.75 (d, J = 9 Hz, 1H), 6.82 (dd, J = 2, 8 Hz, 1H), 6.88 (d, J = 2 Hz, 1H), 6.91 (d, J = 9 Hz, 2H), 7.08 (m, 3H), 7.37 (d, J = 9 Hz, 2H), 8.32 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 503. Anal. Calcd for $C_{29}H_{30}N_2O_6 \cdot 0.5H_2O$: C, 68.09; H, 6.11; N, 5.48. Found: C, 67.98; H, 6.02; N, 5.33.

Compound 8, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[3,4-dimethylphenyl]acetamido)-pyrrolidine-3-carboxylic acid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.15 (s, 3H), 2.22 (s, 3H), 2.95 (d, J = 17 Hz, 1H), 3.05 (t, J = 10 Hz, 1H), 3.18 (dd, J = 9, 10 Hz, 1H), 3.45 (d, J = 17 Hz, 1H), 3.50 (dd, J = 3, 10 Hz, 1H), 3.70 (m, 1H), 3.79 (s, 3H), 3.93 (d, J = 9 Hz, 1H), 5.95 (dd, J = 1, 2 Hz, 2H), 6.75 (d, J = 8 Hz, 1H), 6.83 (dd, J = 1, 8 Hz, 1H), 6.9 (m, 3H), 7.0 (m, 2H), 7.36 (brd d, J = 9 Hz, 2H), 7.65 (d, J = 7 Hz, 1H), 8.68 (brd s, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 503. Anal. Calcd for $C_{29}H_{30}N_2O_6 \cdot 0.8H_2O$: C, 67.38; H, 6.16; N, 5.42. Found: C, 67.24; H, 5.94; N, 5.20.

Compound 9, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[3,5-dimethylphenyl]acetamido)-pyrrolidine-3-carboxylic acid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.29 (s, 6H), 2.89 (d, J = 17 Hz, 1H), 2.98 (t, J = 10 Hz, 1H), 3.18 (dd, J = 9, 10 Hz, 1H), 3.4 (m, 2H), 3.66 (m, 1H), 3.79 (s, 3H), 3.91 (d, J = 9 Hz, 1H), 5.99 (dd, J = 1, 2 Hz, 2H), 6.75 (brd s, 1H), 6.80 (d, J = 8 Hz, 1H), 6.87 (dd, J = 1, 8 Hz, 1H), 6.9 (m, 3H), 7.03 (d, J = 1 Hz, 1H), 7.11 (brd s, 2H), 7.34 (d, J = 7 Hz, 1H), 8.74 (brd s, 1H); MS (DCI/NH_3) $(M + H)^+$ at m/z 503. Anal. Calcd for $C_{29}H_{30}N_2O_6 \cdot 0.5H_2O$: C, 68.09; H, 6.11; N, 5.48. Found: C, 67.93; H, 6.01; N, 5.19.

Compound 17, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2-ethyl-6-methylphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.10 (t, J = 9 Hz, 3H), 2.10 (s, 3H), 2.44 (q, J = 9 Hz, 2H), 3.02 (d, J = 18 Hz, 1H), 3.14 (m, 2H), 3.47 (d, J = 18 Hz, 1H), 3.55 (dd, J = 6,12 Hz, 1H), 3.73 (m, 1H), 3.82 (s, 3H), 3.95 (d, J = 9 Hz, 1H), 5.92 (d, J = 2 Hz, 1H), 5.94 (d, J = 2 Hz, 1H), 6.76 (d, J = 9 Hz, 1H), 6.82 (dd, J = 2, 8 Hz, 1H), 6.87 (d, J = 2 Hz, 1H), 6.92 (d, J = 9 Hz, 2H), 7.20−7.10 (m, 3H), 7.38 (d, J = 9 Hz, 2H), 8.32 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 517. Anal. Calcd for $C_{30}H_{32}N_2O_6 \cdot 0.5H_2O$: C, 68.56; H, 6.33; N, 5.33. Found: C, 68.58; H, 6.29; N, 5.13.

