Selective Endothelin A Receptor Antagonists. 3. Discovery and Structure−Activity Relationships of a Series of 4-Phenoxybutanoic Acid Derivatives

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The third in this series of papers describes our further progress into the discovery of a potent and selective endothelin A (ET_A) receptor antagonist for the potential treatment of diseases in which a pathophysiological role for endothelin has been implicated. These include hypertension, ischemic diseases, and atherosclerosis. In earlier publications we have outlined the discovery and structure−activity relations of two moderately potent series of nonpeptide ET_A receptor antagonists. In this paper, we describe how a pharmacophore model for ET_A receptor binding was developed which enabled these two series of compounds to be merged into a single class of 4-phenoxybutanoic acid derivatives. The subsequent optimization of in vitro activity against the ET_A receptor led to the discovery of (*R*)-4-[2-cyano-5-(3-pyridylmethoxy)phenoxy]-4-(2-methylphenyl)butanoic acid (**12m**). This compound exhibits low-nanomolar binding to the ET_A receptor and a greater than 1000-fold selectivity over the ET_B receptor. Data are presented to demonstrate that **12m** is orally bioavailable in the rat and is a functional antagonist in vitro and in vivo of ET-1-induced vasoconstriction.

Introduction

Endothelin-1 (ET-1) is a 21-amino acid peptide which possesses potent vasoconstrictor properties.[1] Adverse stimuli (such as ischemia/ hypoxia) can stimulate ET-1 production from both human and animal endothelial cells as well as other cell types such as smooth muscle cells, fibroblasts, macrophages, glial cells, mesangial cells, and various cultured cancer cells.[2] A large body of evidence has accumulated in recent years implicating a pathophysiological role for ET-1 in numerous clinical conditions.[2] ET-1 belongs to a family of three isopeptides (ET-1, ET-2, ET-3) found in humans which exert their activities via two specific seven-transmembrane, G-protein-coupled receptors. These receptors have been cloned and designated ET_A and ET_B receptors.[2] The ET_A receptor is located principally on vascular smooth muscle and mediates vasoconstriction and smooth muscle cell proliferation.[3] Stimulation of ET_B receptors located on the endothelium evokes the release of NO and PGI_2 to cause local vasodilation and prevention of platelet aggregation.[4] However, ET_B receptors have also been identified on vascular smooth muscle and can produce contraction upon activation.[5]

Throughout the 1990s the search by the pharmaceutical industry for competitive antagonists of the ET receptors has been very successful, and consequently there are presently a large number of reports describing structurally diverse receptor antagonists with varying potencies and subtype selectivity.[6] Many of the first compounds that were identified were peptides and include the selective ET_A receptor antagonists BQ 123 (**1**)[7] (Chart 1) and FR 139317,[8] the selective ET_B antagonist BQ 788,[9] and the mixed ET_A/ET_B antagonist PD 142893.[10] These peptides have been invaluable in characterizing ET receptors particularly in isolated tissues, but their usefulness has been limited where in vivo studies are concerned. However it is the recent disclosure of several nonpeptidic orally active antagonists[6] that has allowed a more extensive exploration of the role of endothelin in the pathology of animal disease models and which has led to the commencement of clinical studies in humans. Some of these nonpeptide antagonists (Chart 1) include Ro 47-0203 (Bosentan) (**2**),[11] SB 209670 (**3**),[12] PD 156707 (**4**),[13] and A-127722 (**5**).[14] Other compounds from Bristol Myers Squibb, Zeneca, Roussel, Merck, Knoll, and Texas Biotechnology as well as from our own laboratories at Rhone-Poulenc Rorer[15,16] have been disclosed recently.

There is debate about the potential merits of a selective ET_A receptor antagonist compared with those of a mixed ET_A/ET_B antagonist.[17] Potential advantages for a selective antagonist of the ET_A receptor are (a) Vasoconstriction in the human cardiovascular system by ET-1 appears to be predominantly mediated by the ET_A receptor.[17,18] The involvement of the ET_B receptor in the contraction of human tissue is the subject of current research and is only now being elucidated.[19] (b) The mitogenic and proinflammatory effects induced by ET-1 are mediated by ET_A receptor stimulation.[3] (c) Blockade of the ET_B receptor would prevent the putative beneficial effects of ET-1-induced NO and PGI_2 release from the endothelium.[4,20] (d) The ET_B receptor, predominantly in the lung, appears to act as a clearance receptor for ET-1; for example, the ET_B-selective antagonist BQ 788 has been shown to reduce the rate of clearance of exogenous ET-1 from the circulation of the rat.[21] In addition, the mixed receptor antagonist Ro 46-2005 induces a raised plasma level of ET-1, and this

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Chart 1. Endothelin Antagonists

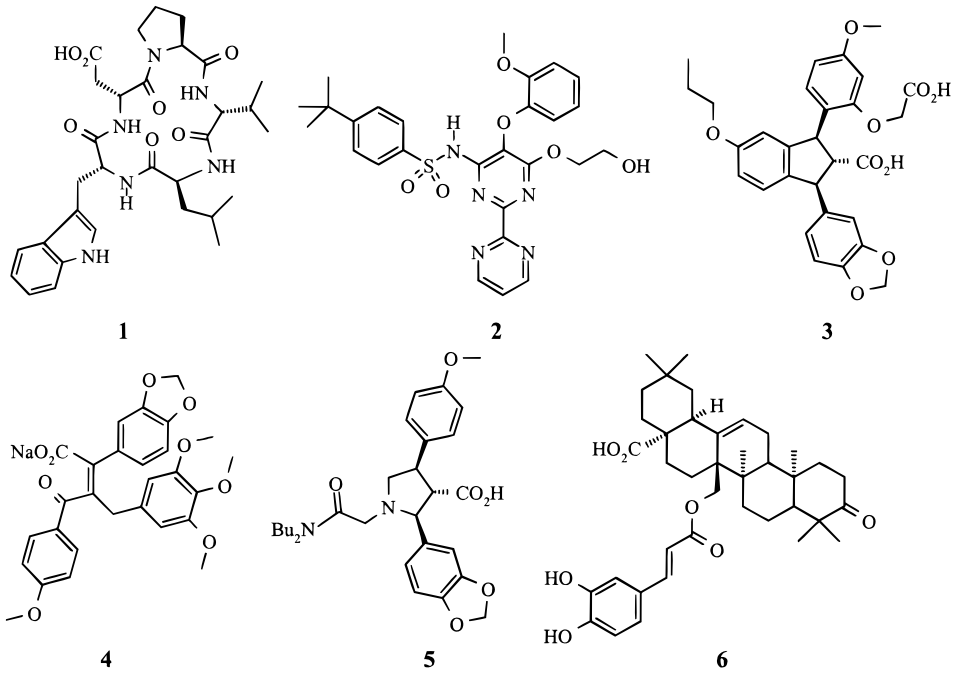
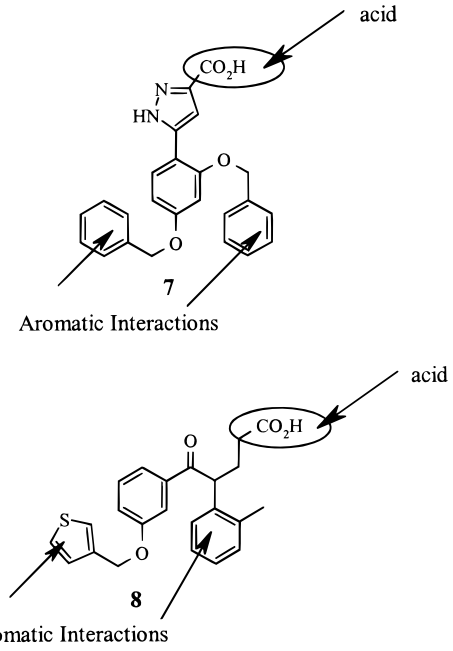


Chart 2. Common Pharmacophoric Features



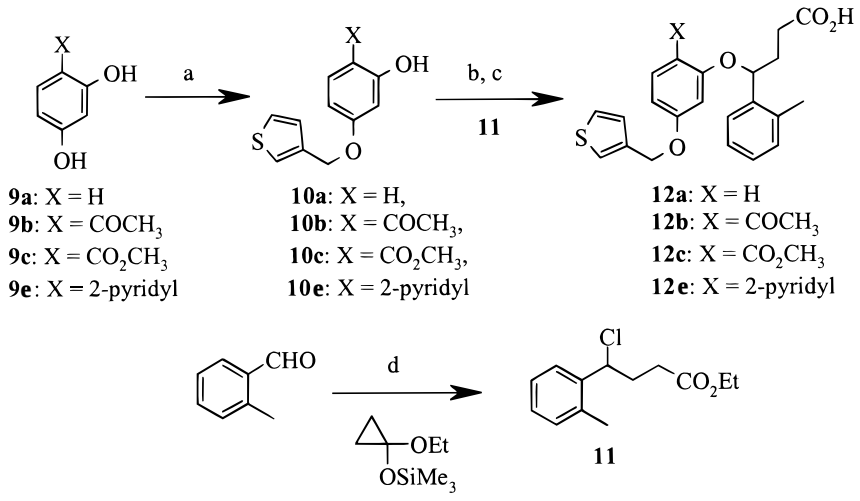
effect would be expected to compromise effective ET_A blockade.[22] It is likely however that the "ideal" subtype selectivity will depend on the tissue/disease being targeted and will only become clear following the release of data from clinical trials in humans. At the outset of our research however, we sought to identify a novel series of nonpeptidic ET_A-selective ligands. The preceding papers in this series[15,16] outlined our lead generation studies[23] based on the known ET_A ligands BQ 123 (**1**)[7] and myriceron caffeoyl ester (**6**)[24] and explicated the discovery and structure−activity relationships of two moderately potent series of ET_A-selective antagonists exemplified by the pyrazole acid **7** (1000 nM ET_A) and the thienyl derivative **8** (90 nM ET_A) (Chart 2). The work presented in this paper will describe how we identified common pharmacophoric elements in these two series of compounds and combined them into one series of potent, selective, bioavailable ET_A receptor antagonists.

