Selective Endothelin A Receptor Antagonists. 4. Discovery and Structure−Activity Relationships of Stilbene Acid and Alcohol Derivatives[1−3]

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This publication describes the synthesis and optimization of a novel series of stilbene endothelin antagonists. Analysis of the SAR established for previous papers in this series prompted the design and synthesis of (*Z*)-4-phenyl-5-(3-benzyloxyphenyl)pent-4-enoic acid **3** which was found to be a moderately active inhibitor of the binding of [^{125}I]ET-1 to ET_A receptors with an IC_{50} of 6 μM. More interestingly, the intermediate compound (*E*)-2-phenyl-3-(3-benzyloxyphenyl)-propenoic acid **5** was equiactive with **3**. Optimization of **5** resulted in the preparation of (*E*)-2-phenyl-3-(2-cyano-5-(thien-3-ylmethoxy))phenylpropenoic acid **18** (RPR111723) which had an IC_{50} in the binding assay of 80 nM on the ET_A receptor and a pK_B of 6.5 in the functional assay, measured on rat aortic strips. Reduction of the acid group of **5** gave the first nonacidic ET_A antagonist in our series, (*E*)-2-phenyl-3-(3-benzyloxyphenoxy)prop2-enol **6** with an IC_{50} of 20 μM. Optimization of **6** resulted in the preparation of 2-(2-methylphenyl)-3-(2-cyano-5-(thien-3-ylmethyl)phenyl)prop-2-enol **33** with an IC_{50} of 300 nM on the ET_A receptor.

Introduction

The endothelins comprise a family of closely related peptides (ET-1, ET-2, and ET-3) whose biological actions are mediated in mammals by two receptor subtypes designated ET_A and ET_B.[4] Endothelin-1 (ET-1) is a 21 amino acid peptide with potent vasoconstrictor properties that was originally identified in conditioned medium from cultured bovine endothelial cells.[5] It has since been found in different animal and human cells including smooth muscle, macrophages, glial cells, and mesangial cells.[6] Both ET_A and ET_B receptor subtypes are also widely distributed in tissues.[7] In the vasculature ET_A receptors were first identified on smooth muscle and were recognized to mediate the ET-1-induced vasoconstrictor actions whereas ET_B receptors were found on endothelial cells and were responsible for the ET-1 induced vasodilatation. Subsequently it was recognized that ET_B receptors were also distributed to smooth muscle and were able to mediate contractions.[8] The relative importance of the ET_A and ET_B vasoconstrictor receptors varies between tissue and species.[9]

Endothelin has been implicated in the pathophysiology of a large number of diseases. Significantly raised levels of ET-1 have been detected in the plasma, and other biological fluids, of patients suffering from congestive heart failure, stable and unstable angina, acute myocardial infarction, primary pulmonary hypertension, acute renal insufficiency/failure, shock (septic and cardiogenic), cerebral ischaemia/stroke (haemorrhagic and nonhaemorrhagic), asthma, and metastatic prostate cancer.[10,11]

The most relevant receptor to target for treatment of these disease states remains controversial; however the ET_B receptor on endothelium may play a beneficial role in certain disease states as it is responsible for the enhanced production of PGI_2 and NO induced by ET-1.[12,13] Since these later effects might be beneficial, it has been suggested that a selective ET_A antagonist may provide some advantage over a non selective agent.[10] In the previous publications (parts 1 and 2 of the present series),[1,2] we described how the first lead compounds were discovered from a 3D search of the RPR corporate database using a pharmacophore query derived from the known ET_A antagonists BQ123[14] and Shionogi 50-235.[15] Optimization of these lead compounds led to the identification of a new series of nonpeptidic ET_A selective endothelin antagonists, which were described in part 3[3] and are exemplified by the butanoic acid derivative **1** (Chart 1).

Chemistry

The physical properties of all compounds for which biological data were available are presented in Tables 1 and 2. The starting benzaldehydes **36**, **37**, and **38** were made by alkylation of the commercially available 3-hydroxybenzaldehyde and 2-nitro-5-hydroxybenzaldehyde, respectively (Scheme 1). Compound **39** was prepared sequentially by bromination of the 3-hydroxybenzaldehyde in dichloromethane[16] to give **35** followed by alkylation, since bromination of the 3-aryloxybenzaldehyde (e.g., **37**) in the same conditions was known to give **35** with concomitant debenzylation.[17] The cyanobenzaldehyde **40** was made by cyanation of the bromo derivative **39** with copper cyanide in DMF following a literature procedure for *m*-bromobenzaldehyde.[18] Perkin condensation of the appropriate benzaldehyde with different arylacetic acids permitted variation of substitution on the α-phenyl group of the stilbene derivatives **5**, **9**−**21**, **23**, and **26** (Scheme 1). Generally this reaction gave mainly the (*E*)-α-phenylcinnamic acid. In the case of the condensation of benzaldehyde with phenylacetic acid, some (*Z*)-α-phenylcinnamic acid **8** was formed and isolated. In the case of the *o*-substituted benzaldehydes (Br, NO_2, and CN) the reaction was rapid and only the (*E*)-α-phenylcinnamic acid was isolated.[19]

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Chart 1

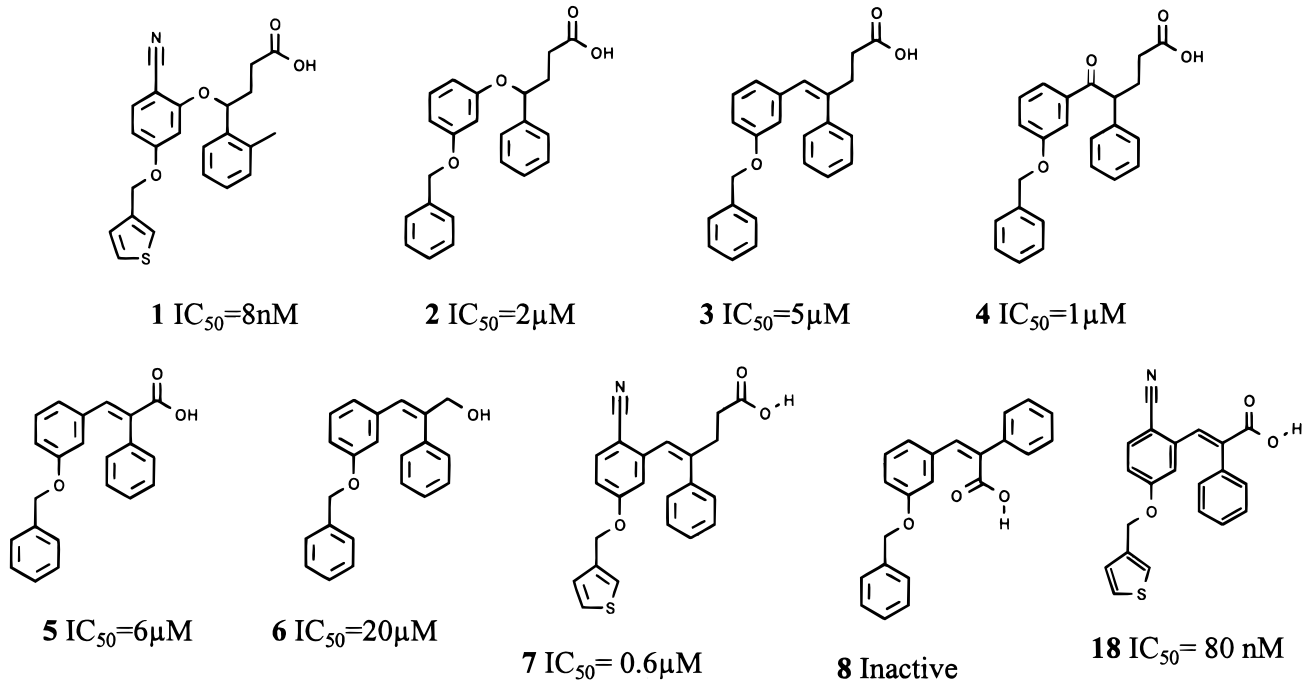
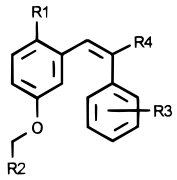