Compound 18, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2-isopropyl-6-methylphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (d, J = 8 Hz, 3H), 1.16 (d, J = 8 Hz, 3H), 2.09 (s, 3H), 2.86 (m, 1H), 3.02 (d, J = 18 Hz, 1H), 3.15 (m, 2H), 3.50 (m, 2H), 3.72 (m, 1H), 3.83 (s, 3H), 3.96 (d, J = 10 Hz, 1H), 5.92 (d, J = 2 Hz, 1H), 5.94 (d, J = 2 Hz, 1H), 6.75 (d, J = 9 Hz, 1H), 6.82 (dd, J = 2, 8 Hz, 1H), 6.86 (d, J = 2 Hz, 1H),

6.92 (d, J = 9 Hz, 2H), 7.07 (dd, J = 2, 9 Hz, 1H), 7.18 (m, 2H), 7.39 (d, J = 9 Hz, 2H), 8.35 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 531. Anal. Calcd for $C_{31}H_{34}N_2O_6 \cdot 0.5H_2O$: C, 69.00; H, 6.54; N, 5.19. Found: C, 69.27; H, 6.67; N, 5.21.

Compound 19, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethylphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (t, J = 7 Hz, 6H), 2.42 (q, J = 7 Hz, 4H), 3.01 (d, J = 16 Hz, 1H), 3.13 (t, J = 10 Hz, 1H), 3.15 (dd, J = 6, 8 Hz, 1H), 3.45 (d, J = 16 Hz, 1H), 3.56 (dd, J = 5, 11 Hz, 1H), 3.70 (ddd, J = 3, 5, 6 Hz, 1H), 3.82 (s, 3H), 3.96 (d, J = 10 Hz, 1H), 5.93 (d, J = 3 Hz, 1H), 5.94 (d, J = 3 Hz, 1H), 6.75 (d, J = 8 Hz, 1H), 6.92 (d, J = 8 Hz, 2H), 7.11 (d, J = 8 Hz, 2H), 7.21 (dd, J = 6, 8 Hz, 1H), 7.39 (d, J = 8 Hz, 2H), 7.82 (dd, J = 2, 8 Hz, 1H), 7.89 (d, J = 3 Hz, 1H), 8.24 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 531. Anal. Calcd for $C_{31}H_{34}N_2O_6$: C, 70.17; H, 6.46; N, 5.28. Found: C, 69.88; H, 6.42; N, 5.09.

Compound 20, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2-ethyl-6-propylphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, J = 8 Hz, 3H), 1.15 (t, J = 8 Hz, 3H), 1.60 (m, 2H), 2.51 (m, 4H), 3.75−3.40 (m, 4H), 3.79 (s, 3H), 3.89 (m, 2H), 4.67 (m, 1H), 5.96 (s, 2H), 7.00−6.70 (m, 7H), 7.41 (m, 3H), 8.84 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 545. Anal. Calcd for $C_{32}H_{36}N_2O_6 \cdot 1.0$TFA: C, 62.00; H, 5.66; N, 4.25. Found C, 61.81; H, 5.90; N, 4.20.

Compound 21, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-dipropylphenyl]acetamido)-pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 8 Hz, 3H), 0.96 (t, J = 8 Hz, 3H), 1.63 (m, 4H), 2.52 (m, 4H), 3.25−2.90 (m, 3H), 3.44 (m, 2H), 3.72 (m, 1H), 3.80 (s, 3H), 3.92 (m, 1H), 5.93 (m, 2H), 6.75 (d, J = 8 Hz, 1H), 6.83 (m, 2H), 6.88 (d, J = 9 Hz, 2H), 7.00 (m, 2H), 7.37 (brd d, J = 9 Hz, 2H), 7.73 (m, 1H), 8.62 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 559. Anal. Calcd for $C_{33}H_{38}N_2O_6 \cdot 0.5H_2O$: C, 69.82; H, 6.92; N, 4.93. Found: C, 69.96; H, 6.93; N, 4.63.