Chemistry

The synthesis of the majority of the compounds described in this paper is depicted in Scheme 1 and begins from readily available resorcinol derivatives. Monoalkylation of the appropriate commercially available resorcinols **9** with 3-(chloromethyl)thiophene proceeded under standard conditions and was facilitated by the presence of a phase-transfer catalyst. Resorcinol itself (**9a**, X = H) yielded the expected mixture of starting, and mono- and dialkylated material from which the desired product **10a** (X = H) could be isolated by chromatography. Where X is electron-withdrawing (COMe, CO_2Me), the alkylation of **9b**,**c** proceeded quite chemoselectively to yield almost entirely the desired monoalkylated products **10b**,**c**, respectively. The butanoic acid side chain was then introduced by a second alkylation with the 4-chlorobutanoic ester **11** followed by hydrolysis using methanolic KOH to yield the 4-phenoxybutanoic acid derivatives **12a**−**e** (Table 1). The chloro ester **11** (and analogues) was most efficiently prepared by the titanium-mediated "homoenolate" reaction[25] of silyl ether **13** with an aryl aldehyde (Scheme 1). The synthesis of the non commercially available 4-(2-pyridyl)resorcinol (**9e**) is outlined in Scheme 2 and relies on the palladium(II)-mediated coupling of 2-bromopyridine with the organomagnesium compound derived from bromide **14**. The resultant biaryl **15** was bisdemethylated to furnish **9e** which was carried through to target compound **12e** as described above.

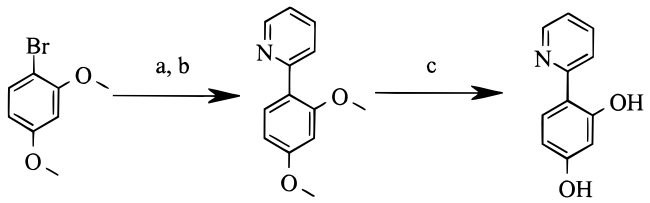
The ester group in **10c** (X = CO_2Me) could be further elaborated to amides and to heterocyclic functionalities (e.g., oxazole and imidazole) as shown in Scheme 3. Thus treatment of **10c** (X = CO_2Me) with ethanolic ammonia under high pressure yielded the amide **10d** (X = $CONH_2$) which was taken on to target compound **12d**

Scheme 1. General Synthesis of 4-Phenoxybutanoic Acid Derivatives[a]



[a] (a) $ArCH_2Cl$, K_2CO_3, KI, Bu_4NBr, MEK; (b) K_2CO_3, KI, MEK **11**; (c) 10% KOH, MeOH; (d) $TiCl_4$, CH_2Cl_2, [(1-ethoxycyclopropyl)oxy]trimethylsilane (**13**).

Scheme 2. Synthesis of 4-(2-Pyridyl)resorcinol (**9e**)[a]



[a] (a) Mg, THF, reflux; (b) $(Ph_3P)PdCl_2$, 2-bromopyridine, THF, reflux; (c) Pyr·HCl, 160 °C.

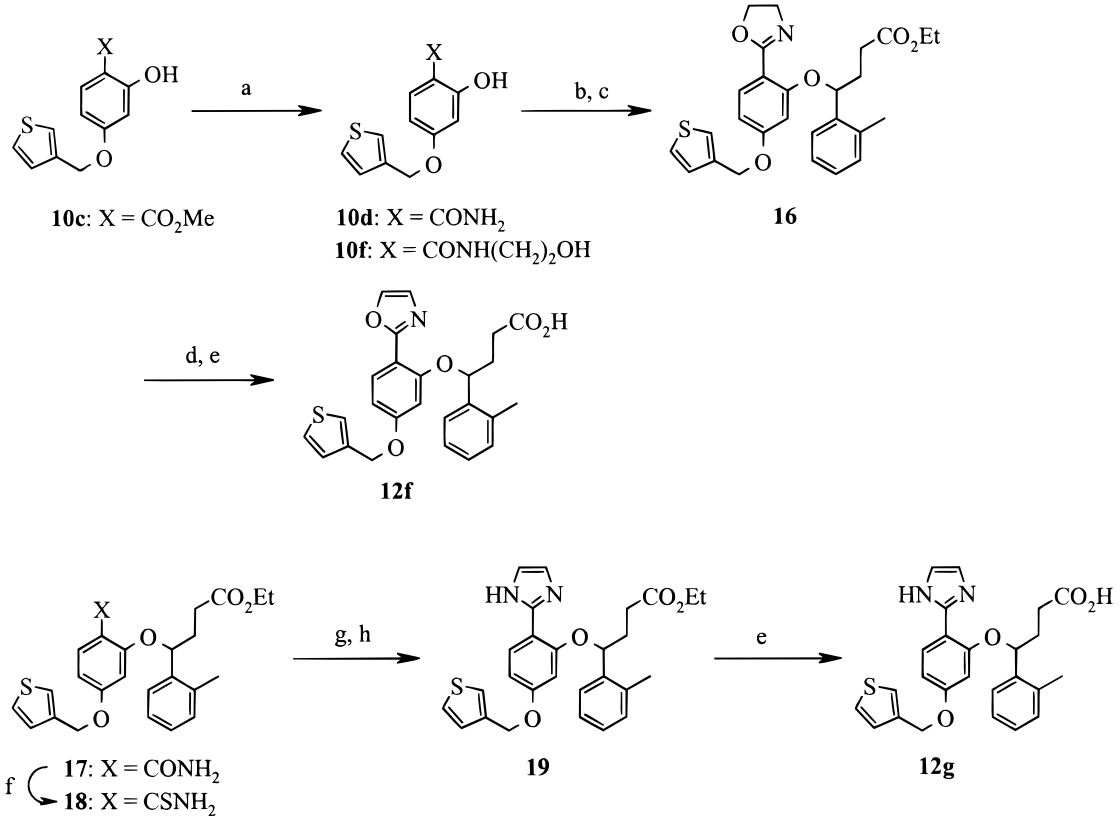
as described above. In a similar fashion, the ester **10c** (X = CO_2Me) was refluxed with ethanolamine to give the amide **10f** (X = $CONHCH_2CH_2OH$). This compound was alkylated on the phenolic oxygen with chloro ester **11** and treated with the Burgess dehydrating agent to afford the oxazoline **16**. Oxidation of the oxazoline ring was effected using nickel oxide with subsequent ester hydrolysis producing the oxazole **12f**. The amide intermediate **17** (X = $CONH_2$) was transformed to the corresponding thioamide **18** using Lawesson's reagent (Scheme 3). Conversion of this to the *S*-methyl derivative was effected with methyl iodide, and subsequent condensation with aminoacetaldehyde diethyl acetal followed by acid hydrolysis yielded the imidazole **19**. The imidazole acid **12g** was then obtained after saponification.

Scheme 4 shows how the acetyl-substituted ester **20** was converted into the pyrazoles **12h**,**i**. The acetyl group was deprotonated with sodium hydride and acylated with either diethyl oxalate or ethyl formate. Treatment of the resultant 1,3-dicarbonyl compounds with hydrazine afforded the pyrazoles **21h** (R = CO_2Et) and **21i** (R = H); ester hydrolysis then yielded the pyrazole diacid **12h** and the pyrazole **12i**.

Where the target compounds contained nitrile groups at the X position which were strongly electron-withdrawing, we were able to take advantage of the nucleophilic aromatic substitution reaction of an ortho fluoride ion with an alkoxide group. This was particularly useful for the synthesis of enantiomerically pure compounds utilizing chiral alkoxide anions. This process is exemplified in Scheme 5 for the asymmetric synthesis of the cyano derivative **12k** (Table 1). The keto ester **22** was prepared by standard transformations, a *tert*-butyl ester being employed to suppress lactonization in the subsequent reduction step. Asymmetric reduction of **22** to the chiral hydroxy ester **23** was attempted using a variety of chiral reagents, both stoichiometric and catalytic.[26] The most successful in terms of yield and enantiomeric excess of **23** was Brown's *B*-chlorodiisopinocampheylborane (DIP-chloride).[27] Thus treatment of ketone **22** with (+)-DIP-chloride in ether at −20 °C followed by workup with diethanolamine gave the (4*R*)-hydroxy ester **23** in >98% ee. The enantiomeric excess was determined in the following manner using Mosher's ester. Esterification of **23** with (+)-Mosher's acid chloride/pyridine and ^{1}H NMR analysis of the resultant crude ester **24** showed the methine C*H*O proton to appear as a doublet of doublets at δ 6.06. The presence of the other possible diastereoisomer derived from the (*S*)-enantiomer of **23** was not detected. To check this, the keto ester **22** was reduced with borane:THF and the resultant racemic alcohol esterified with (+)-Mosher's acid chloride/pyridine. The ^{1}H NMR spectrum of the diastereomeric mixture clearly showed two distinct double doublets for the methine C*H*O protons at δ 6.06 and 6.12.

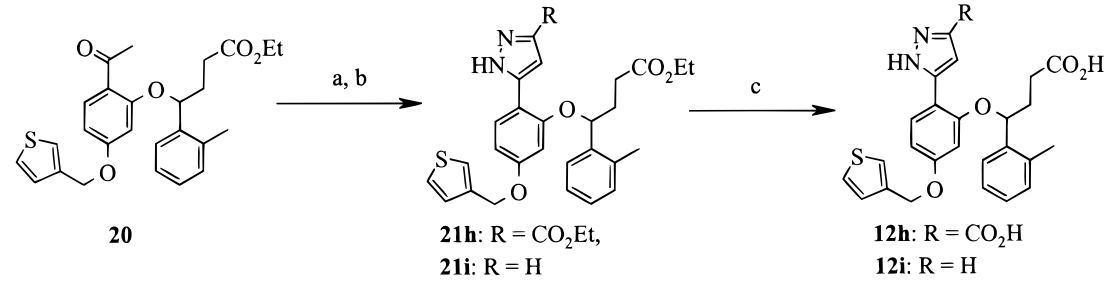
Exposure of chiral alcohol **23** to base unfortunately resulted in immediate lactonization. This problem was circumvented by saponification of **23** at this stage and isolation of the sodium salt **25** (Scheme 5). We were able to further deprotonate **25** with sodium hydride, and the resultant dianion was found to react chemoselectively with the appropriately substituted 2-fluorophenol **26** affording the (4*R*)-phenoxybutanoic acid **12k**. The enantiomeric excess of **12k** was found to be >99% ee by chiral HPLC analysis (see the Experimental Section) following a single recrystallization. The (*S*)-enantiomeric compounds (e.g., **12l**) were obtained by utilizing (−)-DIP-chloride in Scheme 5. The (3-thienyl)methyl group in fluoride **26** was introduced by alkylating the commercially available cyanophenol **27** with 3-(chloromethyl)thiophene (Scheme 5). Alkylation of **27** with

Scheme 3. Synthesis of Oxazole and Imidazole Analogues **12f**,**g**[a]



[a] (a) NH_3, EtOH, 100 °C or ethanolamine, reflux; (b) NaH, THF, **11**; (c) (methoxycarbonylsulfamoyl)triethylammonium hydroxide (Burgess reagent), THF, reflux; (d) NiO_2, toluene, 100 °C; (e) 10% KOH, MeOH; (f) Lawesson's reagent, THF; (g) NaH, MeI, THF; (h) (i) aminoacetaldehyde diethyl acetal, AcOH, THF, reflux, (ii) 5.0 M aq HCl.