Table 1. Binding Activity of the Stilbene Acids and Related Compounds



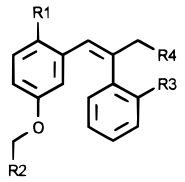
compd	R1	R2[a]	R3	R4	method	mp (°C)	formula	analysis	IC_{50} (μM)[b]
3	H	Ph	H	$CH_2CH_2CO_2H$		71−74	$C_{24}H_{22}O_3$	C, H	5.3, 4.3
5	H	Ph	H	CO_2H	A	175−178	$C_{22}H_{18}O_3$	C, H	6.0, 6.8
7	CN	3-Th	H	$CH_2CH_2CO_2H$		111−112	$C_{23}H_{19}NO_3S{\cdot}0.15H_2O$	C, H, N	0.5, 0.7
9[c]	NO_2	Ph	H	CO_2H	A	221−223	$C_{22}H_{16}NNaO_5{\cdot}3H_2O$, NaOH	C, H, N	0.6
10	NO_2	Ph	2-F	CO_2H	A	157−158	$C_{22}H_{16}FNO_5$	C, H, N	0.9
11	NO_2	3-Th	2-F	CO_2H	A	165−166	$C_{20}H_{14}FNO_5S$	C, H, N	0.6
12	NO_2	3-Th	2-Cl	CO_2H	A	176−178	$C_{20}H_{14}ClNO_5S{\cdot}1.25H_2O$	C, H, N[d]	1.3, 1.1
13	NO_2	3-Th	2-F, 6-Cl	CO_2H	A	204−205	$C_{20}H_{14}ClFNO_5S{\cdot}1.25H_2O$	C, H, N	2
14	NO_2	3-Th	H	CO_2H	A	148−150	$C_{20}H_{15}NO_5S$	C, H, N	0.5
15	NH_2	3-Th	2-F	CO_2H	A[e]	200−202	$C_{20}H_{16}FNO_5S$	C, H, N	IA[f]
16	Br	3-Th	H	CO_2H	A	144−147	$C_{20}H_{15}BrO_3S$	C, H	0.7
17	Br	3-Th	2-CH_3	CO_2H	A	125−128	$C_{21}H_{17}BrO_3S$	C, H	3
18	CN	3-Th	H	CO_2H	A	179−181	$C_{21}H_{15}NO_3S{\cdot}0.2H_2O$	C, H, N	0.08, 0.05
19	CN	3-Th	3,4-OCH_2O-	CO_2H	A	181−183	$C_{22}H_{15}NO_5S$	C, H, N	0.2, 0.2
20	CN	3-Th	2-Cl	CO_2H	A	185−188	$C_{21}H_{14}ClNO_3S$	C, H, N	0.2, 0.2
21	CN	3-Th	2-CH_3	CO_2H	A	155−157	$C_{22}H_{17}NO_3S{\cdot}0.2H_2O$	C, H, N	0.3, 0.3
22	CN	3,4-OCH_2OPh	H	CO_2H	B	176−177	$C_{24}H_{17}NO_5$	C, H, N	1.3
23	CN	3-Th	3-OCH_3	CO_2H	A	147−150	$C_{22}H_{17}NO_4S$	C, H, N	1.1
24	CN	H	H	CO_2H	A[g]	180−182	$C_{17}H_{13}NO_3$	C, H, N	IA[f]
25	CN	3-Py	H	CO_2H	B	226−229	$C_{22}H_{16}N_2O_3{\cdot}0.45\ CH_2Cl_2$	C, H, N	0.15−0.11
26	CN	3-Th	4-OCH_3	CO_2H	A	188−190	$C_{22}H_{17}NO_4S$	C, H, N	IA[f]
27	CN	3-Th	H	$CONH_2$		169−172	$C_{21}H_{16}N_2O_2S$	C, H, N	3.1−2.9

[a] 3-Th = thiophen-3-yl. [b] Inhibition of Et-1 binding to ET_A receptor on rat A10 cells (see Experimental Section). [c] Compound isolated as the sodium salt. [d] H: calcd 3.79 found 3.28. [e] Obtained by reduction of compound **11** (see Experimental Section). [f] IA = inactive (i.e., inhibition <20% at 30 μM). [g] Prepared from phenylacetic acid and compound **44** (Scheme 2).

The preparation of the stilbene alcohols **6**, **28**−**33** was achieved in one step by reduction of the corresponding acid. One to two equivalents of diborane was found to reduce selectively the acid group in the presence of a nitro, a cyano, a double bond, and a benzyloxy group, but a larger excess of reagent resulted in reduction of the double bond. Diborane in THF is a very efficient reagent when fresh, but borane−methyl sulfide complex (BMS) was found to be a more reliable reagent.[20] The amine **15** was prepared by reducing the nitro **11** with iron in ethanol following a literature procedure.[21] Compound **34** was prepared by alkylation of **33** with methyl iodide. Compound **27** was made from **18** in two steps, using successively thionyl chloride and ammonia in DMF (Scheme 1).

Variation of the arylmethyloxy group of the β-aryl was

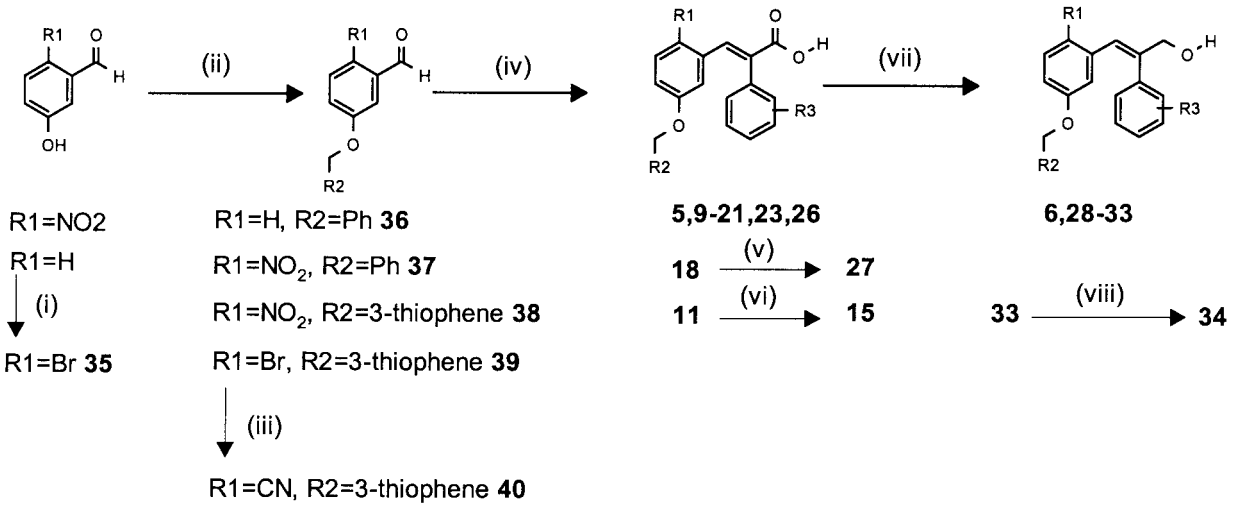
Table 2. Binding Activity of the Stilbene Alcohols and Related Compound