Compound 22, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diisopropylphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (d, J = 8 Hz, 6H), 1.16 (d, J = 8 Hz, 6H), 2.84 (m, 2H), 3.01 (d, J = 18 Hz, 1H), 3.14 (m, 2H), 3.50 (d, J = 18 Hz, 1H), 3.55 (dd, J = 6, 12 Hz, 1H), 3.73 (m, 1H), 3.83 (s, 3H), 3.96 (d, J = 10 Hz, 1H), 5.91 (d, J = 2 Hz, 1H), 5.93 (d, J = 2 Hz, 1H), 6.74 (d, J = 9 Hz, 1H), 6.83 (dd, J = 2, 8 Hz, 1H), 6.85 (d, J = 2 Hz, 1H), 6.93 (d, J = 9 Hz, 2H), 7.15 (d, J = 9 Hz, 2H), 7.29 (m, 1H), 7.39 (d, J = 9 Hz, 2H), 8.29 (brs, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 559. Anal. Calcd for $C_{33}H_{38}N_2O_6 \cdot 0.5H_2O$: C, 69.82; H, 6.92; N, 4.93. Found: C, 69.69; H, 6.63; N, 4.89.

Compound 23, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-methyl-N-[2,6-diethylphenyl]acetamido)-pyrrolidine-3-carboxylic acid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.03 (t, J = 7 Hz, 3H), 1.13 (t, J = 7 Hz, 3H), 2.1−2.4 (m, 4H), 2.46 (d, J = 16 Hz, 1H), 2.84 (t, J = 9 Hz, 1H), 2.97 (d, J = 16 Hz, 1H), 3.06 (t, J = 10 Hz, 1H), 3.11 (s, 3H), 3.5−3.6 (m, 2H), 3.77 (s, 3H), 3.93 (d, J = 10 Hz, 1H), 5.93 (dd, J = 1, 2 Hz, 2H), 6.7−6.8 (m, 3H), 7.0−7.2 (m, 6H); MS (DCI, NH_3) $(M + H)^+$ at m/z 545. Anal. Calcd for $C_{32}H_{36}N_2O_6$: C, 70.57; H, 6.66; N, 5.14. Found: C, 70.26; H, 6.52; N, 4.88.

Compound 24, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2-ethyl-6-methoxyphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, CD_3OD) δ 1.10 (t, J = 8 Hz, 3H), 2.48 (d, J = 8 Hz, 2H), 3.4−3.9 (m, 7H), 3.73 (s, 3H), 3.84 (s, 3H), 5.93 (s, 2H), 6.80 (d, J = 8 Hz, 1H), 6.86 (d, J = 8 Hz, 2H), 6.93 (dd, J = 2, 8 Hz, 1H), 7.03 (brd d, J = 9 Hz, 2H), 7.07 (d, J = 2 Hz, 1H), 7.23 (t, J = 8 Hz, 1H), 7.53 (brd d, J = 9 Hz, 2H); MS (APCI) $(M + H)^+$ at m/z 533. Anal. Calcd for $C_{30}H_{32}N_2O_7 \cdot 0.7$TFA: C, 61.59; H, 5.38; N, 4.57. Found: C, 61.27; H, 5.44; N, 4.61.

Compound 25, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-dimethoxyphenyl]acetamido)-pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.85 (brd d, J = 18 Hz, 1H), 3.03 (m, 2H), 3.49 (brd d, J = 15 Hz, 1H), 3.70 (m, 2H), 3.71 (s, 6H), 3.81 (s, 3H), 3.88 (brd, J = 10 Hz, 1H), 5.93 (s, 2H), 6.56 (d, J = 9 Hz, 2H), 6.75 (d, J = 9 Hz, 1H), 6.86 (d, J = 2 Hz, 1H), 6.90 (d, J = 9 Hz, 2H), 6.99 (d, J = 2 Hz, 1H), 7.17 (t, J = 9 Hz, 1H), 7.39 (brd d, J = 9 Hz, 2H), 8.18 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 535. Anal. Calcd for $C_{29}H_{30}N_2O_8 \cdot 0.75$AcOH: C, 63.20; H, 5.74; N, 4.83. Found: C, 63.18; H, 5.34; N, 4.79.