Scheme 4. Synthesis of Pyrazole Analogues **12h**,**i**[a]



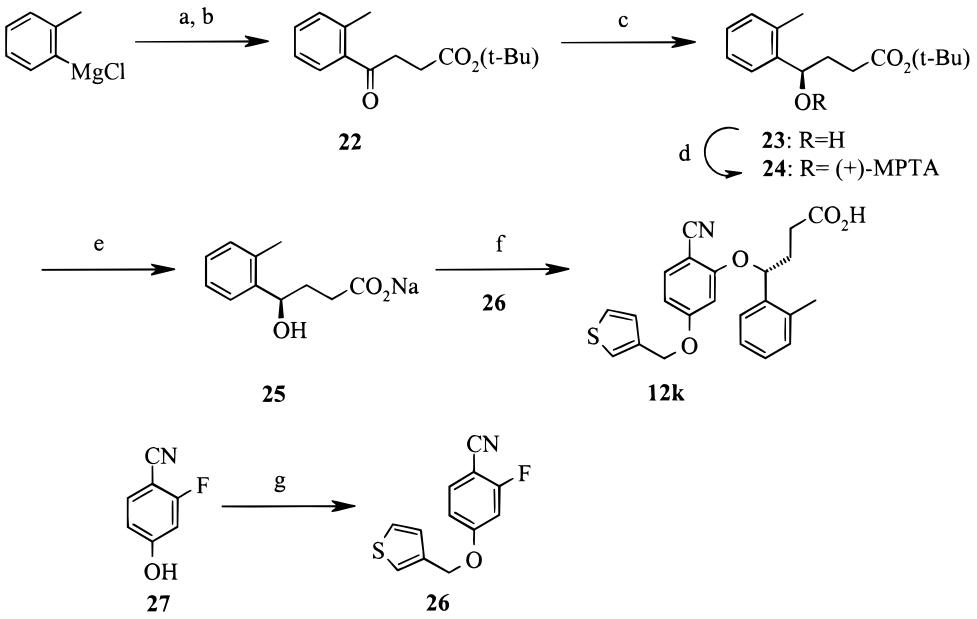
[a] (a) NaH, diethyl oxalate (for **21h**) or ethyl formate (for **21i**), toluene, reflux; (b) hydrazine hydrate, EtOH, reflux; (c) 10% KOH, MeOH.

the appropriate chloromethyl-substituted heteroaromatics allowed this position to be further varied giving rise to compounds **12m**−**p** (Table 2). These chloromethyl compounds were either commercially available or prepared using published procedures.[28]

Results and Discussion

Identification of the Phenoxybutanoic Acid Series. The previous papers in this series described the discovery and structure−activity relationships of two series of selective ET_A receptor antagonists exemplified by the pyrazole **7**[15] and the ketone **8**[16] (Chart 2). It was hypothesized that these two series bound to the same local site in the receptor since they had been generated from the same 3D database search.[23] The relationship between the two series was not obvious; however, several features of the molecules seemed to be common: (i) a carboxylic acid group, (ii) a central 1,3-substituted phenyl ring with (iii) a pendant aryloxymethoxy, and (iv) a phenyl ring attached through a two-atom spacer. No direct superimposition was possible; invariably three of the features fitted reasonably well but not the fourth. At this stage the concept of group interaction sites was introduced. It is not necessary to have all features of molecules superimposed for them to interact with the same binding groups of a receptor site. A basic receptor center, say a lysine group, can accept an interaction with an acid group from many directions. In such a case it would be the interaction center, and not the groups themselves, which would be considered to superimpose. To enable testing of this concept a dummy atom, representing an interaction site, was bonded to an oxygen of each carboxylic acid, in the direction of the lone pair and at 2.8 Å. The two molecules were then subjected to conformational analysis. Prior to the analysis the central phenyl rings

Scheme 5. Asymmetric Synthesis of **12k** via Fluoride Displacement[a]



[a] (a) Succinic anhydride; (b) isobutylene, CH_2Cl_2, concentrated H_2SO_4, (*t*-Bu)OH; (c) (+)-DIP-chloride, ether, −20 °C; (d) (+)-Mosher's acid chloride, pyridine; (e) 5.0 M NaOH; (f) **26**, NaH, THF; (g) 3-(chloromethyl)thiophene, K_2CO_3, KI, Bu_4NBr, MEK.

were superimposed and the pendant arylmethoxy group was locked into a low-energy conformation; the primary aim of the work was then to compare the positions of the remaining aryl rings and the acid interaction points. The analysis was carried out in a 3D box, and distances of the key groups to the corners of the box were used to define the conformational space. A comparison of the molecules was then made looking at the positions of the aromatic rings and acid interaction points within this 3D box and at the energy cutoff employed; only one region of conformational space was occupied. Representative conformations from this region are shown in Figure 1; the cationic receptor interaction center is clearly revealed. From this superimposition the design of new compounds was a matter of combining features from both series in a single molecule. The first such molecule designed was the pyrazole diacid **12h** which incorporated both acid functions enabling a bidentate interaction with the putative receptor cationic center (Figure 2). The low-nanomolar activity of this molecule prompted us to further explore this series of 4-phenoxybutanoic acids (see below).

Structure−Activity Relationships for the 4-Phenoxybutanoic Acid Series. All test compounds were assayed in vitro for their ability to antagonize the binding of [^{125}I]ET-1 to rat aortic A10 cell ET_A receptors and rat cerebellum ET_B receptors. Functional activity of selected molecules was measured as the inhibition of ET-1-induced contraction of isolated rat aortic rings denuded of the endothelium (an ET_A-mediated process).[29] In all cases, the compounds showed no agonist activity but antagonized the ET-1-induced contractions in a concentration-dependent manner; the concentration−response curves of ET-1 were shifted to the right in a parallel fashion by increasing concentrations of compound with no significant reduction in the maximal response. In vitro data is shown in Tables 1 and 2, the ET_A-selective cyclic peptide BQ 123 (**1**) and the lead compounds **7** and **8** are included for purposes of comparison.

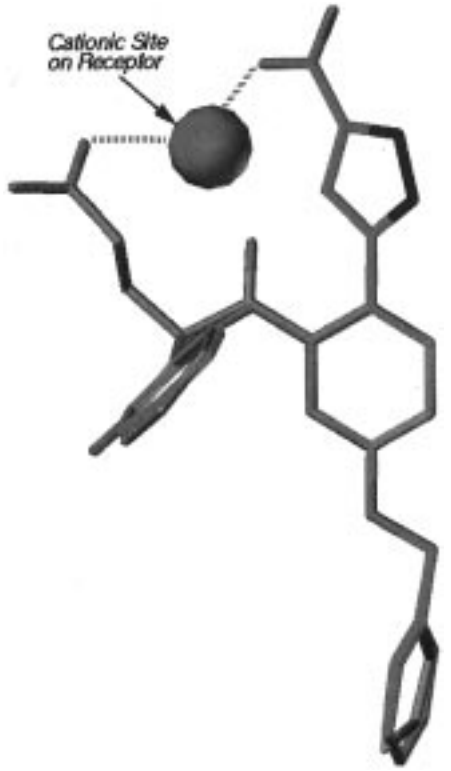


Figure 1. Superimposition of **7** (blue) and **8** (yellow) in the unified model.

The pyrazole diacid **12h**, designed from the cation binding model described above, was found to have IC_{50}'s of 7.0 and 3000 nM in the ET_A and ET_B receptor binding assays, respectively. This was an encouraging result which supported our binding model and represented a >10-fold improvement in potency at the ET_A receptor. **12h** was a functional endothelin-1 antagonist having a pK_B of 7.3 on rat aorta, a figure identical to that of the standard antagonist BQ 123 (**1**) which was the starting point for our studies. Unfortunately, **12h** possessed negligible oral bioavailability in the rat following a 30

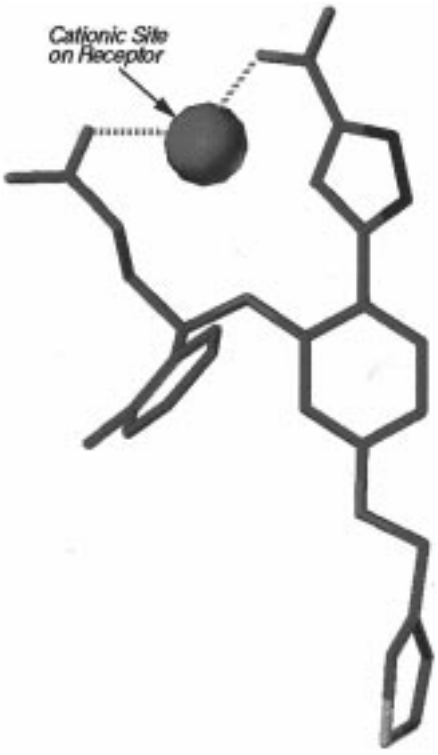


Figure 2. Pyrazole diacid **12h** (green) shown fitting the unified model.

mg/kg dose (data not shown); the poor absorption being ascribed to the presence of two anionic acid functionalities in the molecule.