compd	R1	R2[a]	R3	R4	method	mp (°C)	formula	analysis	IC_{50} (μM)[b]
6	H	Ph	H	OH	C	73−74	$C_{22}H_{20}O_2 \cdot 0.2H_2O$	C, H	20
28	NO_2	3-Th	Cl	OH	C	85−87	$C_{20}H_{16}ClNO_4S$	C, H, N	0.3, 0.4
29	NO2	3-Th	H	OH	C	92−94	$C_{20}H_{17}NO_4S \cdot 0.5H_2O$	C, H, N	1.4
30	Br	3-Th	H	OH	C	57−59	$C_{20}H_{17}BrO_2S$	C, H	2.1, 2.0
31	Br	3-Th	CH_3	OH	C	oil	$C_{20}H_{17}NO_4S \cdot 0.5H_2O$	C, H	0.6
32	CN	3-Th	H	OH	C	104−107	$C_{21}H_{17}NO_2S$	C, H, N	0.8, 0.6
33	CN	3-Th	CH_3	OH	C	99−102	$C_{22}H_{19}NO_2S$	C, H, N	0.3, 0.3
34	CN	3-Th	CH_3	OCH_3	C[c]	84−86	$C_{23}H_{21}NO_2S$	C, H, N	IA[d]

[a] 3-Th = thiophen-3-yl. [b] Inhibition of Et-1 binding to ET_A receptor on rat A10 cells. [c] Obtained by methylation of compound **33** (see Experimental Section). [d] IA = inactive (i.e. inhibition <20% at 30 μM).

Scheme 1[a]



[a] Reagents: (i) bromine, CH_2Cl_2, room temperature; (ii) $ArCH_2Br$, NaH, DMF, room temperature; (iii) CuCN, DMF, reflux; (iv) $ArCH_2COOH$, AcOH, Ac_2O, Et_3N, reflux; (v) $SOCl_2$, CH_2Cl_2, room temperature, then ammonia, DMF, room temperature; (vi) iron powder, NH_4Cl, EtOH, reflux; (vii) BMS, THF, room temperature; (viii) NaH, DMF, MeI, room temperature

achieved by an alternative method developed in Scheme 2, in which the cyano derivative was isolated as the stable acetal **43**, hydrolyzed, and condensed with phenylacetic acid to give **24**. Demethylation gave compound **45** which was in turn alkylated to provide **22** and **25**.

The two chain extended acids **3** and **7** were both prepared following similar methodologies (Scheme 3) starting from the two alcohols **6** and **32**, respectively. The starting alcohols were transformed into the bromide with CBr_4 and PPh_3 in dichloromethane, following a literature procedure,[22] and reacted with the sodium salt of dimethyl malonate. The resulting diester was then hydrolyzed to the bis acid which was in turn decarboxylated by heating (170−190 °C) to give **3** and **7**, respectively.

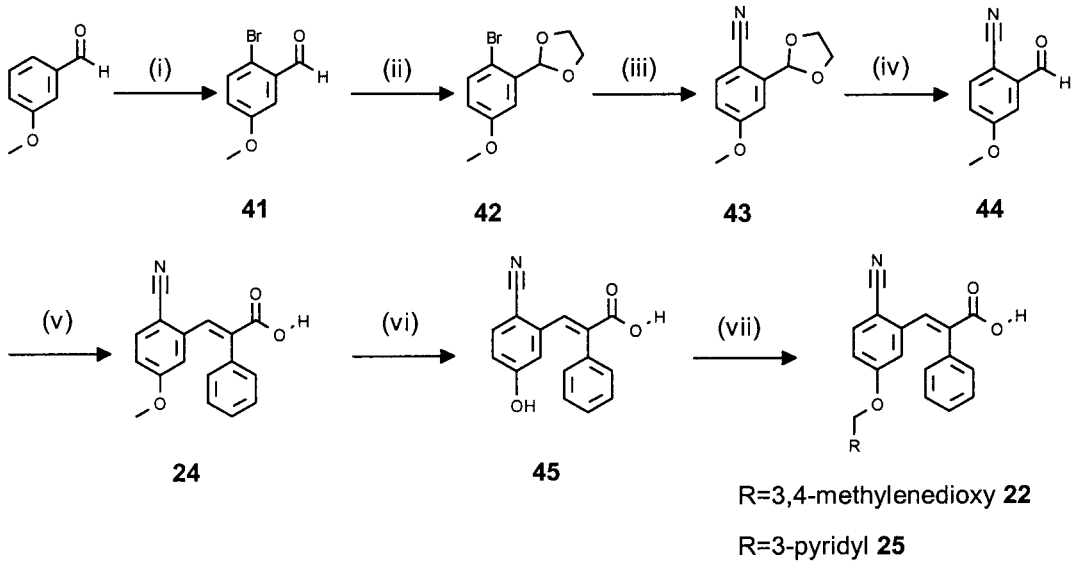
Results and Discussion

This work was instigated as part of the exploration of the SAR of the ether series of compounds reported previously (e.g., **1**).[3] The objective was to investigate replacement of the ether with the less labile and more rigid olefinic linker. The first such compound synthesized (compound **3**) was the direct analogue of the simpler ether derivative **2** and was found to show a 3-fold loss in activity compared to the activity of **2.** Introduction of the cyano and the thiophene groups, found highly beneficial features in compound **1**, into compound **3** gave compound **7** (Table 1). The resultant improvement in activity was only a factor of 10, as compared to 250 for the ether series (comparing compounds **1** and **2**). It should be noted that compound **1** also possessed an *o*-methyl group, which in our experience conferred a 2.5-fold increase in activity.[3] However, even after making allowance for this it seemed that the more potent extended stilbene acids were intrinsically less active than the ether series by more than a log unit. The most likely reason for this was the lack of a hydrogen bond acceptor which was not only present in **2** (ether linker)[3] but also in the carbonyl linker series as in **4**.[2]

Interestingly, during the synthesis of compound **3** we identified two simple intermediates with unexpectedly good activities: compounds **5** and **6**.

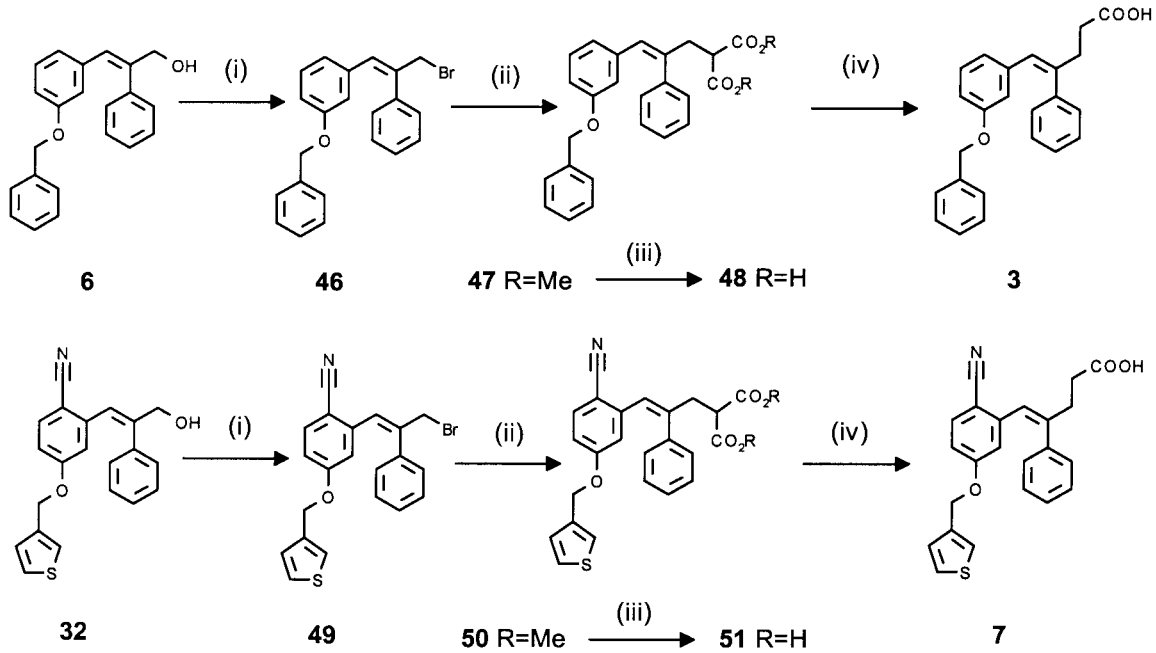
The structure−activity relationships for **5** were first explored. The activity of this *E*-isomer **5** (IC_{50} = 6 μM) was compared with the *Z*-isomer **8** (inactive, i.e., inhibi-