Compound 26, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-dibromophenyl]acetamido)-pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.01 (brd d, J = 18 Hz, 1H), 3.13 (m, 2H), 3.55 (brd d, J = 15 Hz, 1H), 3.73 (m, 2H), 3.81 (s, 3H), 3.98 (brd d, J = 10 Hz, 1H), 5.93 (s, 2H), 6.76 (d, J = 9 Hz, 1H), 6.86 (m, 1H), 6.91 (d, J = 9 Hz, 2H), 7.02 (t, J = 9 Hz, 1H), 7.40 (brd d, J = 10 Hz, 2H), 7.58 (d, J = 9 Hz, 2H), 8.58 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 633. Anal. Calcd for $C_{27}H_{24}\,Br_2N_2O_6 \cdot 0.3H_2O$: C, 50.85; H, 3.89; N, 4.39. Found: C, 50.45; H, 3.48; N, 4.22.

Compound 27, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethyl-4-fluorophenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, DMSO) δ 1.01 (t, J = 7.6 Hz, 3H), 2.39 (q, J = 7.5 Hz, 4H), 2.83 (t, J = 9.7 Hz, 1H), 2.91 (d, J = 15.9 Hz, 1H), 3.09 (t, J = 9.7 Hz, 1H), 3.19 (d, J = 15.9 Hz, 1H), 3.47 (m, 1H), 3.59 (m, 1H), 3.76 (s, 3H), 3.90 (d, J = 9.8 Hz, 1H), 5.98 (s, 2H), 6.80 (d, J = 7.8 Hz, 1H), 6.84 (dd, J = 1.4, 8.1 Hz, 1H), 6.91 (m, 2H), 6.92 (d, J = 9.5 Hz, 2H), 7.27 (d, J = 1.0 Hz, 1H), 7.52 (m, 2H), 9.24 (s, 1H); MS (DCI) $(M + H)^+$ at m/z 549. Anal. Calcd for $C_{31}H_{33}FN_2O_6 \cdot 0.3$EtOAc: C, 67.26; H, 6.20; N, 4.87. Found: C, 67.23; H, 6.21; N, 4.80.

Compound 31, *trans,trans*-2-(4-Ethoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethylphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (t, J = 9 Hz, 6H), 1.42 (t, J = 9 Hz, 3H), 2.43 (q, J = 9 Hz, 4H), 3.02 (d J = 18 Hz, 1H), 3.15 (m, 2H), 3.60−3.40 (m, 2H), 3.71 (m, 1H), 4.10−3.90 (m, 3H), 5.92 (d, J = 2 Hz, 1H), 5.94 (d, J = 2 Hz, 1H), 6.73 (d, J = 9 Hz, 1H), 6.83 (dd, J = 2, 8 Hz, 1H), 6.86 (d, J = 2 Hz, 1H), 6.90 (d, J = 9 Hz, 2H), 7.11 (d, J = 10 Hz, 2H), 7.21 (m, 1H), 7.36 (d, J = 9 Hz, 2H), 8.26 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 545. Anal. Calcd for $C_{32}H_{36}N_2O_6 \cdot 0.5\ H_2O$: C, 69.42; H, 6.74; N, 5.06. Found: C, 69.52; H, 6.52; N, 4.89.

Compound 32, *trans,trans*-2-(4-Propoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethylphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (t, J = 7 Hz, 3H), 1.08 (t, J = 7 Hz, 6H), 1.82 (sext, J = 7 Hz, 2H), 2.43 (q, J = 7 Hz, 4H), 3.01 (d, J = 16 Hz, 1H), 3.13 (t, J = 10 Hz, 1H), 3.15 (dd, J = 6, 8 Hz, 1H), 3.48 (d, J = 16 Hz, 1H), 3.55 (dd, J = 5, 11 Hz, 1H), 3.70 (ddd, J = 3, 5, 6 Hz, 1H), 3.85 (q, J = 7 Hz, 2H), 3.96 (d, J = 10 Hz, 1H), 5.92 (d, J = 3 Hz, 1H), 5.93 (d, J = 3 Hz, 1H), 6.75 (d, J = 8 Hz, 1H), 6.90 (d, J = 8 Hz, 2H), 7.11 (d, J = 8 Hz, 2H), 7.21 (dd, 6, 8 Hz, 1H), 7.37 (d, J = 8 Hz, 2H), 7.83 (dd, J = 2, 8 Hz, 1H), 7.86 (d, J = 3 Hz, 1H), 8.27 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 559. Anal. Calcd for $C_{33}H_{38}N_2O_6 \cdot 0.25H_2O$: C, 70.38; H, 6.89; N, 4.97. Found: C, 70.49; H, 6.85; N, 4.68.

