Structure−Activity Relationships of *N* [2]-Aryl-3-(isoxazolylsulfamoyl)-2-thiophenecarboxamides as Selective Endothelin Receptor-A Antagonists[1]

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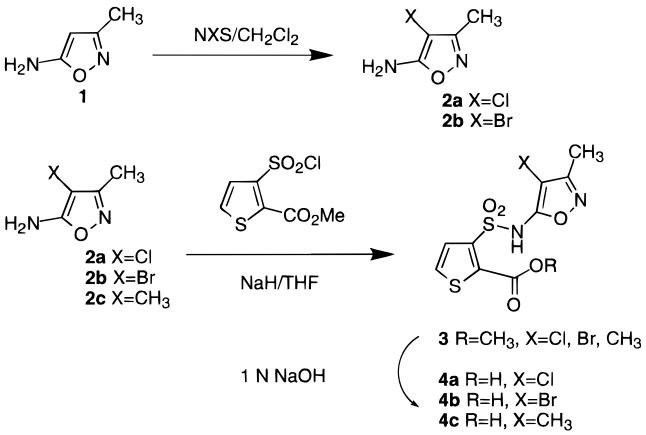
We report here that *N* [2]-aryl-3-(isoxazolylsulfamoyl)-2-thiophenecarboxamides are potent and selective small molecule ET_A receptor antagonists. The aryl group was subjected to extensive structural modification. With monosubstitution, the *para* position was most useful in increasing potency, with methyl being preferred. With disubstitution, 2,4-disubstitution further enhanced activity with methyl or cyano groups being preferred at the 2-position. In this series, a benzo[*d*][1,3]dioxole group is equivalent to a 4-methyl group in *in vitro* activity and afforded the compounds with both *in vivo* activity and moderate half-lives.

Introduction

The potent vasoactive endothelins (ET-1, ET-2, ET-3), isolated in 1988 by Yanagisawa and co-workers, are a family of peptides with 21 amino acid residues and two disulfide bridges.[2] The endothelins have been implicated in many physiological and pathological processes such as hypertension, congestive heart failure, renal failure, cerebral vasospasm, atherosclerosis, restenosis, myocardial infarction, pulmonary disorders, and subarachnoid hemorrhage.[3−9] The endothelins exert their biological activity through cell surface receptors. Two receptors subtypes have been cloned: the ET-1 selective ET_A receptor and the nonselective ET_B receptor.[3,10−14] In the past few years, efforts at various laboratories have resulted in both peptide and nonpeptide endothelin receptor antagonists being reported: The ET_A selective compounds include BQ-123,[15,16] FR-139317,[17] BMS-182874,[18,19] PD-155080,[20] and A-127722.[21] The nonselective inhibitors include SB-209670,[22] L-749329,[23,24] Ro-46-2005,[9] and bosentan,[25] while the ET_B selective compounds reported are BQ-788,[26] IRL-1038,[27] Ro-46-8443,[28,29] and IPI-950.[30] In continuation of our work in the sulfonamide ET antagonists,[30−34] we have turned our attention to thiophenesulfonamides.[35,36] In this paper, we present the structure−activity relationships of one particular thiophenesulfonamide series: *N* [2]-aryl-3-(isoxazolylsulfamoyl)-2-thiophenecarboxamides. These compounds consist of three units: an isoxazlole, a thiophene, and an aryl moiety. The isoxazole and the thiophene moieties are tethered by a sulfonamide group, while the aryl group and the thiophene are connected by an amide linkage. Studies in our laboratory and others have shown that isoxazoles with dimethyl substituents are preferred for binding affinity.[18,19,34] A halogen substitution on the 4-position has been shown to increase the binding affinity by 3−5-fold.[32,33] Therefore in this study, we prepared isoxazolyl sulfonamides with chloro, bromo, or, in a few cases, methyl groups at the 4-position of the isoxazole ring. We report the discovery that *N* [2]-aryl-3-[(4-chloro-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamides are high-affinity, selective ET_A receptor antagonists.

Scheme 1. Synthesis of the Intermediate Acid **4**



Synthesis

Most of the sulfonamides were synthesized by coupling the desired aniline with a carboxylic acid **4** (Scheme 1): 5-Amino-3-methylisoxazole **1** was treated with NCS or NBS in dichloromethane at 0 °C to give haloisoxazole **2** (87%). The amine was then coupled with the commercially available 2-(methoxycarbonyl)-3-thiophenesulfonyl chloride using sodium hydride in THF at 0 °C. The resulting ester **3** was usually not isolated but directly hydrolyzed in 1 N NaOH to the corresponding acid **4** (45%, two steps).

Coupling of the acid **4** with anilines was achieved by three general methods (Scheme 2): either (A) conversion of acid **4** using carbonyldiimidazole to the corresponding acylimidazolide which was then treated with strongly nucleophilic anilines with or without heating, (B) in the case of weakly nucleophilic anilines, sodium hydride was used to deprotonate the aniline at 0 °C prior to the coupling with the acylimidazolide, or (C) treatment of a mixture of acid **4** and an aniline in THF with phosphonitrilic chloride trimer.