Synthesis of compound **12i** in which the acid is removed from the pyrazole ring demonstrated that only one acidic functionality is necessary for low-nanomolar activity, the IC_{50} (ET_A) being 20 nM. This compound was still selective, being some 500-fold less active at the ET_B receptor. Removing the pyrazole group altogether (compound **12a**) reduced potency 40-fold. The effect of introducing other heterocyclic rings at this position was explored; the oxazole **12f** and the 2-pyridyl derivative **12e** were equiactive with **12i**. An imidazole ring was less tolerated, this analogue **12g** being 10-fold less potent than **12i** at the ET_A receptor. We postulated that the pyrazole ring was interacting with the receptor as an H-bond acceptor and proceeded to introduce a series of groups at this position that would have this property. Thus the acetyl and amido analogues (**12b**,**d**, respectively) were bioisosteric with the pyrazole ring having IC_{50}'s of ~30 nM; a carboxylic acid (**12c**) was however less tolerated. The most effective H-bond acceptor group introduced was a nitrile as in **12j** which possessed an IC_{50} of 7.0 nM (ET_A) and was still 500-fold selective. Preparation of the individual enantiomers of this compound revealed that the (*R*)-isomer **12k** was the eutomeric component (IC_{50} 5.0 nM). The (*S*)- isomer **12l** was however only 10-fold less active perhaps reflecting the flexibility of the propanoic side chain. **12k** was examined in the functional assay and had a pK_B of 7.4 on rat aorta. Most importantly, this compound demonstrated good oral bioavailability (60%) in the rat (data not shown).

The 3-thienyl and 2-methylphenyl substituents present in this series of antagonists had been identified as optimum from our earlier studies.[16] The heteroaromatic ring was thought to be a possible position to modulate the pharmacokinetics of this series, and so a number of additional heterocyclic analogues of **12k** were prepared (Table 2). These compounds **(12m−o)** were all essentially equiactive and were potent functional antagonists on rat aorta (pK_B ~7.0), the exception being the piperonyl derivative **12p** which dropped 10-fold in binding potency. We introduced this ring as it was observed to be a key pharmacophore in many other published endothelin antagonists (Chart 1). The 3-pyridyl derivative **12m** (IC_{50} 5.0 nM) was selected for further in vivo study on account of its superior pharmacokinetics (see below).

Pharmacokinetics of 12m. The pharmacokinetics of **12m** in the rat following iv and oral administration is illustrated in Figure 3. Single-dose (3 mg/kg) intravenous pharmacokinetics of **12m** in rats gave area under the curve (from time 0 to infinity, 19.8% extrapolated) of 5.39 μg h/mL, a terminal phase half-life of 3.6 h, a volume of distribution at steady state of 1.2 L/kg, and a total plasma clearance of 0.6 L/h/kg. After the single oral dose (30 mg/kg), a mean peak plasma concentration of 17.2 ± standard deviation 6.6 μg/mL was achieved at 1 h, and area under the curve (from time 0 to infinity, 5.4% extrapolated) was 44.1 μg h/mL. The terminal phase half-life was 3.3 h. Absolute oral bioavailability was calculated to be 82%.

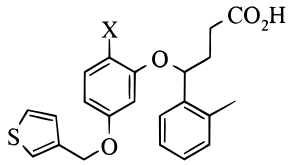
Effect of 12m on Vasoconstriction in the Pithed Rat. The ability of **12m** to block the pressor response to ET-1 in the pithed rat[30] was examined along with the selective ET_A antagonist BQ 123 (**1**) for purposes of comparison. Animals were pretreated with the selective ET_B antagonist BQ 788 to ensure pressor effects were mediated only by the ET_A receptor and to abolish the initial ET_B-mediated depressor response to ET-1 observed in this model. The diastolic blood pressure of the pithed rat increases in a dose-dependent fashion following bolus injections of ET-1 (see the Experimental Section). The log dose−response curves to ET-1 are shifted to the right from vehicle control in a parallel, dose-related manner following increasing iv doses of **12m** and **1** (see Figure 4 and data in Table 3) with no significant reduction in the maximal reponse.

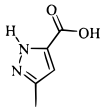
These experiments in the pithed rat model clearly demonstrate the ability of **12m** to antagonize the pressor response to ET-1 in vivo in a dose-dependent manner. **12m** appears some 5-fold less potent than BQ 123 (**1**) in this model, however, despite the two compounds being equipotent in our in vitro binding and functional assays (see Table 1). This disparity in the pithed rat model has also been observed for the Shionogi ET_A antagonist 97-139[31] and was attributed to plasma protein binding of the compound. The effect of protein binding on the in vitro and in vivo activities of this series of compounds will be the subject of a future publication from these laboratories.

Conclusion

The use of molecular modeling to superimpose two previously discovered series of endothelin antagonists led to the discovery of a novel class of phenoxybutanoic acids containing a key H-bond acceptor group. Variation of this substituent culminated in the synthesis of

Table 1. Variation of H-Bond Acceptor Group X: Physical Properties and in Vitro Activity



Compound	X	Formula[a]	mp (°C)	ET_A IC_{50} (nM)[b]	ET_B IC_{50} (nM)[c]	pK_B[d] (n)
BQ 123 (**1**)	-	-	-	12.0, 14.0	>30000	6.9 ± 0.1 (3)
7	-	-	-	1000, 900	>30000	-
8	-	-	-	90, 110	>30000	5.7, 5.7
12a	H	$C_{22}H_{22}O_4S$	100-4	871, 814	>30000	-
12b	$COCH_3$	$C_{24}H_{24}O_5S$[e]	116-21	26 ± 1.0 (3)	10000	-
12c	CO_2H	$C_{23}H_{22}O_6S$	123-7	158, 207	>10000	-
12d	$CONH_2$	$C_{23}H_{23}NO_4S$	203-5	29 ± 2.0 (3)	10000	-
12e	2-pyridyl	$C_{27}H_{25}NO_4S$	65-7	40, 37	10000	-
12f		$C_{25}H_{23}NO_5S$	112-4	20, 17	10000	-
12g		$C_{25}H_{24}N_2O_4S$[f]	165-7	163, 200	>10000	-
12h		$C_{26}H_{24}N_2O_6S$: $0.5H_2O$	209-10	7.0 ± 1.0 (3)	3000	7.3 ± 0.4 (4)
12i		$C_{25}H_{24}N_2O_4S$	189-90	20 ± 1.0 (3)	10000	-
12j	CN	$C_{23}H_{21}NO_4S$	167-9	7.0 ± 0.0 (3)	5000	-
12k (*R*-isomer)	CN	$C_{23}H_{21}NO_4S$	143	5.0 ± 1.0 (3)	>10000	7.4 ± 0.4 (7)
12l (*S*-isomer)	CN	$C_{23}H_{21}NO_4S$	143	113 ± 8.0 (3)	10000	-

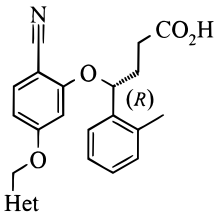
[a] Satisfactory microanalyses were obtained (±0.4%) for C, H, N, S unless otherwise stated. [b] Inhibition of [^{125}I]ET-1 binding in vitro to rat A10 cell ET_A receptors (mean of *n* experiments ± SEM; both values if performed in duplicate). [c] Inhibition of [^{125}I]ET-1 binding in vitro to rat cerebellum ET_B receptors (single experiment). [d] Antagonism of ET-1-mediated contraction of isolated de-endothelialized rat aortic rings (mean of *n* experiments ± SEM). [e] C: calcd, 67.9; found, 68.4. [f] C: calcd, 66.9; found, 65.3.

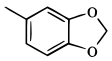
the cyano derivative **12m** (RPR 111844) which exhibited an IC_{50} of 5.0 nM at the rat ET_A receptor and 1000-fold selectivity over the ET_B receptor. This level of potency and selectivity was maintained when the compound was screened against human ET_A and ET_B receptors expressed on CHO cells (data not shown). **12m** also demonstrated in vivo functional activity in a rat model of ET-1-induced vasoconstriction. The promising pharmacokinetics of **12m** (>80% in rat, terminal $t_{1/2}$ = 3.3 h) has prompted us to examine the effects of this compound in preclinical models of cardiovascular disease.

Experimental Section

Chemical Methods. Reagents, starting materials, and solvents were purchased from common commercial suppliers and used as received or purified by standard methods. All organic solutions were dried over magnesium sulfate and concentrated at reduced pressure under aspirator vacuum using a Buchi rotary evaporator. Reaction products were purified, when necessary, by flash chromatography on silica gel (40−63 μm) eluting with the solvent system indicated. Yields are not optimized. Melting points were determined on a Gallenkamp 595 apparatus and are uncorrected. ^{1}H NMR spectra were acquired on a Varian VXR-400 spectrometer; peak positions are reported in parts per million relative to

Table 2. Variation of Heterocycle: Physical Properties and in Vitro Activity



Compd	Het	Formula[a]	mp (°C)	ET_A IC_{50} (nM)[b]	ET_B IC_{50} (nM)[c]	pK_B[d] (n)
12k	3-thienyl	$C_{23}H_{21}NO_4S$	143	5.0 ± 1.0 (3)	>10000	7.4 ± 0.4 (7)
12m	3-pyridyl	$C_{24}H_{22}N_2O_4$	58-60	5.0 ± 1.0 (4)	10000	6.8 ± 0.1 (7)
12n	4-pyridyl	$C_{24}H_{22}N_2O_4$	159-60	20, 22	10000	7.1 ± 0.2 (7)
12o		$C_{22}H_{20}N_2O_4S$	oil	7.0, 7.0	10000	7.3 ± 0.3 (5)
12p		$C_{26}H_{23}NO_6$	138-40	69, 54	10000	-

[a–d] See footnotes in Table 1.