Compound 33, *trans,trans*-2-(4-Isopropoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethylphenyl]acetamido)-pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.09 (t, J = 7 Hz, 6H), 1.33 (dd, J = 2, 7 Hz, 6H), 2.47 (q, J = 7 Hz, 4H), 3.55 (m, 2H), 3.80 (m, 3H), 4.64 (septet, J = 7 Hz, 1H), 5.92 (d, J = 3 Hz, 1H), 5.93 (d, J = 3 Hz, 1H), 6.78 (d, J = 8 Hz, 1H), 6.91 (dd, J = 2, 8 Hz, 1H), 6.99 (brd d, J = 9 Hz, 2H), 7.05 (d, J = 2 Hz, 1H), 7.11 (dd, J = 1, 8 Hz, 1H), 7.13 (s, 1H), 7.22 (dd, J = 8, 9 Hz, 1H), 7.51 (brd d, J = 9 Hz, 2H); MS (ESI+) $(M + H)^+$ at m/z 559. Anal. Calcd for $C_{33}H_{38}N_2O_6 \cdot 0.7$TFA: C, 64.71; H, 6.11; N, 4.39. Found: C, 64.54; H, 5.78; N, 4.21.

Compound 34, *trans,trans*-2-(4-[2-Methoxyethoxy]phenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethylphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (t, J = 7 Hz, 6H), 2.43 (q, J = 7 Hz, 4H), 3.00 (d, J = 11 Hz, 1H), 3.05−3.15 (m, 2H), 3.44 (s, 3H), 3.46 (d, J = 11 Hz, 1H), 3.45−3.55 (m, 1H), 3.65−3.75 (m, 1H), 3.75−

3.80 (m, 2H), 3.93 (d, J = 7 Hz, 1H), 4.12−4.17 (m, 2H), 5.94 (dd, J = 2, 4 Hz, 2H), 6.75 (d, J = 8 Hz, 1H), 6.82 (dd, J = 2, 9 Hz, 1H), 6.87 (d, J = 2 Hz, 1H), 6.95 (d, J = 8 Hz, 1H), 7.10 (d, J = 6 Hz, 2H), 7.19−7.24 (m, 1H), 7.37 (d, J = 8 Hz, 2H), 8.29 (s, 1H); MS (APCI+) $(M + H)^+$ at m/z 575. Anal. Calcd for $C_{33}H_{38}N_2O_7$: C, 68.97; H, 6.67; N, 4.87. Found: C, 68.92; H, 6.83; N, 4.77.

Compound 35, *trans,trans*-2-(4-[2-Methoxyethyl]phenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethylphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, DMSO) δ 1.00 (t, J = 7.5 Hz, 6H), 2.40 (q, J = 7.5 Hz, 4H), 2.66 (t, J = 8.3 Hz, 1H), 2.79 (t, J = 6.8 Hz, 2H), 2.87 (d, J = 15.6 Hz, 1H), 3.00 (t, J = 9.2 Hz, 1H), 3.20 (d, J = 15.9 Hz, 1H), 3.24 (s, 3H), 3.42−3.47 (m, 1H), 3.53 (t, J = 6.9 Hz, 1H), 3.60 (m, 1H), 3.91 (d, J = 9.5 Hz, 1H), 5.94 (s, 2H), 6.76 (d, J = 7.8 Hz, 1H), 6.84 (dd, J = 1.4, 8.1 Hz, 1H), 7.08 (d, J = 7.5 Hz, 2H), 7.17 (d, J = 6.4 Hz, 3H), 7.22 (d, J = 1.4 Hz, 1H), 7.51 (d, J = 8.1 Hz, 2H), 9.21 (s, 1H); MS (DCI) $(M + H)^+$ at m/z 559. Anal. Calcd for $C_{33}H_{38}N_2O_6 \cdot 0.2H_3PO_4$: C, 68.54; H, 6.73; N, 4.84. Found C, 68.28; H, 6.46; N, 4.82.