Scheme 2[a]



[a] Reagents: (i) bromine, CH_2Cl_2, room temperature; (ii) ethylene glycol, toluene, PTSA, reflux; (iii) CuCN, DMF, reflux; (iv) PTSA, acetone, water, reflux; (v) phenylacetic acid, Et_2N, acetic anhydride, reflux; (vi) pyridine hydrochloride, 160 °C; (vii) $ArCH_2Cl$, K_2CO_3, acetone, reflux.

Scheme 3[a]



[a] Reagents: (i) CBr_4, PPh_3, CH_2Cl_2, 10 °C; (ii) dimethyl malonate, NaOMe, MeOH, reflux; (iii) NaOH, H_2O, EtOH, reflux; (iv) 170−190 °C, neat.

tion <20% at 30 μM) and found to be in full agreement with the preferred "cisoid" bioactive conformation defined by a receptor model developed and described previously for the earlier nonstilbene members of this series[3] (e.g., see Figure 1). This model, which in addition to defining the bioactive conformation also identifies the position of a critical cationic receptor group, has proved extremely valuable in the design of potent analogues such as **1**.[3] It was used in this work to compare these stilbene analogues with compounds derived from earlier series.

Interestingly, the screening of compound **18**, which was a synthetic intermediate for **7**, gave a still more active compound with an IC_{50} of 80 nM (Table 1). It can be seen (Figure 1) that **18** fits the receptor site model very well with the acid−cationic group interaction at a slightly shallower angle than for the more potent derivative **1**. A rapid study of this readily accessible stilbene acid series showed that an electron-withdrawing group (which can also act as a H-bond acceptor[23]) at the ortho position of the β-phenyl group was beneficial for activity (Table 1). Indeed reduction of the nitro group of **11** to the amine **15** abolished the activity completely. The cyano derivatives showed better activity than the nitro derivatives (e.g., **18** and **14**). This may be due to steric repulsion in the bioactive conformation between the olefinic proton and the nearby oxygen of the nitro group. Such an interaction could

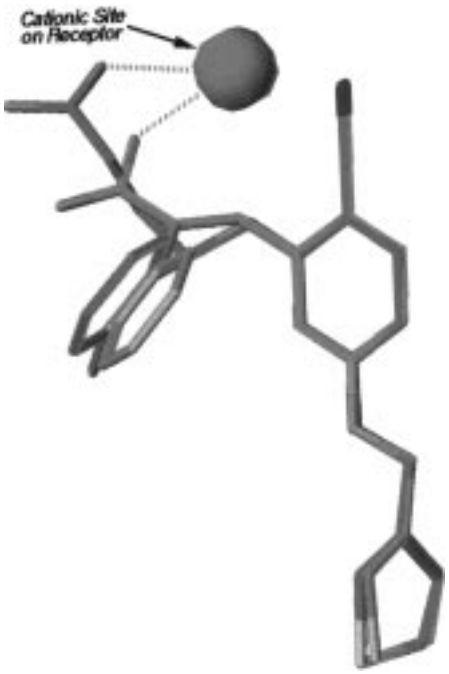


Figure 1. Superposition of compounds **1** (in green) and **18** (in cyan) showing the acid groups pointing toward the putative cationic center (in magenta).

cause a rotation about the bond linking the nitroaryl to the olefinic group, thus disfavoring the bioactive conformation.[24] The presence of an arylmethyloxy group on the β-phenyl ring was also important for activity. Absence of the ring resulted in complete loss of activity (e.g., the methoxy compound **24**). Isosteric replacement with a thiophene ring was acceptable, but replacement with an electron rich aromatic group **22** or an electron deficient aromatic group **25** caused a reduction in activity. Substitution of the α-phenyl with an ortho chloro or methyl group led to a 3-fold loss in activity (compare **18** with **20** and **21**). A methoxy group in the meta position **23** was tolerated, whereas the para methoxy **26** was inactive.

The compound **6** ($IC_{50} = 20\ \mu M$) was the first nonacidic compound with ET_A binding activity we had observed, with an activity only 3-fold lower than the acid **5**. Clearly an explanation for this is that while the acid group interacts with the cationic center through a strong ionic interaction, the alcohol interaction is through the acceptance of a weaker H-bonding interaction. However methylation of the alcohol abolished the activity (compound **34**, Table 2) despite the ether, according to literature values,[23] being of similar strength to the alcohol as a H-bond acceptor. The reason for this was unclear, but it may be simply that in the conformation required for receptor interaction the methyl group infringes the steric constraints of the receptor site.

It is interesting to notice that the SAR seen for the acid series correlated with the SAR obtained with the alcohol series with the exception of the effect of the ortho substituent on the α-phenyl ring. For the acid series the ortho methyl group decreased activity by a factor of about 2.5 (compare **18** and **21**) while in the alcohol series the effect is positive by a similar factor (compare **32** and **33**), and in this the SAR of the alcohol series was very similar to that of the earlier published series related to **1**.[3]

It is noteworthy that the amide compound **27** also retained some activity ($IC_{50} = 3\ \mu M$) but much less than the alcohol **32**.

Conclusion

While developing a rigid analogue to the lead compound **1** we identified a new series of small and easily accessible endothelin antagonists. The most potent compound in this series was the of (*E*)-2-phenyl-3-(2-cyano-5-(thien-3-ylmethoxy))phenylpropenoic acid **18** (RPR111723) with an IC_{50} of 80 nM which also showed good functional activity in the rat aorta with a pK_B of 6.5 (see the Experimental Section). It is noteworthy that RPR111723 is comparable in potency to the ET_A selective antagonist BMS-182874 ($IC_{50} = 150$ nM, $pK_B = 6.3$).[24] Compound **18** was selective for ET_A receptor and failed to inhibit binding of ET-1 to ET_B receptors at concentrations up to 30 μM. Although this stilbene series was not developed further, the observation that the simple stilbene **18** was 8-fold more active than the chain extended analogue **7** suggested that a similar relationship should exist for the more potent ether series (e.g., **1**) and preparation of such short chain compounds will be the subject of a future publication.

Experimental Section

Chemical Methods. Melting points were taken on an Electrothermal melting point apparatus and are uncorrected. Infrared spectra were recorded in potassium bromide on a Nicolet 205XB FI spectrometer. Proton NMR spectra were recorded using a Varian VXR 400 (or a Varian XL 200 when specified) spectrometer; peak positions are reported in parts per million relative to internal tetramethylsilane on the δ scale. Mass spectra were recorded on a VG 7070E/250 spectrometer. Microanalyses were performed on a Carlo-Erba 1106 microanalyzer. Where analyses are indicated by the symbols of the elements, results obtained were within 0.4% of the theoretical values. All the reactions were performed at room temperature unless otherwise stated. All organic solutions were dried with magnesium sulfate. Yields are not optimized.