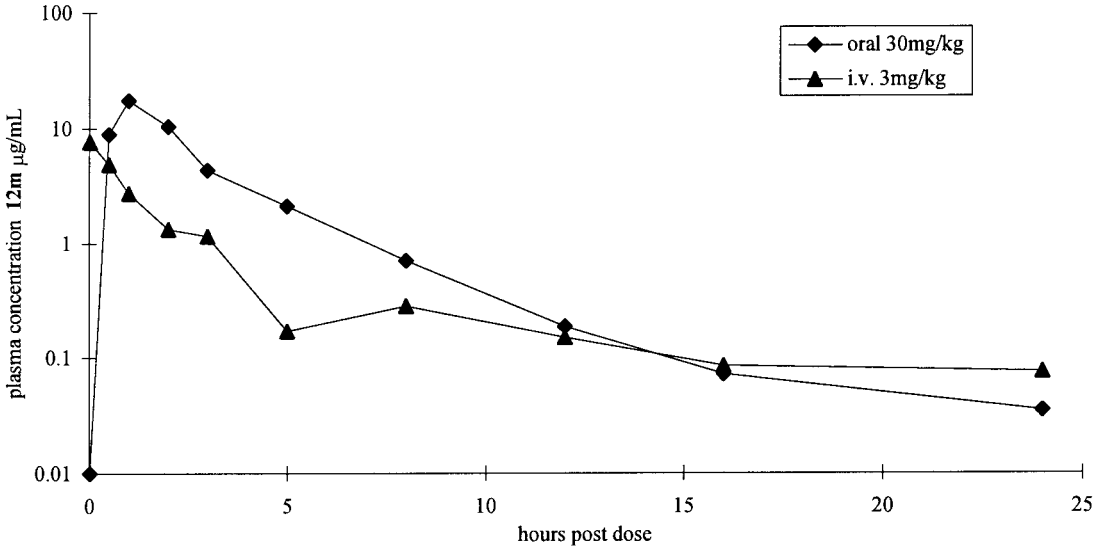


Figure 3. Pharmacokinetics of **12m** in the rat.

internal tetramethylsilane on the δ scale. Elemental analyses were performed by the Physical Chemistry Department at Rhone-Poulenc Rorer. The structure and purity of all compounds were confirmed by microanalytical and/or spectroscopic methods. Satisfactory microanalyses (± 0.4%) were obtained for C, H, N unless otherwise stated.

(*R*/*S*)-4-[3-(3-Thienylmethoxy)phenoxy]-4-(2-methylphenyl)butanoic Acid (12a). A suspension of **10a** (X = H) (3 g, 15 mmol), 4-chloro-4-(2-methylphenyl)butyric acid ethyl ester (**11**) (4.53 g, 20 mmol), potassium carbonate (4.14 g, 30 mmol), and potassiun iodide (0.5 g) in methyl ethyl ketone (100 mL) was stirred and heated to reflux for 48 h. After cooling the reaction mixture was evaporated to dryness and partitioned between ethyl acetate (200 mL) and water (200 mL). The organic phase was washed with water (100 mL), dried, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with ethyl acetate and cyclohexane (5:95 v/v) to give (*R*/*S*)-4-[(3-benzyloxy)phenoxy]-4-phenylbutanoic acid ethyl ester (1.0 g, 17%) as a yellow oil. This material (1.0 g, 2.94 mmol) was dissolved in methanol (50 mL) and treated with 10% aqueous potassium hydroxide solution (5 mL), and the reaction mixture was stirred and heated to reflux for 30 min. After cooling, the reaction mixture was partitioned between ethyl acetate (100 mL) and 1.0 M hydrochloric acid (50 mL). The organic layer was washed with water (50 mL), dried, and concentrated. The residue was recrystallized from cyclohexane to give **12a** (0.55 g, 57%) as a beige solid, mp 100−4 °C. ^{1}H NMR ($CDCl_3$): 2.35 (m, 2H), 2.41 (s, 3H), 2.65−2.75 (m, 2H), 4.85 (br s, 2H), 5.46 (m, 1H), 6.80 (m, 2H), 7.1−7.25 (m, 5H), 7.34 (m, 1H), 7.38−7.49 (m, 2H), 7.52 (m, 1H). Anal. ($C_{22}H_{22}O_4S$) C, H, N.

3-(3-Thienylmethoxy)phenol (10a: X = H). A suspen-

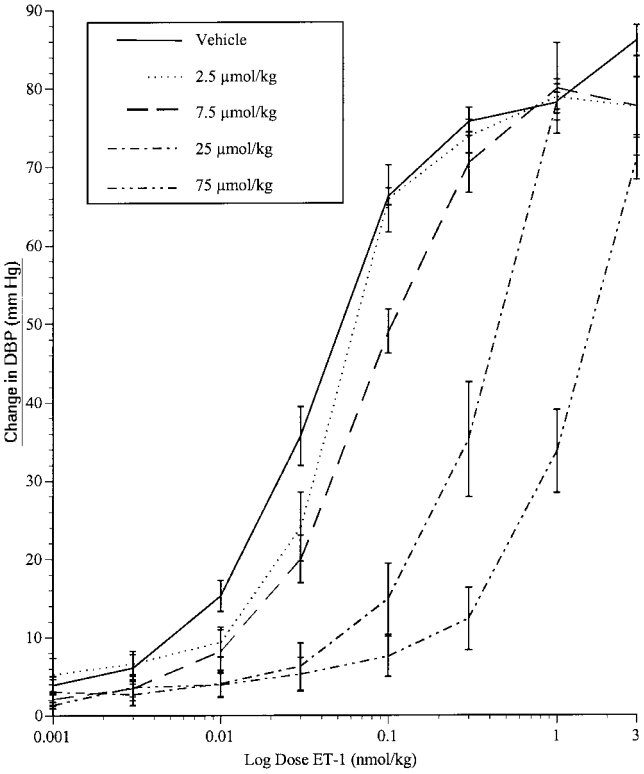


Figure 4. Effects of **12m** on ET-1-induced changes in diastolic blood pressure in the pithed rat (pretreated with BQ 788 (3 mg/kg)).

Table 3. Effects of **12m** and BQ 123 (**1**) on ET-1-Induced Changes in Diastolic Blood Pressure in the Pithed Rat Pretreated with BQ 788 (3 mg/Kg)

compd	iv dose (μm/kg)	no. of rats (n)	shift[a]
12m	2.5	6	ns[b]
12m	7.5	5	2.27[c]
12m	25	6	10.48[c]
12m	75	6	36.58[c]
1	2.5	6	15.06[c]
1	25	4	57.79[c]

[a] Degree of rightfold shift of log dose of ET-1−response curve (mean of n experiments). [b] Not significant. [c] Significantly different from vehicle-treated animals.

sion of resorcinol (**9a**: X = H) (18.3 g, 166 mmol), benzyl chloride (21 g, 166 mmol), potassium carbonate (23.3 g, 169 mmol), potassium iodide (1.0 g), and tetra-*n*-butylammonium bromide (1.0 g) in methyl ethyl ketone (500 mL) was stirred and heated to reflux for 5 h. After completion, the reaction mixture was filtered and evaporated to dryness. Water (500 mL) was added to the residue, and the reaction mixture was extracted with diethyl ether (3 × 300 mL). The organic phase was washed with water (250 mL), and the product was extracted with 10% aqueous sodium hydroxide (3 × 50 mL). Solid carbon dioxide was added until the pH of the solution was 5.0, and a brown oil was liberated. This was extracted with diethyl ether (3 × 100 mL), dried, and concentrated in vacuo to leave an oil. Purification by flash chromatography on silica gel, eluting with a mixture of ethyl acetate and cyclohexane (1:9 v/v), gave **10a** (X = H) as a colorless solid (9 g, 27%). ^{1}H NMR ($CDCl_3$): 5.00 (s, 2H), 6.80 (m, 2H), 7.34 (m, 1H), 7.38−7.49 (m, 3H), 7.52 (m, 1H).

4-Chloro-4-(2-methylphenyl)butyric Acid Ethyl Ester (11). A solution of *o*-tolualdehyde (22.8 g, 190 mmol) in dry dichloromethane (50 mL) was cooled to 0 °C and titanium(IV) tetrachloride (190 mL of a 1.0 M solution in dichloromethane, 190 mmol) was added dropwise giving a yellow suspension which was stirred at this temperature for 15 min. [(1-Ethoxycyclopropyl)oxy]trimethylsilane (**13**) (30 g, 170 mmol) was then added to the suspension, keeping the temperature below 10 °C. After complete addition, the reaction mixture was allowed to warm to room temperature and stirred for a further 2 h. The reaction mixture was quenched with water (100 mL) and extracted with ethyl acetate (5 × 100 mL). The combined organics were then washed with brine (100 mL), dried, and evaporated in vacuo to leave a brown oil, which was purified by fractional distillation to give **11** (23.1 g, 51%) as a pale-yellow oil, bp 115−20 °C/1 mbar. ^{1}H NMR ($CDCl_3$): 1.28 (t, J = 8 Hz, 3H), 2.38 (m, 2H), 2.40 (s, 3H), 2.55 (m, 2H), 4.15 (q, J = 8 Hz, 2H), 5.25 (dd, J = 12, 4 Hz, 1H), 7.23−7.28 (m, 3H), 7.50 (m, 1H).

(*R*/*S*)-4-[2-Acetyl-5-(3-thienylmethoxy)phenoxy]-4-(2-methylphenyl)butanoic Acid (12b). A mixture of 2,4-dihydroxyacetophenone (**9b**: X = $COCH_3$) (5 g, 33 mmol), 3-thienyl chloride (5.3 g, 40 mmol), potassium carbonate (5.3 g, 40 mmol), potassium iodide (0.1 g, 0.6 mmol), and tetra-*n*-butylammonium bromide (0.1 g) in methyl ethyl ketone (100 mL) was stirred and heated to reflux for 6 h. After cooling, the reaction mixture was partitioned between ethyl acetate (200 mL) and 1.0 M aqueous hydrochloric acid (200 mL). The organics were washed with water (100 mL), dried, and concentrated to dryness. The residue was recrystallized (with charcoal) from cyclohexane to afford **10b** (X = $COCH_3$) as a colorless solid (2 g, 24%), mp 99−102 °C. ^{1}H NMR ($CDCl_3$): 2.58 (s, 3H), 5.10 (s, 2H), 6.51 (m, 2H), 7.14 (dd, J = 4, 2 Hz, 1H), 7.35 (m, 2H), 7.65 (m, 1H).