Compound 28, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethyl-4-methylphenyl]acetamido)pyrrolidine-3-carboxylic acid. Ethyl *trans,trans*-2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(4-bromo-2,6-diethylphenyl)aminocarbonylmethyl)pyrrolidine-3-carboxylate was prepared according to the procedures described above. This compound (200 mg, 0.31 mmol), in solution in 8 mL of THF, was added to a mixture (purged with nitrogen) of [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1:1 complex with dichloromethane) (13 mg) and cesium carbonate (307 mg, 0.942 mmol) in anhydrous *N,N*-dimethylformamide (2 mL) at ambient temperature. After stirring the mixture for 10 min at ambient temperature, 1.0 M trimethylborane (0.471 mL, 0.471 mmol) in tetrahydrofuran was added. The reaction was stirred overnight at 65 °C under nitrogen. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with water (2 × 30 mL) and brine. The organic phase was dried with sodium sulfate, and the solvents were removed in vacuo . The residue was chromatographed on silica gel eluting with 3:1 hexanes−ethyl acetate to give ethyl *trans,trans*-2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethyl-4-methylphenyl]acetamido)pyrrolidine-3-carboxylate (110 mg, 60% yield). This material was hydrolyzed as described above to give the title compound: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (t, J = 9 Hz, 6H), 2.28 (s, 3H), 2.39 (q, J = 9 Hz, 4H), 3.00 (d, J = 18 Hz, 1H), 3.13 (m, 2H), 3.45 (d, J = 18 Hz, 1H), 3.55 (dd, J = 2, 9 Hz, 1H), 3.72 (m, 1H), 3.81 (s, 3H), 3.95 (d, J = 10 Hz, 1H), 5.91 (d, J = 2 Hz, 1H), 5.93 (d, J = 2 Hz, 1H), 6.75 (d, J = 9 Hz, 1H), 6.82 (dd, J = 2, 8 Hz, 1H), 6.86 (d, J = 2 Hz, 1H), 6.92 (m, 4H), 7.38 (d, J = 9 Hz, 2H), 8.20 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 545. Anal. Calcd for $C_{32}H_{36}N_2O_6 \cdot 0.5\ H_2O$: C, 69.42; H, 6.74; N, 5.06. Found: C, 69.43; H, 6.57; N, 4.94.

The following compounds were prepared using the above procedure.

Compound 29, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethyl-4-ethylphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (t, J = 9 Hz, 6H), 1.22 (t, J = 9 Hz, 3H), 2.40 (q, J = 9 Hz, 4H), 2.60 (q, J = 9 Hz, 2H), 3.00 (d, J = 18 Hz, 1H), 3.13 (m, 2H), 3.46 (d, J = 18 Hz, 1H), 3.52 (dd, J = 2, 9 Hz, 1H), 3.71 (m, 1H), 3.82 (s, 3H), 3.95 (d, J = 10 Hz, 1H), 5.91 (d, J = 2 Hz, 1H), 5.93 (d, J = 2 Hz, 1H), 6.75 (d, J = 9 Hz, 1H), 6.82 (dd, J = 2, 8 Hz, 1H), 6.84 (d, J = 2 Hz, 1H), 6.91 (d, J = 9 Hz, 2H), 6.95 (s, 2H), 7.38 (d, J = 9 Hz, 2H), 8.22 (brd s, 1H); MS (DCI, NH_3) $(M + H)^+$ at m/z 559. Anal. Calcd for $C_{33}H_{38}N_2O_6 \cdot 0.25\ H_2O$: C, 70.38; H, 6.89; N, 4.97. Found: C, 70.18; H, 7.14; N, 4.63.