(*Z*)-4-Phenyl-5-(3-benzyloxy)phenylpenten-4-oic Acid 3. Compound **48** (3.5 g, 87 mmol) was heated neat to 170 °C for 20 min. The melt was then treated with charcoal in hot cylohexane and filtered. Upon cooling a white solid precipitated which was filtered to give **3** (2.7 g, 87%), having mp 71−74 °C. ^{1}H NMR ($CDCl_3$) 2.46 (2H, t, $J = 8$ Hz, $CH_2CH_2CO_2H$), 2.84 (2H, t, $J = 8$ Hz, $CH_2CH_2CO_2H$), 4.68 (2H, s, CH_2), 6.48 (1H, s, olefin-H), 6.51 (1H, d, $J = 2$ Hz, ArH), 6.56 (1H, d, $J = 8$ Hz, ArH), 6.69 (1H, dt, $J = 2$ Hz, $J = 8$ Hz, ArH), 7.02 (1H, t, $J = 8$ Hz, ArH), 7.14−7.38 (10H, m, ArH); MS (FAB) *m*/*z* 359 $[MH]^+$. Anal. ($C_{24}H_{22}O_3$) C, H.

Method A. General Method for the Preparation of Compounds 5, 9−14, 16−21, 23, 24, and 26. This procedure is illustrated for the preparation of (*E*)-2-phenyl-3-(3-benzyloxy)phenylpropenoic acid **5**. 3-Benzyloxybenzaldehyde (49 g, 230 mmol), phenylacetic acid (45.4 g, 340 mmol), acetic anhydride (115 mL), and triethylamine (32 mL, 230 mmol) were refluxed for 20 min. The solution was cooled to 90 °C, water (115 mL) was added to decompose the excess anhydride, and a solid precipitated which was filtered. Trituration of the solid with diethyl ether gave a white solid which was filtered and dried to give **5** (64 g, 85%), having mp 175−178 °C. ^{1}H NMR ($CDCl_3$) 4.63 (2H, s, CH_2), 6.52−7.41 (14H, m, ArH), 7.82 (1H, s, olefin-H); MS (FAB) *m*/*z* 331 $[MH]^+$. Anal. ($C_{22}H_{18}O_3$) C, H.

(*Z*)-4-Phenyl-5-(2-cyano-5-(thiophen-3-ylmethoxy)phenyl)penten-4-oic Acid 7. Compound **51** (0.85 g, 2 mmol) was heated neat to 190 °C for 10 min. The melt was treated with K_2CO_3 in water (50 mL) and washed with diethyl ether (15

mL). The aqueous phase was acidified to pH 1 with concentrated aqueous HCl (1 mL), and the mixture was extracted twice with ethyl acetate (30 mL). The gathered organic extracts were washed with water, dried, and distilled under reduced pressure. The resulting gum was triturated with hot cyclohexane. Upon cooling a white solid precipitated which was filtered to give **7** (0.47 g, 61%), having mp 111−112 °C. ^{1}H NMR ($CDCl_3$) 2.51 (2H, t, J = 8 Hz, CH_2CH$_2$CO$_2$H), 2.92 (2H, t, J = 8 Hz, CH$_2$CH_2CO$_2$H), 4.53 (2H, s, CH$_2$), 6.38 (1H, d, J = 4 Hz, ArH), 6.62 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 6.75 (1H, s, olefin-H), 6.95 (1H, dd, J = 2 Hz, J = 4 Hz, ArH), 7.10−7.17 (3H, m, ArH), 7.24−7.36 (4H, m, ArH), 7.45 (1H, d, J = 8 Hz, ArH); MS (FAB) m/z 390 [MH]$^+$. Anal. ($C_{23}H_{19}NO_3S\cdot0.15H_2O$) C, H, N.

(*Z*)-2-Phenyl3-(3-benzyloxy)phenylpropenoic Acid 8. The filtrates of the preparation of compound **5** were concentrated and columned on silica gel (eluant CH_2CL_2/MeOH, 95/5) to give compound **8** (1.5 g, 2%) as a gum. ^{1}H NMR ($CDCl_3$) 5.06 (2H, s, CH$_2$), 6.99−7.51 (15H, m, ArH, olefin-H); MS (FAB) m/z 331 [MH]$^+$. Anal. ($C_{22}H_{18}O_3$) C, H.

(*E*)-2-(2-Fluorophenyl)-3-(2-amino-5-(3-thiophen-3-yl)-methyloxy)propenoic Acid 15. Compound **11** (2 g, 5 mmol), iron powder (2 g, slight excess), and ammonium chloride (1.5 g, 28 mmol), in aqueous methanol (50 mL, 50/50), were refluxed for 90 min. The mixture was filtered through Celite and the filter washed with hot MeOH. The gathered filtrate was basified and the compound extracted with ethyl acetate to give **15** (0.7 g, 37%) as an off-white solid, having mp 200−202 °C. ^{1}H NMR (DMSO-d_6) 4.40 (2H, s, CH$_2$), 5.10 (2H, bs, NH$_2$), 6.08 (1H, d, J = 4 Hz, ArH), 6.60−6.68 (2H, m, ArH), 6.92 (1H, dd, J = 2 Hz, J = 4 Hz, thiophen-H), 7.10−7.51 (6H, m, ArH) 7.85 (1H, s, olefin-H). Anal. ($C_{20}H_{16}FNO_3S$) C, H, N.

Method B. General Method for the Preparation of Compounds 22 and 25. This procedure is illustrated for the preparation of (*E*)-2-phenyl-3-(2-cyano-5-(pyridin-3-ylmethoxy)phenyl)propenoic acid **25.** Compound **45** (0.44 g, 1.7 mmol), 3-picolyl chloride hydrochloride (0.44 g, 1.7 mmol), potassium carbonate (1.5 g, 10.9 mmol), potassium iodide (0.01 g, cat.) and tetrabutylammonium bromide (2 mg, cat.), in methyl ethyl ketone (25 mL) was refluxed for 20 h. The mixture was cooled to room temperature, and the precipitated inorganics were filtered. The filtrate was evaporated under reduced pressure and the residue taken up into ethanol (25 mL) and treated with 1 N sodium hydroxide solution (2 mL) at reflux for 2 h. The solvent was distilled under reduced pressure and the residue dissolved into water (75 mL), washed with diethyl ether, and acidified with 1 N HCl to pH 5. A solid was obtained which was filtered and recrystallized from 2-ethoxyethanol to give **25** as a white solid (0.21 g, 35%) having mp 230−232 °C. ^{1}H NMR (DMSO-d_6) 4.72 (2H, s, CH$_2$) 6.51 (1H, d, J = 4 Hz, ArH), 7.06 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.13−7.20 (2H, m, ArH), 7.32−7.44 (4H, m, ArH), 7.63−7.70 (1H, m, ArH), 7.85 (1H, d, J = 8 Hz, ArH), 7.79 (1H, s, olefin-H) 8.47−8.58 (2H, m, ArH); MS (FAB) m/z 357 [MH]$^+$. Anal. ($C_{22}H_{16}N_2O_3$, 0.45 mol CH_2Cl_2) C, H, N.