A portion of this material (1.0 g, 4 mmol), 4-chloro-4-phenylbutyric acid ethyl ester (**11**) (1.13 g, 5 mmol), potassium carbonate (1.03 g, 7.5 mmol), and potassium iodide (0.1 g) in methyl ethyl ketone (100 mL) was stirred and heated to reflux for 48 h. After cooling, the reaction mixture was partitioned between ethyl acetate (100 mL) and water (100 mL); the organics were washed with water (50 mL), dried, and concentrated to dryness. The residue was purified by flash chromatography on silica gel, eluting with a mixture of ethyl acetate and cyclohexane (1:9 v/v) to give (*R*/*S*)-4-[2-acetyl-5-(3-thienylmethoxy)phenoxy]-4-(2-methylphenyl)butanoic acid ethyl ester (**20**) (1.0 g, 55%) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.27 (t, J = 8 Hz, 2H), 2.25 (m, 2H), 2.47 (s, 3H), 2.50−2.60 (m, 2H), 2.73 (s, 3H), 4.10 (q, J = 8 Hz, 2H), 4.90 (m, 2H), 5.49 (m, 1H), 6.13 (m, 1H), 6.50 (m, 1H), 7.01 (m, 1H), 7.15 (m, 1H), 7.19 (m, 3H), 7.30 (m, 2H), 7.79 (m, 1H).

This material (1.0 g, 2.21 mmol) was dissolved in methanol (50 mL), treated with 10% aqueous potassium hydroxide solution (2 mL), and heated to reflux for 1 h. After cooling, the reaction mixture was evaporated to dryness, and the residue was partitioned between ethyl acetate (50 mL) and 1.0 M hydrochloric acid (50 mL). The organic layer was washed with water (50 mL), dried, and concentrated in vacuo to leave a residue, which was recrystallized from ethyl acetate and cyclohexane to give **12b** (0.4 g, 43%) in a form of a white solid, mp 116−21 °C. ^{1}H NMR ($CDCl_3$): 2.36 (m, 2H), 2.41 (s, 3H), 2.65−2.75 (m, 2H), 2.72 (s, 3H), 4.90 (m, 2H), 5.51 (m, 1H), 6.14 (m, 1H), 6.50 (m, 1H), 7.03 (m, 1H), 7.15−7.20 (m, 4H), 7.30 (m, 1H), 7.38 (m, 1H), 7.81 (m, 1H). Anal. ($C_{24}H_{24}O_5S$) C, H, N.

2-(Aminocarbonyl)-5-(3-thienylmethoxy)phenol (10d: X = $CONH_2$). A solution of 2-(methoxycarbonyl)-5-(3-thienylmethoxy)phenol (**10c**: X = CO_2Me) (15 g, 57 mmol) in ethanolic ammonia solution (150 mL) was placed in a bomb and heated on a steam bath for 24 h. After cooling, the solvent was evaporated, and the residue was taken up in dichloromethane (100 mL), filtered, and concentrated to dryness. Purification of the residue by flash chromatography on silica gel, eluting with a mixture of ethyl acetate and dichloromethane (1:4 v/v), gave **10d** (X = $CONH_2$) (3.75 g, 26%) as a white solid, mp 174−7 °C. ^{1}H NMR (DMSO-d_6): 5.13 (s, 2H), 6.50 (m, 2H), 7.19 (m, 1H), 7.58 (m, 2H), 7.77 (br m, 1H), 7.79 (m, 1H), 8.21 (br s, 1H).

2-(2,4-Dimethoxyphenyl)pyridine (15). To a mechanically stirred suspension of magnesium turnings (8.11 g, 333 mmol) in dry tetrahydrofuran (300 mL), containing a few crystals of iodine under gentle reflux, was added dropwise a

solution of 1-bromo-2,4-dimethoxybenzene (48.02 mL, 333 mmol) in dry tetrahydrofuran (100 mL). The reaction mixture was stirred and heated to reflux for a further 30 min. After cooling, the above reagent was added dropwise to a refluxing solution of 2-bromopyridine (50.3 g, 333 mmol) and bis-(triphenylphosphine)palladium(II) chloride (3.51 g, 5 mmol) in dry tetrahydrofuran (100 mL). The reaction mixture was refluxed for a further 6 h, then filtered, and concentrated to dryness in vacuo; 150 mL of water was added, and the reaction mixture was extracted with ethyl acetate (3 × 50 mL), dried, and evaporated to dryness to give a black oil. This was purified by flash chromatography on silica gel, eluting with a mixture of diethyl ether and pentane (1:2 v/v) to give **15** (28 g, 39%) as a light-yellow solid, after trituration with pentane, mp 58−60 °C. ^{1}H NMR ($CDCl_3$): 3.63 (s, 3H), 3.41 (s, 3H), 6.48 (dd, J = 8, 2 Hz, 1H), 6.54 (d, J = 2 Hz, 1H), 7.14 (m, 1H), 7.68 (d, J = 8 Hz, 1H), 7.75 (m, 2H), 8.41 (m, 1H). Anal. ($C_{13}H_{13}NO_2$) C, H, N.

2-(2,4-Dihydroxyphenyl)pyridine (9e). A mixture of **15** (14 g, 65 mmol) and pyridine hydrochloride (121 g, 1000 mmol) was heated to 160 °C for 7 h. After cooling, the residue was dissolved in dichloromethane (300 mL) and washed with 2.0 M hydrochloric acid (3 × 300 mL). The combined organics were washed with water (150 mL), dried, and concentrated in vacuo to furnish an oil. This was purified by flash chromatography on silica gel, eluting with ethyl acetate and pentane (1:1 v/v) to give **9e** (8 g, 66%) as a white solid, mp 170−172 °C. ^{1}H NMR ($CDCl_3$): 6.48 (dd, J = 8, 2 Hz, 1H), 6.54 (d, J = 2 Hz, 1H), 7.14 (m, 1H), 7.68 (d, J = 8 Hz, 1H), 7.75 (m, 2H), 8.41 (m, 1H), 12.00 (br, 1H). Anal. ($C_{11}H_9NO_2$) C, H, N.

(*R/S*)-4-[2-(Oxazol-2-yl)-5-(3-thienylmethoxy)phenoxy]-4-(2-methylphenyl)butanoic Acid (12f). A solution of **10f** (X = $CONHCH_2CH_2OH$) (2.5 g, 5 mmol) in dry tetrahydrofuran (50 mL) was treated with Burgess reagent (1.3 g, 5.5 mmol), and the mixture was heated to reflux for 30 min. After cooling, the reaction mixture was partitioned between ethyl acetate (100 mL) and water (100 mL); the organic layer was dried and evaporated to dryness. The residue was purified by flash chromatography on silica gel, eluting with ethyl acetate and cyclohexane (1:1 v/v) to give (*R/S*)-4-[2-(oxazolin-2-yl)-5-(3-thienylmethoxy)phenoxy]-4-(2-methylphenyl)butanoic acid ethyl ester (**16**) (1.5 g, 63%) as a colorless oil. A stirred solution of **16** (0.9 g, 1.88 mmol) in toluene (50 mL) was treated with nickel peroxide (5.04 g, 56 mmol) and heated at 100 °C for 24 h. The reaction mixture was filtered through a pad of diatomaceous earth and concentrated to dryness. The residue was subjected to purification by flash chromatography on silica gel, eluting with a mixture of ethyl acetate and cyclohexane (15:85 v/v) to give (*R/S*)-4-[2-(oxazol-2-yl)-5-(3-thienylmethoxy)-phenoxy]-4-(2-methylphenyl)butanoic acid ethyl ester as a colorless oil (140 mg, 16%). This material was hydrolyzed as for example **12a** above to afford **12f** (70 mg, 54%) as a colorless solid, mp 112−114 °C, after recrystallization from ethyl acetate/hexane. ^{1}H NMR (DMSO-d_6): 2.05 (m, 2H), 2.40 (s, 3H), 2.41 (m, 1H), 2.58 (m, 1H), 5.00 (m, 2H), 5.62 (m, 1H), 6.38 (s, 1H), 6.64 (m, 1H), 7.10−7.20 (m, 4H), 7.37 (m, 2H), 7.43 (m, 1H), 7.56 (m, 1H), 7.78 (m, 1H), 8.20 (1H). Anal. ($C_{25}H_{23}NO_5S$) C, H, N.

(*R/S*)-4-[2-(Imidazol-2-yl)-5-(3-thienylmethoxy)phenoxy]-4-(2-methylphenyl)butanoic Acid Ethyl Ester (19). To a stirred solution of **18** (X = $CSNH_2$) (1.63 g, 3.5 mmol) in dry tetrahydrofuran (25 mL) was added sodium hydride (0.14 g of a 60% dispersion w/w in mineral oil, 3.5 mmol), followed by methyl iodide (0.33 mL, 5.2 mmol). The suspension was stirred at room temperature for 3.5 h to form a clear orange solution, and the reaction mixture was concentrated to dryness. The residue was dissolved in ethyl acetate (20 mL), washed with water (2 × 10 mL), dried, and concentrated to dryness. The residue was purified by flash chromatography on silica gel, eluting with a mixture of ethyl acetate and pentane (1:3 v/v) to yield (*R/S*)-4-[2-(methylthioimidato-2)-5-(3-thienylmethoxy)phenyl]-4-(2-methylphenyl)butanoic acid ethyl ester (0.91 g, 54%) as a yellow solid, mp 97−9 °C. ^{1}H NMR ($CDCl_3$): 1.20 (t, J = 8 Hz, 3H), 2.18 (m, 2H), 2.42 (s, 3H), 2.50 (s, 3H), 2.58−2.61 (m, 2H), 4.10 (q, J = 8 Hz, 2H), 4.85 (m, 2H), 5.40 (m, 1H), 6.08 (m, 1H), 6.24 (m, 1H), 7.00 (m, 1H), 7.18 (m, 4H), 7.32 (m, 2H), 7.41 (m, 1H), 9.60 (br s, 1H).

To a portion of this material (0.2 g, 0.41 mmol) in dry tetrahydrofuran (2 mL) and glacial acetic acid (0.05 mL, 0.82 mmol) was added aminoacetaldehyde diethyl acetal (0.055 mL, 0.41 mmol), and the mixture was heated on a steam bath for 2 h. After the mixture cooled, 5.0 M hydrochloric acid (0.166 mL, 0.82 mmol) was added, and the reaction mixture was allowed to stir at room temperature for 2 h. The reaction mixture was basified to pH 8.0 with solid sodium bicarbonate, diluted with 10 mL water, extracted with ethyl acetate (3 × 10 mL), dried, and concentrated to dryness. The residue was purified by flash chromatography on silica gel, eluting with ethyl acetate and pentane (1:1 v/v) to give **19** (0.07 g, 35%) in a form of a white solid. ^{1}H NMR ($CDCl_3$): 1.18 (t, J = 8 Hz, 3H), 2.20 (m, 1H), 2.42 (s, 3H), 2.50 (m, 3H), 4.10 (m, 2H), 4.93 (m, 2H), 5.58 (m, 1H), 6.30 (m, 1H), 6.60 (m, 1H), 7.03 (m, 1H), 7.16 (m, 1H), 7.20 (m, 4H), 7.22 (m, 2H), 7.30 (m, 1H), 8.21 (m, 1H). Anal. ($C_{27}H_{28}N_2O_4S$) C, H, N.