Compound 30, *trans,trans*-2-(4-Methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*-[2,6-diethyl-4-carboxyphenyl]acetamido)pyrrolidine-3-carboxylic acid: ^{1}H NMR (300 MHz, DMSO) δ 1.04 (t, J = 9 Hz, 6H), 2.44 (q, J = 9 Hz, 4H), 3.00−2.80 (m, 3H), 3.45−3.00 (m, 2H), 3.62 (m, 1H), 3.76 (s, 3H), 3.92 (d, J = 9 Hz, 1H), 5.98 (s, 2H), 6.83 (m, 2H), 6.93 (d, J = 9 Hz, 2H), 7.27 (m, 2H), 7.54 (d, J = 9 Hz, 2H), 7.68 (brd s, 2H); MS (DCI, NH_3) $(M + H)^+$ at m/z 575. Anal. Calcd for $C_{32}H_{34}N_2O_8 \cdot 0.5H_2O$: C, 65.85; H, 6.04; N, 4.80. Found: C, 66.03; H, 5.84; N, 4.67.

Preparation of Enantiomerically Pure Antagonists. Core pyrrolidines **14** were resolved into individual enantiomers as follows: for R_3 = OCH_3 or OEt, through preparation of a salt with mandelic acid, followed by multiple recrystallizations; for R_3 = OPr or $OCH_2CH_2OCH_3$, through preparative HPLC on a chiral Regis Whelk-O2 column. Alkylation and hydrolysis of the resulting chiral cores led to compounds **36**−**39**, which were analyzed by chiral HPLC using a Regis analytical Whelk-O column. Compounds **36**−**39** were all determined to be >99% enantiopure.

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 were 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, 5 μg/mL pepstatin A, and 0.025% bacitracin) using a microultrasonic 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 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 were calculated using an average of at least two separate determinations.

Phosphoinositol Hydrolysis Assays. ET_A: MMQ cells (0.4 × 106 cells/mL) were labeled with 10 μCi/mL [^{3}H]-myoinositol in RPMI for 16 h. The cells were washed with PBS, 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 as described by Berridge.[9] 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 [^{3}H]myoinositol in F-12 media + 10% FBS + 1xP/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 incubated for 30 min at 37 °C. The cells were then solubilized by the addition of 50 μL of 1 N NaOH and 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 were calculated using an average of at least two separate determinations.

Pharmacokinetic Analysis. The pharmacokinetic behavior of compound **16h** was 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 mol equiv of sodium hydroxide. Groups of rats (n = 4/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[10] were obtained with the program CSTRIP.[11] Area under the curve (AUC) values were calculated by the trapezoidal rule over the time course of the study. 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).

Pseudoefficacy Studies. Briefly, rats were anesthetized with methoxyflurane (Penthrane, USP), and a peripheral vein and artery were catheterized with polyurethane tubing (Microrenathane, Braintree Scientific, Braintree, MA) for drug or ET peptide administration and for measurement of mean systemic arterial pressure (MAP), respectively. Following recovery to consciousness (>45 min), rats were dosed orally by gavage with either vehicle or A-192621 (dose volumes = 2 mL/kg) and placed unrestrained in individual cages. Their arterial catheters were coupled to fluid-filled strain-gauge transducers connected to a digital data acquisition system (Mi2, Malvern, PA) for determination of blood pressure and heart rate (HR). ET-1 (0.3 nmol/kg, i.v. in 0.1% BSA−saline) was administered as a bolus 1 h after the oral dose of A-192621 (30 mg/kg) or vehicle. Baseline blood pressures and HR (sampled at 2−5-min intervals) were averaged for 30 min prior to infusion of the ET peptide. The peak value of the transient vasodepressor response to an ET peptide was determined from the calibrated strip chart of recording using the algorithm: MAP = 1/3(systolic − diastolic) + diastolic. The pressor response to the ET peptide was quantified as the peak change in MAP relative to baseline: peak ΔMAP = maximum change of MAP following ET-1 challenge. The time-dependent vasopressor effect was calculated from the AUV of MAP versus time and expressed as a percent change from baseline in each animal: AUC_{bp} = % ΔMAP × time.

Long-Term Treatment Study. In a subchronic study, A-192621 (30 mg/kg/day) or vehicle (tap water) was administered to rats for 3 days in the diet (mixed in meal, #5002, PMI Nutrition International, St. Louis, MO). On the third day of dosing, rats were instrumented and caged as described above. MAP and HR were determined over 90 min (averaged), after which blood samples were drawn for determination of plasma ET-1 and drug concentrations.

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