(*E*)-2-Phenyl-3-(2-cyano-5-(3-thiophen-3-yl)methyloxy)phenylpropenamide 27. Compound **18** (1 g, 2.8 mmol) in dichloromethane (20 mL) was treated with thionyl chloride (0.4 mL) at reflux for 1 h. The solvent was distilled under reduced pressure and the residue taken up in DMF (10 mL) and treated with concentrated aqueous NH_4OH (1 mL) for 1 h. The mixture was concentrated in vacuo and the residue taken up in ethyl acetate and washed with water. Distillation of the solvent gave an off white solid which was recrystallized from 2-propanol to give **27** (0.26 g, 25%) as a white solid having mp 169−172 °C. ^{1}H NMR (DMSO-d_6) 4.75 (2H, s, CH$_2$), 6.44 (1H, d, J = 4 Hz, ArH), 6.92−7.03 (2H, m, ArH), 7.10−7.18 (3H, m, ArH) 7.36−7.43 (3H, m, ArH), 7.51−7.57 (2H, m, ArH, olefinic-H), 7.53 (1H, d, J = 8 Hz, ArH); MS (FAB) m/z 361 [MH]$^+$. Anal. ($C_{21}H_{16}NO_2S$) C, H, N.

Method C. General Method for the Preparation of Compounds 6 and 28−33. This procedure is illustrated for the preparation of (*Z*)-2-phenyl-3-(2-cyano-5-(3-thiophen-3-yl)-methyloxyphenyl)propenol **32**. To a solution of **18** (2.55 g, 7 mmol) in THF (30 mL), under N_2, at 0 °C, was added borane in THF (11 mL, 11 mmol, 1.0 M solution in THF or 5.5 mL, 11 mmol, 2 M solution of BMS in THF), and the solution was allowed to stir at room temperature for 2 h. The reaction mixture was then cooled again to 0 °C and quenched sequentially with water (25 mL) and sodium hydroxide (30 mL, 1 N). The reaction mixture was stirred at room temperature for 30 min and concentrated in vacuo. The residue was dissolved in ethyl acetate (50 mL), washed twice with water (50 mL), dried, and evaporated to dryness. The gummy residue was triturated with pentane to afford **32** (2 g, 83%) as a fawn-colored solid having mp 104−107 °C. ^{1}H NMR ($CDCl_3$) 1.91 (1H, t, J = 6 Hz, OH), 4.53 (2H, d, J = 6 Hz, CH_2OH), 4.56 (2H, s, CH$_2$), 6.46 (1H, d, J = 4 Hz, ArH), 6.75 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 6.94−6.98 (2H, m, ArH), 7.10−7.47 (7H, m, ArH, olefin-H), 7.48 (1H, d, J = 8 Hz, ArH); MS (FAB) m/z 348 [MH]$^+$. Anal. ($C_{21}H_{17}NO_2S$) C, H, N.

(*Z*)-2-(2-Methylphenyl)-3-(2-cyano-5-(thiophen-3-yl)-methyloxyphenyl)propenol Methyl Ether 34. To a solution of **33** (1 g, 2.8 mmol), in DMF (20 mL), was added at room temperature, under nitrogen, sodium hydride (0.12 g, 3 mmol, 60% dispersion in oil), and the mixture was stirred for 30 min. Iodomethane (0.22 mL, 3.46 mmol) was added at once, and the reaction mixture was stirred at room temperature for 3 h. The solution was then poured into water (200 mL) and extracted twice with ethyl acetate (50 mL). The gathered organic phases were washed with water (50 mL) and brine (25 mL), dried, and distilled under reduced pressure. The residue was taken up into toluene and purified by flash column chromatography (eluant, toluene) to give, after trituration with pentane, **34** (0.22 g, 21%) as a white solid having mp 84−86 °C. ^{1}H NMR ($CDCl_3$) 2.27 (3H, s, CH$_3$), 3.72 (3H, s, OCH$_3$), 3.93 (2H, s, CH$_2$), 5.00 (2H, s, CH_2OCH$_3$), 6.07 (1H, s, olefin-H), 6.75 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 6.84 (1H, d, J = 4 Hz, ArH), 6.87 (1H, d, J = 8 Hz, ArH), 6.98−7.15 (3H, m, ArH), 7.25−7.36 (2H, m, ArH), 7.41 (1H, d, J = 8 Hz, ArH); MS (EI) m/z 375 [M]$^+$. Anal. ($C_{23}H_{21}NO_2S\cdot0.5H_2O$) C, H, N.

2-Bromo-5-hydroxybenzaldehyde 35. A solution of bromine (5 mL, 0.1 mol.) in dichloromethane (50 mL) was added dropwise at room temperature to a solution of 3-hydroxybenzaldehyde in dichloromethane (400 mL). The red solution was stirred for 3 h and the solvent distilled under reduced pressure. The solid was dissolved into water (250 mL) with potassium carbonate (2 equiv). The greenish suspension was filtered and the solution cautiously acidified to pH 1 with concentrated aqueous HCl. A solid separated which was filtered and recrystallized from acetic acid to give **35** as a white solid (9 g, 45%) having mp 131−132 °C (lit.[16] mp = 133 °C). ^{1}H NMR ($CDCl_3$) 6.99 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.38 (1H, d, J = 4 Hz, ArH), 7.44 (1H, d, J = 8 Hz, ArH), 9.51 (1H, s, OH), 10.26 (1H, s, ArCHO).

3-Benzyloxybenzaldehyde 36. To a solution of 3-hydroxybenzaldehyde (10 g, 82 mmol) in DMF (250 mL) was added potassium *tert*-butoxide (9.2 g, 82 mmol) at room temperature under N_2. The mixture was stirred 15 min and the benzyl bromide (14 g, 82 mmol) added at once. The mixture was stirred for 3 h at room temperature and the solvent distilled under reduced pressure. The residue was partitioned between water and ethyl acetate, the phases were separated, and the organics were concentrated. The residue was purified by distillation (bp 180 °C, 0.1 mbar) to give **36** (14.8 g, 80%) as a colorless oil which solidified on standing mp 52−54 °C (lit.[26] mp 54 °C). ^{1}H NMR ($CDCl_3$) 5.13 (2H, s, CH$_2$) 7.23−7.51 (9H, m, ArH) 9.98 (1H, s, CHO).

5-Benzyloxy-2-nitrobenzaldehyde 37. Following the same procedure as above, but replacing the 3-hydroxybenzaldehyde with 5-hydroxy-2-nitrobenzaldehyde, compound **37** was obtained in 69% yield as a yellow oil, after purification by filtration through silica (eluant, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) 5.21 (2H, s, CH$_2$), 7.21 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.32−7.45 (6H, m, ArH), 8.16 (1H, d, J = 8 Hz, ArH), 10.52 (1H, s, CHO).

5-(3-Thiophen-3-yl)-methyloxy-2-nitrobenzaldehyde 38. To a solution of 5-hydroxy-2-nitrobenzaldehyde (6 g, 36 mmol) in DMF (150 mL) was added potassium *tert*-butoxide (4.3 g, 38 mmol) at room temperature under N_2. The mixture was stirred 15 min and the 3-(chloromethyl)-thiophene[3] (5 g, 38 mmol) added dropwise over 3 min. The mixture was stirred for 6 h at room temperature and the solvent distilled under reduced pressure. The residue was partitioned between water and ethyl acetate, the phases were separated, and the organics were concentrated. The residue was purified by filtration through silica (eluant CH_2Cl_2) to give **38** (3.6 g, 38%) as a yellow oil. ^{1}H NMR ($CDCl_3$) 5.22 (2H, s, CH_2), 7.14 (1H, dd, J = 2 Hz, J = 4 Hz, thiophen-H), 7.21 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.36−7.43 (3H, m, aromatic-H), 8.16 (1H, d, J = 8 Hz, ArH), 10.48 (1H, s, CHO).