(*R/S*)-4-[2-(Thioimidato-2)-5-(3-thienylmethoxy)phenyl]-4-(2-methylphenyl)butanoic Acid Ethyl Ester (18: X = $CSNH_2$). A solution of (*R/S*)-4-[2-(aminocarbonyl)-5-(3-thienylmethoxy)phenyl]-4-(2-methylphenyl)butanoic acid ethyl ester (**17**) (X = $CONH_2$) (4.99 g, 11 mmol) in dry tetrahydrofuran (100 mL) was cooled in an ice bath, and Lawesson's reagent (2.22 g, 5.5 mmol) was added dropwise. The yellow suspension was allowed to warm to room temperature and was stirred for 5 h to result in a clear solution. The reaction mixture was concentrated to dryness and purified by flash chromatography, eluting with a mixture of ethyl acetate and pentane (1:2 v/v). Trituration of the oily residue with diethyl ether afforded **18** (X = $CSNH_2$) (2.42 g, 47%) as a light-green solid, mp 134−5 °C. ^{1}H NMR ($CDCl_3$): 1.20 (t, J = 8 Hz, 3H), 2.18−2.30 (m, 2H), 2.42 (s, 3H), 2.53−2.58 (m, 2H), 4.12 (q, J = 8 Hz, 2H), 4.95 (m, 2H), 5.58 (m, 1H), 6.21 (m, 1H), 6.58 (m, 1H), 7.01 (m, 1H), 7.20 (m, 4H), 7.25 (m, 1H), 7.30 (m, 1H), 7.94 (br s, 1H), 8.62 (m, 1H), 9.20 (br s, 1H).

(±)-5-{2-[3-Carboxy-1-(2-methylphenyl)propoxy]-4-(3-thienylmethoxy)phenyl}pyrazole-3-carboxylic Acid Hemihydrate (12h). A mixture of **20** (18.89 g, 44 mmol) and diethyl oxalate (19.27 g, 132 mmol) in dry toluene (100 mL) was added dropwise to a stirred solution of sodium hydride (1.76 g of a 60% dispersion w/w in mineral oil, 44 mmol) in dry toluene (400 mL) under a nitrogen atmosphere. The reaction mixture was heated to reflux for 16 h to result in a pale-red solution. After the mixture cooled, the toluene was evaporated, and ethanol (500 mL) and glacial acetic acid (10.57 g, 176 mmol) followed by hydrazine hydrate (2.2 g, 44 mmol) were added. The reaction mixture was heated to reflux for a further 90 min and cooled, and the ethanol removed in vacuo. The residue was partitioned between ethyl acetate (250 mL); and water (250 mL), the organic layer was washed with brine (200 mL), dried, and concentrated to dryness to give an oil. This was purified by flash chromatography on silica gel, eluting with pentane and methyl *tert*-butyl ether (2:1 v/v) to yield diester **21h** (7.2 g, 36%) as a pale-yellow glass. ^{1}H NMR ($CDCl_3$): 1.21 (t, J = 8 Hz, 3H), 1.42 (t, J = 8 Hz, 3H), 2.24 (m, 1H), 2.28 (m, 1H), 2.42 (s, 3H), 2.58 (m, 2H), 4.10 (q, J = 8 Hz, 2H), 4.42 (q, J = 8 Hz, 2H), 4.95 (m, 2H), 5.60 (m, 1H), 6.39 (m, 1H), 6.60 (m, 1H), 7.03 (m, 1H), 7.10 (s, 1H), 7.18 (m, 4H), 7.20 (m, 1H), 7.34 (m, 1H), 7.58 (m, 1H).

This material was hydrolyzed as for example **12a** above to give **12h** (6.22 g, 90%) as a white solid, after recrystallization from ethanol, mp 209−210 °C. ^{1}H NMR (DMSO-d_6): 2.10 (m, 2H), 2.40 (s, 3H), 2.50 (m, 2H), 4.98 (m, 2H), 5.60 (m, 1H), 6.30 (m, 1H), 6.62 (m, 1H), 7.05 (m, 1H), 7.10−7.25 (m, 5H), 7.41 (m, 1H), 7.52 (m, 1H), 7.70 (m, 1H). Anal. ($C_{26}H_{24}N_2O_6S\cdot 0.5H_2O$) C, H, N.

(*R*)-4-[2-Cyano-5-(3-thienylmethoxy)phenoxy]-4-(2-methylphenyl)butanoic Acid (12k). A solution of sodium (*R*)-4-hydroxy-4-(2-methylphenyl)butanoate (**25**) (3.0 g, 15 mmol) in dry tetrahydrofuran (50 mL) was treated portionwise with sodium hydride (1.5 g of a 60% dispersion w/w in mineral

oil, 37 mmol) at room temperature. After complete addition, the reaction mixture was stirred at ambient temperature for 1 h and warmed to 55 °C, and **26** (3.0 g, 13 mmol) was added in one portion. The reaction mixture was stirred, heated to 55 °C for 15 h, cooled, diluted with 1 N hydrochloric acid (200 mL), and extracted with ethyl acetate (4 × 100 mL). The combined organic extracts were dried and concentrated to leave an oily residue which was boiled in isopropyl ether (50 mL), filtered while hot, and allowed to cool. The yellow solid which separated was collected and recrystallized from a mixture of ethyl acetate and hexane to give **12k** (1.5 g, 24%) as a white fluffy solid, mp 143 °C, $[\alpha]_D = -53°$ ($c = 0.01$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 2.34 (m, 2H), 2.41 (s, 3H), 2.62 (m, 1H), 2.78 (m, 1H), 4.89 (m, 2H), 5.48 (dd, $J = 12$, 4 Hz, 1H), 6.15 (d, $J = 2$ Hz, 1H), 6.50 (dd, $J = 12$, 2 Hz, 1H), 7.00 (dd, $J = 4$, 1 Hz, 1H), 7.14−7.20 (m, 4H), 7.31 (dd, $J = 6$, 4 Hz, 1H), 7.38 (m, 1H), 7.42 (d, $J = 8$ Hz, 1H). Anal. ($C_{23}H_{21}NO_4S$) C, H, N. Chiral HPLC on a Chiralcel OD column (heptane/ethanol/glacial acetic acid, 980/20/2, flow rate 1 mL/min) showed **12k** to have a >98% ee. A typical retention time for the (*R*)-isomer **12k** was 81 min and for the (*S*)-isomer **12l**, 91 min.

2-Fluoro-4-(3-thiopheneylmethoxy)benzonitrile (26). A mixture of 4-cyano-3-fluorophenol (**27**) (4.8 g, 35 mmol), 3-thienyl chloride (5.3 g, 40 mmol), potassium carbonate (5.3 g, 4 mmol), potassium iodide (0.1 g, 0.6 mmol) and tetra-*n*-butylammonium bromide (0.1 g) in methyl ethyl ketone (100 mL) was stirred and heated to reflux for 18 h. After cooling, the reaction mixture was filtered, concentrated, taken up in ethyl acetate (500 mL), and washed with 1.0 M aqueous hydrochloric acid (200 mL) and 1.0 M aqueous sodium hydroxide (200 mL), followed by water (200 mL). The organic layer was dried and concentrated in vacuo to leave an oil which was passed through a short pad of silica gel with the aid of dichloromethane. The filtrate was evaporated to leave an oil, which crystallized on standing. Recrystallization from isopropyl ether gave **26** (6 g, 50%) as a white solid, mp 63−5 °C. ^{1}H NMR ($CDCl_3$): 5.11 (s, 2H), 6.80 (m, 2H), 7.12 (m, 1H), 7.34 (m, 1H), 7.38 (m, 1H), 7.52 (m, 1H).

***tert*-Butyl 4-(2-Methylphenyl)-4-oxobutanoate (22).** To a cooled (−78 °C) solution of succinic anhydride (75 g, 750 mmol) in dry tetrahydrofuran (800 mL) was added a solution of *o*-tolylmagnesium chloride (800 mL of a 1.0 M solution in toluene, 800 mmol), and the reaction mixture stirred at this temperature for 2 h. The reaction mixture was warmed to room temperature, poured into 1.0 M hydrochloric acid (1 L), and extracted with diethyl ether (4 × 500 mL). The combined organics were washed with water (2 × 500 mL), dried, and concentrated to leave 4-(2-methylphenyl)-4-oxobutanoic acid as a colorless oil (110 g, 75%). ^{1}H NMR ($CDCl_3$): 2.41 (s, 3H), 2.80 (t, $J = 8$ Hz, 2H), 3.30 (t, $J = 8$ Hz, 2H), 7.4−7.60 (m, 4H), 10.0 (br, 1H).