2-Bromo-5-(3-thiophen-3-yl-methyloxy)benzaldehyde 39. To a solution of 2-bromo-5-hydroxy-benzaldehyde **35** (12.5 g, 62 mmol) in DMF (150 mL) was added potassium *tert*-butoxide (7.3 g, 65 mmol) at room temperature under N_2. The mixture was stirred 15 min and the 3-(chloromethyl)-thiophene[3] (8.7 g, 65 mmol) added dropwise over 3 min. The mixture was stirred for 6 h at room temperature and left to stand overnight. The solvent was distilled under reduced pressure, the residue was partitioned between water and ethyl acetate, the phases were separated, and the organics were concentrated to give **39** (18 g, 100%) as a crude oil. ^{1}H NMR ($CDCl_3$) 5.10 (2H, s, CH_2), 7.09 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.14 (1H, dd, J = 2 Hz, J = 4 Hz, thiophen-H), 7.36−7.43 (2H, m, thiophen-H), 7.49 (1H, d, J = 4 Hz, ArH), 7.53 (1H, d, J = 8 Hz, ArH), 10.30 (1H, s, CHO).

2-Cyano-5-(3-thiophen-3-yl)methyloxybenzaldehyde 40. A stirred solution of compound **39** (5 g, 17 mmol) in DMF (100 mL) under N_2 was treated with copper(I) cyanide (3 g, 34 mmol). The resulting mixture was refluxed for 90 min. The cooled reaction mixture was added to water (500 mL) and extracted twice with ethyl acetate (250 mL). The combined organic extracts were washed twice with water (100 mL), dried, filtered, and evaporated to dryness. The residual oil was taken up into dichloromethane and purified by filtration through silica. Distillation of the eluant gave compound **40** (2 g, 48%) as an amber oil which solidified on standing to give a waxy solid. ^{1}H NMR ($CDCl_3$) 5.21 (2H, s, CH_2), 7.14 (1H, dd, J = 2 Hz, J = 4 Hz, thiophen-H), 7.28 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.36−7.41 (2H, m, thiophen-H), 7.58 (1H, d, J = 4 Hz, ArH), 7.75 (1H, d, J = 8 Hz, ArH), 10.33 (1H, s, CHO).

2-Bromo-5-methoxybenzaldehyde 41. To a solution of 3-methoxybenzaldehyde (334 g, 2.45 mol) in dichloromethane (3 L), maintained at 0 °C, was added a solution of bromine (393 g, 2.45mol) in dichloromethane (500 mL) dropwise over 2.5 h. After addition, the reaction mixture was allowed to reach room temperature overnight. The solvent was then distilled under reduced pressure and the residue recrystallized from cyclohexane to give **41** as a brown solid (405 g, 76%) having mp 73−76 °C (lit.[27] mp 75−76 °C). ^{1}H NMR ($CDCl_3$) 3.85 (3H, s, CH_3), 7.04 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.42 (1H, d, J = 4 Hz, ArH), 7.53 (1H, d, J = 8 Hz, ArH), 10.31 (1H, s, CHO).

2-(2-Bromo-5-methoxy-phenyl)dioxolane 42. A mixture of **41** (305 g, 1.4 mol), ethylene glycol (260 g, 4.2 mol), and *p*-toluenesulfonic acid (5 g) in toluene (2.4 L) was refluxed with a Dean−Stark for 3 h. The solvent was then distilled under reduced pressure and the residue partitioned between ethyl acetate and water. The aqueous layer was washed twice with ethyl acetate, and the gathered organic phase was washed in turn with water, dried over magnesium sulfate, and distilled under reduced pressure to give **42** (375 g, 100% crude) as a brown oil. ^{1}H NMR ($CDCl_3$) 3.81 (3H, s, CH_3), 4.05−4.18 (4H, m, OCH_2CH_2O), 6.03 (1H, s, CH), 6.79 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.15 (1H, d, J = 4 Hz, ArH), 7.43 (1H, d, J = 8 Hz, ArH).

2-(2-Cyano-5-methoxy-phenyl)dioxolane 43. A mixture of **42** (53 g, 0.2 mol) and cuprous cyanide (17.8 g, 0.2 mol) in dimethylformamide (50 mL) was refluxed for 1 h. The cooled mixture was then poured into aqueous ammonia (made from concentrated aqueous ammonia, 200 mL, and water, 200 mL) and extracted twice with ethyl acetate (200 mL). The organic phase was in turn washed with ammonia until the blue coloration faded. The organics were washed with water, dried, and distilled under reduced pressure to give **43** as a brown oil (40 g, 100% crude). ^{1}H NMR ($CDCl_3$) 3.88 (3H, s, CH_3), 4.06−4.24 (4H, m, OCH_2CH_2O), 5.86 (1H, s, CH), 6.94 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.12 (1H, d, J = 4 Hz, ArH), 7.62 (1H, d, J = 8 Hz, ArH).

2-Cyano-5-methoxybenzaldehyde 44. A solution of **43** (10 g, 43 mmol) in aqueous acetic acid (50%, 80 mL) was heated to 100 °C for 2 h. Water (100 mL) was then added and the solution left to cool to 10 °C in ice. The solid formed was isolated by filtration and purified by recrystallization from toluene to give **44** (3.9 g, 55%) as a white solid having mp 105−109 °C. ^{1}H NMR ($CDCl_3$) 3.94 (3H, s, CH_3), 7.22 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.51 (1H, d, J = 4 Hz, ArH), 7.74 (1H, d, J = 8 Hz, ArH), 10.33 (1H, s, CHO).

(*E*)-2-Phenyl-3-(2-cyano-5-hydroxy)phenylpropenoic Acid 45. A mixture of **24** (1.4 g, 5 mmol) and pyridine hydrochloride (14 g, 10 equiv w/w) was heated at 160 °C for 17 h. The cooled reaction mixture was partitioned between ethyl acetate (40 mL) and 1 N HCl (40 mL). The aqueous layer was washed with ethyl acetate, and the gathered organic extracts were washed with water and dried. Distillation of the solvent gave a residue which was purified by column chromatography (eluant CH_2Cl_2/MeOH, 19/1) to give **45** as a waxy solid. ^{1}H NMR (DMSO-d_6) 6.26 (1H, d, J = 4 Hz, ArH), 6.77 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.10−7.16 (2H, m, ArH), 7.31−7.36 (3H, m, ArH), 7.13 (1H, d, J = 8 Hz, ArH), 7.84 (1H, s, olefin-H); MS (FAB) *m*/*z* 366 $[MH]^+$. Anal. ($C_{16}H_{11}NO_3$) C, H, N.

(*E*)-4-Phenyl-5-(3-benzyloxy)phenyl-1-bromoprop-2-ene 46. To a solution of **6** (11.75 g, 35 mmol) in dichloromethane (275 mL), cooled to 10 °C, was added carbon tetrabromide (15.4 g, 46 mmol) and triphenylphosphine (14.5 g, 55 mmol). The solution was stirred at that temperature for 1 h and filtered through a pad of silica with dichloromethane. Distillation of the filtrate gave **46** (13 g, 100%) as a crude oil. ^{1}H NMR ($CDCl_3$) 4.38 (2H, s, CH_2Br), 4.59 (2H, s, CH_2), 6.55 (1H, t, J = 2 Hz, ArH), 6.53 (1H, d, J = 8 Hz, ArH), 6.75 (1H, dt, J = 2 Hz, J = 8 Hz, ArH), 6.78 (1H, s, olefin-H), 7.05 (1H, t, J = 8 Hz, ArH), 7.26−7.72 (10H, m, ArH).

(*Z*)-Methyl 4-Phenyl-5-(3-benzyloxy)phenyl-2-methoxycarbonylpenten-4-oate 47. To a solution of sodium methoxide (2 g, 37 mmol) in anhydrous methanol (200 mL) was added dimethyl malonate (5.1 g, 38.5 mmol) at room temperature. After 15 min, compound **46** (13 g, 34 mmol) was added in solution into methanol (75 mL). The reaction mixture was refluxed for 1 h and the solvent distilled under reduced pressure. The residue was then partitioned between ethyl acetate (150 mL) and 1 N HCl (50 mL). The aqueous layer was washed with ethyl acetate (50 mL), and the gathered organic layers were washed in turn with water, dried, and distilled under reduced pressure. Purification of the residue with flash column chromatography (eluant pentane/ethyl acetate, 3/1) gave **47** (10.8 g, 73%) as an amber oil. ^{1}H NMR ($CDCl_3$) 3.10 (2H, d, J = 8 Hz, CH_2CH), 3.46 (1H, t, J = 8 Hz, CH_2CH), 3.70 (6H, s, CH_3), 4.68 (2H, s, CH_2), 6.48 (2H, m, ArH, olefin-H), 6.53 (1H, d, J = 8 Hz, ArH), 6.75 (1H, dt, J = 2 Hz, J = 8 Hz, ArH), 7.02 (1H, t, J = 8 Hz, ArH), 7.13−7.48 (10H, m, ArH).

(*Z*)-4-Phenyl-5-(3-benzyloxy)phenyl-2-carboxypenten-4-oic Acid 48. A mixture of **47** (7 g, 16.3 mmol), sodium hydroxide (2.25 g, 56 mmol), methanol (150 mL), and water (100 mL) was heated at reflux for 3 h. The solvent was distilled under reduced pressure and the residue dissolved into water (100 mL) and acidified to pH 1 with concentrated aqueous HCl. The precipitate was extracted twice with ethyl acetate (100 mL), and the gathered organic extracts were washed with water, dried, and distilled under reduced pressure. The residue was triturated with cyclohexane to give **48**

(3.5 g, 53%) as a white solid having mp 112−116 °C dec. ^{1}H NMR ($CDCl_3$) 3.13 (2H, d, J = 8 Hz, CH_2CH), 3.50 (1H, t, J = 8 Hz, CH_2CH), 4.66 (2H, s, CH_2), 6.48−6.55 (3H, m, ArH, olefin-H), 6.69 (1H, dt, J = 2 Hz, J = 8 Hz, ArH), 6.98 (1H, t, J = 8 Hz, ArH), 7.12−7.37 (10H, m, ArH); MS (FAB) m/z 403 $[MH]^+$. Anal. ($C_{25}H_{22}O_5$, 0.3 C_6H_{12}) C, H.

***(E)*-4-Phenyl-5-(2-cyano-5-(thiophen-3-yl)phenyl)-1-bromoprop-2-ene 49.** To a solution of **32** (2.65 g, 7 mmol), in dichloromethane (75 mL), cooled to 10 °C, were added carbon tetrabromide (3.3 g, 9.9 mmol) and triphenylphosphine (3 g, 11.3 mmol). The reaction mixture was stirred at this temperature for 1 h and allowed to reach room temperature overnight. The solution was then filtered on a pad of silica (dichloromethane) and the solvent distilled under reduced pressure to give **49** (2.8 g, 98%) as an amber oil. ^{1}H NMR ($CDCl_3$) 4.38 (2H, s, CH_2Br), 4.53 (2H, s, CH_2), 6.48 (1H, d, J = 2 Hz, ArH), 6.53 (1H, dd, J = 2 Hz, J = 8 Hz, ArH), 6.75−7.50 (10H, m, olefin-H, ArH).

***(Z)*-Methyl 4-Phenyl-5-(2-cyano-5-(thiophen-3-ylmethoxy)phenyl)-2-methoxycarbonylpenten-4-oate 50.** To a solution of sodium methoxide (0.48 g, 8.9 mmol) in anhydrous methanol (32 mL) was added dimethyl malonate (1.3 g, 9.6 mmol) at room temperature. After 15 min, compound **49** (2.8 g, 6.8 mmol) was added in solution into methanol (20 mL). The reaction mixture was refluxed for 5 h and the solvent distilled under reduced pressure. The residue was then partitioned between ethyl acetate (50 mL) and 1 N HCl (10 mL). The aqueous layer was washed with ethyl acetate (20 mL), and the gathered organic layers were washed in turn with water, dried, and distilled under reduced pressure. Purification of the residue with flash column chromatography (eluant pentane/ethyl acetate, 3/1) gave **50** (1.5 g, 49%) as a pale yellow gum. ^{1}H NMR ($CDCl_3$) 3.19 (2H, d, J = 8 Hz, CH_2CH), 3.47 (1H, t, J = 8 Hz, CH_2CH), 3.75 (6H, s, CH_3), 4.51 (2H, s, CH_2), 6.34 (1H, d, J = 4 Hz, ArH), 6.71−6.77 (2H, m, olefin-H, ArH), 6.95 (1H, dd, J = 2 Hz, J = 4 Hz, ArH), 7.10−7.37 (7H, m, ArH), 7.45 (1H, d, J = 8 Hz, ArH).

***(Z)*-4-Phenyl-5-(2-cyano-5-(thiophen-3-ylmethoxy)phenyl)-2-carboxypenten-4-oic Acid 51.** A mixture of **50** (1.5 g, 3.3 mmol), sodium hydroxide (0.33 g, 8.3 mmol), methanol (50 mL), and water (10 mL) was heated at reflux for 5 h. The solvent was distilled under reduced pressure and the residue dissolved into water (150 mL) and acidified to pH 1 with concentrated aqueous HCl. The precipitate was extracted twice with ethyl acetate (75 mL), and the gathered organic extracts were washed with water, dried, and distilled under reduced pressure. The residue was triturated with dichloromethane and recrystallized from toluene/ethyl acetate to give **51** (0.7 g, 51%) as a white solid having mp 164−168 °C dec. ^{1}H NMR (DMSO-d_6) 3.02 (2H, d, J = 8 Hz, CH_2CH), 3.17 (1H, t, J = 8 Hz, CH_2CH), 4.21 (2H, s, CH_2), 6.35 (1H, d, J = 4 Hz, ArH), 6.68 (1H, s, olefin-H), 6.90 (1H, dd, J = 4 Hz, J = 8 Hz, ArH), 7.01 (1H, dd, J = 2 Hz, J = 4 Hz, ArH), 7.12−7.18 (2H, m, ArH), 7.29−7.43 (4H, m, ArH), 7.53 (1H, m, ArH), 7.64 (1H, d, J = 8 Hz, ArH); MS (FAB) m/z 434 $[MH]^+$. Anal. ($C_{24}H_{19}NO_5S\cdot1.25H_2O$) C, H, N.

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 foetal 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 140000 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 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 phenyl methenyl sulfonyl fluoride (0.1 mM). After homogenization using a glass/Teflon manual homogenizer, the samples were centrifuged at 4 °C for 17 min at 1000g, and the resulting supernatants were retained. This material was centrifuged at 40000g 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 γ-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, 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. Compounds are referred to IA (inactive) when the inhibition is inferior to 20% at 30 μM.

Functional Assay. Male Sprague−Dawley rats were sacrificed, the aorta removed, de-endothelialized and placed in organ baths containing oxygenated Krebs at 37 °C. The tissues were equilibrated under 2 g tension and exposed to phenylephrine (30 nM). A maximum contractile response to 3 μM phenylephrine was then measured. Tissues were incubated for 30 min with vehicle (DMSO) or antagonist in the presence of protease inhibitors leupeptin (1 μM), thiorphan (1 μM), bestatin (1 μM), bacitracin (750 units l-1), and captopril (1 μM). A cumulative concentration response curve to ET-1 (0.01−1000 nM) in 0.1% BSA was generated. All results are expressed as percentage of maximal PE response and pK_B values calculated.

Molecular Modeling. All structures were initially created using Concord 3D builder (distributed by Tripos Inc., 1699 Hanley Road, 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, UK). Charges were first set using the Gasteiger method, and conformational analysis and energy calculations were carried out using the default Chem-X force field.

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