A mixture of 4-(2-methylphenyl)-4-oxobutanoic acid (40 g, 208 mmol), concentrated sulfuric acid (4 mL), and *tert*-butyl alcohol (4 mL, 42 mmol) in dichloromethane (200 mL) was cooled to 0 °C, and isobutylene (~400 mL) was condensed into the reaction. The reaction mixture was stirred at 0 °C until the TLC showed complete reaction (~8 h) and the excess isobutylene was allowed to evaporate. Saturated sodium bicarbonate (200 mL) was carefully added with vigorous stirring. The organic layer was separated, filtered through a pad of silica gel with the aid of dichloromethane, and concentrated to give **22** (50 g, 97%) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): 1.46 (s, 9H), 2.40 (s, 3H), 2.76 (t, $J = 8$ Hz, 2H), 3.28 (t, $J = 8$ Hz, 2H), 7.4−7.60 (m, 4H),

***tert*-Butyl (*R*)-4-Hydroxy-4-(2-methylphenyl)butanoate (23).** A solution of **22** (12 g, 48 mmol) in dry diethyl ether (50 mL) was dried over 10 g of 4 Å molecular sieves for 3 h and was then transferred to a dry flask and placed under an argon atmosphere. The solution was cooled to −20 °C, treated rapidly with (+)-*B*-chlorodiisopinocampheylborane ((+)-DIP-chloride; 25 g, 78 mmol), and sealed under an atmosphere of argon. The reaction mixture was stored at −20 °C for 48 h, allowed to warm to room temperature, and diluted with diethyl ether (50 mL), before addition of diethanolamine (10 g, 95 mmol). The reaction mixture was stirred vigorously at ambient temperature for 5 h, poured into pentane (500 mL) and filtered through a pad of diatomaceous earth. The filtrate was concentrated and pumped under high vacuum (0.5 mmHg) with warming to 50 °C, and the residue was purified by flash chromatography on silica gel, eluting with a mixture of methanol and dichloromethane (5:95 v/v) to give **23** (10 g, 84%) as a colorless oil, bp 175 °C/0.2 mmHg, $[\alpha]_D = +49°$ ($c = 0.01$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 1.46 (s, 9H), 2.00 (m, 2H), 2.34 (s, 3H), 2.41 (m, 2H), 4.99 (dd, $J = 8$, 4 Hz, 1H), 7.1−7.25 (m, 3H), 7.49 (m, 1H). The ee was determined to be >99% by a ^{1}H NMR method in $CDCl_3$ using the derivative **24** formed from (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (see the Chemistry section).

Sodium (*R*)-4-Hydroxy-4-(2-methylphenyl)butanoate (25). A solution of **23** (0.5 g, 2 mmol) in tetrahydrofuran (3 mL) and methanol (3 mL) was treated with 5.0 M aqueous sodium hydroxide (0.42 mL) and stirred at ambient temperature for 8 h. The reaction was concentrated to dryness, the residue was stirred for 30 min in 2-propanol (30 mL), and the resultant precipitate was collected and washed with isopropyl ether to give **25** (300 mg, 70%) in a form of a white solid, mp >250 °C, $[\alpha]_D = +34°$ ($c = 0.01$, MeOH). ^{1}H NMR (DMSO-d_6): 1.65 (m, 2H), 2.11 (m, 2H), 2.22 (s, 3H), 4.75 (dd, $J = 8$, 4 Hz, 1H), 7.05 (m, 2H), 7.23 (m, 1H), 7.48 (m, 1H).

Biological Methods. (i) Radioligand Binding Assays (IC_{50} Determination): Preparation of ET_A Receptors. A10 cells, a rat aortic smooth muscle cell line (ATCC number CRL-1476), were grown to confluence in Dulbecco's modified Eagle's medium containing 10% v/v fetal bovine serum. Two days after the final medium change cells were scraped from the base of the flask and centrifuged at 1000g for 10 min at 4 °C. The resulting pellets were washed twice in 50 mM Hepes buffer (pH 7.3) containing calcium chloride (1 mM) and magnesium chloride (5 mM) and resuspended at a density of 140 000 cells/mL in 50 mM Hepes buffer (pH 7.3). Aliquots of 5 mL were snap-frozen using a mixture of methanol and solid carbon dioxide and stored at −20 °C until required. For use in the binding assay, cells were diluted to the required density with 50 mM Hepes buffer (pH 7.3).

Preparation of ET_B Receptors. Rats were killed by cervical dislocation, and the cerebellum tissue was removed into ice-cold 50 mM Tris buffer (pH 7.4) containing sucrose (0.25 M), ethylenediaminetetraacetic acid (3 mM), aprotinin (0.5 μg/mL), pepstatin A (10 μg/mL), leupeptin (10 μg/mL), and phenylmethanesulfonyl fluoride (0.1 mM). After homogenization using a glass/Teflon manual homogenizer, the samples were centrifuged at 4 °C for 17 min at 1000*g* and the resulting supernatants retained. This material was centrifuged at 40000*g* for 35 min at 4 °C, the pellets were resuspended in 50 mM Tris buffer (pH 7.4), and the protein content was determined. Aliquots of 100 μL were snap-frozen using a mixture of methanol and solid carbon dioxide and stored at −20 °C until required. Samples were diluted as necessary with 50 mM Tris buffer (pH 7.4) containing 0.1% w/v bovine serum albumin for use in the assay.

Binding Assay. Binding assays were performed in Millipore 0.22-μm 96-well multiscreen plates and consisted of 20 pM [^{125}I]ET-1, test compound or vehicle, and A10 cells or cerebellum protein in a final volume of 250 μL. Nonspecific binding was measured using 500 nM unlabeled ET-1. [^{125}I]-ET-1 was prepared in 50 mM Tris buffer (pH 7.4) containing 0.1% w/v BSA, and test compounds were prepared in the same, supplemented with dimethyl sulfoxide at 5% v/v final assay concentration. Reactions were started by the addition of cells or cerebellum protein and allowed to proceed for 2 h at 37 °C before being terminated by vacuum filtration. The filters were then washed and collected for ψ-counting.

Data Analysis. Data from binding assays was corrected for nonspecific binding and expressed as a percentage of total [^{125}I]ET-1 binding in the presence of vehicle. IC_{50} values were obtained by curve fitting. In a minority of cases when duplicate IC_{50} values were not obtained, the results of IC_{50}

measurements were confirmed by retesting a single concentration selected to fall on the concentration-response curve.

(ii) In Vitro Functional Assay (pK_B Determination): This was carried out as previously described[15] using de-endothelialized rat aortic rings.

(iii) In Vivo Functional Assay (Pithed Rat Model): The experimental methods have been described in detail by RPR researchers.[30] A brief protocol is as follows: Male Sprague−Dawley rats (250−350 g) were anesthetized with isoflurane, pithed with a stainless steel rod, and artificially respired. A jugular vein and carotid artery were cannulated for administration of vehicle or test compound and measurement of blood pressure (via a pressure transducer). BQ 788 (3 mg/kg) and test compound (2.5, 7.5, 25, 75 μg/kg) were given iv 10 min prior to measurement of the dose− pressor response curve following bolus injection of ET-1 (0.01−10 nmol/kg). Test compounds caused a rightward, parallel dose-dependent shift of the dose−reponse curve from vehicle control. The shift from vehicle for each antagonist was calculated using the dose of ET-1 which caused a 40 mmHg change in diastolic blood pressure.

Pharmacokinetics in the Rat. The pharmacokinetics of **12m** were investigated following the administration of single doses by the oral and intravenous routes in male Sprague−Dawley rats. **12m** was formulated as a 7.5 mg/mL solution/suspension in 1% w/v carboxymethyl cellulose/0.2% v/v Tween 80 in deionized water for the oral route and as a 3.75 mg/mL solution in dimethyl sulfoxide for the intravenous route. Three individual rats per time point (selected randomly from 57 animals, mean body weight 331 g, standard deviation 22 g) were dosed by gavage at 30 mg/kg (4 mL of vehicle/kg) or at 3 mg/kg (0.8 mL of vehicle/kg) by slow bolus injection into the jugular vein while under light isoflurane anesthesia. Heparinized blood samples were taken by cardiac puncture from individual rats postsacrifice by carbon dioxide asphyxiation at 0.5, 1, 2, 3, 5, 8, 12, 16, and 24 h postdose for the oral groups and at 0.08, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, and 8 h postdose for the intravenous groups. Plasma samples obtained from blood by centrifugation (4 °C) were analyzed by reverse-phase HPLC using an internal standard (1 mL/min 45% methanol/35% water, pH 3/20% tetrahydrofuran, 25-cm × 4-mm i.d. LichrocartRP column, UV detection 250 nm). Retention times of **12m** and internal standard were 6.5 and 11 min, respectively. The limit of detection was 15 ng of **12m**/mL of plasma. Pharmacokinetic parameters were derived from the mean plasma concentrations at each time point using the SIPHAR PK computer program (SIPHAR, SIMED, France).

Molecular Modeling. All structures were initially created using Concord 3D builder (distributed by Tripos Inc., 1699 Hanley Rd, Suite 303, St. Louis, MO 63144). The remaining modeling was carried out within Chem-X (developed and distributed by Chemical Design Ltd., Roundway House, Cromwell Park, Chipping Norton, Oxfordshire OX7 5SR, U.K.). Charges were first set using the Gasteiger method. The acid group interaction points were introduced by conversion of the existing carboxylic acid hydrogen atom to a dynamic dummy atom and extension of the dummy−oxygen bond length to 2.8 Å. The theoretical VDW radius for this dummy was set to zero as was its charge. The charges on the remaining oxygens were modified using the equation $((e1 + e2) - e3)/2$, where *e1* and *e2* were the original charges on the oxygens and *e3* was the charge on the original attached hydrogen. This procedure allowed removal of the hydrogen and equivalencing of the charges on the oxygens but prevented the creation of a full negative charge which would have a distorting effect on energy calculations carried out in vacuo. The molecules were superimposed using the central phenyl template and the 1,3-attachment points. The pendant arylmethoxy groups were frozen in an identical low-energy conformation consistent with SAR. A further dummy was placed at the center of the remaining aryl ring. The 3D box was created around the molecules using a command procedure which placed dummies at defined coordinate points; four of these dummies were designated D1, D2, D3, and D4. The conformational analysis was carried for all unfrozen bonds within -X using 3-point rotation about sp^3-sp^3 bonds, 6-point rotation about sp^3-sp^2 bonds, and 2-point rotation about conjugated bonds and the bond connecting the acid dummy oxygen to the acid group carbon. Energy minimization of all conformations generated was carried out using the torsional minimizer setting. Conformations generated were then plotted using the following distance variables: acid group dummy−D1, D2, D3, and D4; aryl group dummy−D1, D2, D3, and D4; and the ortho hydrogen of the Aryl group−D1, D2, D3, and D4. The ortho hydrogen was introduced to allow the fixing of the ring plane. Four corners of the box were used to ensure complete specification of the 3D position of each group. The plots for each molecule studied were combined in the order **8** then **7** using the AND logical operator. A tolerance of 10% was used along with an energy cutoff of 8 kcal above the found minimum for each conformation. The conformational space containing the remaining conformations for the three compounds was then visualized; the remaining conformations were listed and retrieved from the conformational database. Display of the compounds is shown in Figure 1. This superimposition was used for all subsequent design work.

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