Compounds **6h**, **6i**, and **7** were generated by saponification of the corresponding esters **5h**, **5i**, and **5c** using

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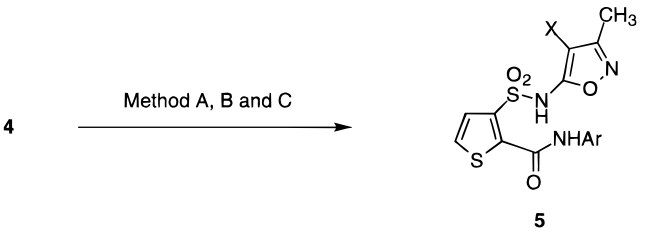
Scheme 2. General Methods for Synthesizing the Amides[a]



[a] Method A: carbonyldiimidazole/$ArNH_2$/THF or DMF. Method B: (1) carbonyldiimidazole/THF or DMF, (2) $ArNH_2$/NaH. Method C: phosphonitrilic chloride trimer/$ArNH_2$/Et_3N/THF.

1 N NaOH at room temperature in essentially quantitative yields (Scheme 3). The amide **8** was made by coupling the acid **7** with *in situ* generated ammonia (66%). The amide in **5e** was reduced with borane·THF[37] to give the corresponding amine **9** (12%). Piperazine **11** was prepared by deprotonating the amide **10** with sodium hydride followed by heating of the dianion with 3-chloro-6-methoxypiperazine in DMF. The methoxy group on the piperazine ring was hydrolyzed during acidic workup to give compound **11**(15%).

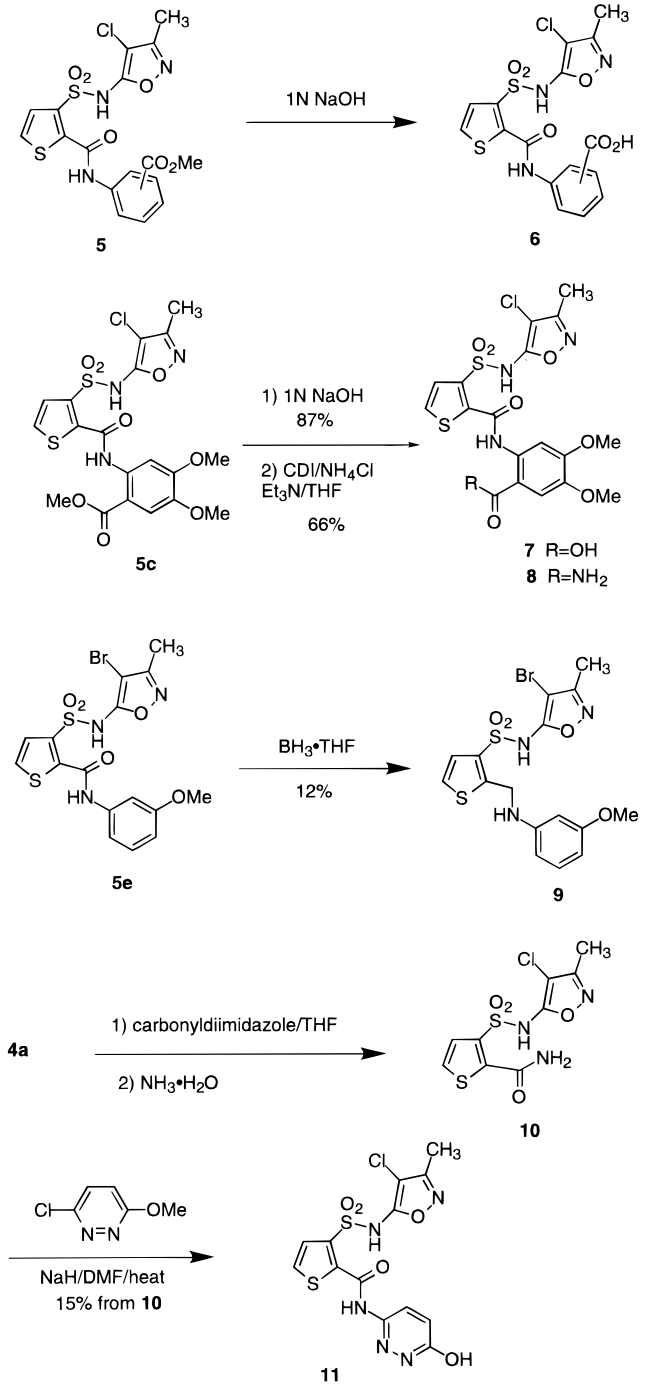
Scheme 4 shows the syntheses of several anilines required for generation of the corresponding sulfonamides. The aniline **13** used to make **5nn** was prepared by nitrating 5-cyanobenzo[*d*][1,3]dioxole with nitric acid (87%) followed by the catalytic hydrogenation of **12** to give a mixture of the desired aminobenzonitrile **13** and aminobenzamide **14**. The pure aniline **13** was obtained by recrystallizing the mixture from aqueous methanol (33%). The (hydroxyalkyl)aniline **17** was prepared by reduction of the commercial acid **15** with borane·THF. The resulting alcohol was nitrated with nitric acid, and subsequent catalytic hydrogenation gave the corresponding aniline **17** (64%, three steps). The methanesulfonamidoaniline **18** was synthesized by mesylating piperonylamine followed by nitration and catalytic hydrogenation (18%, three steps). The (cyanomethyl)-aniline **19** was derived by nitrating 5-(cyanomethyl)-benzo[*d*][1,3]dioxole followed by catalytic hydrogenation (30%).

Results and Discussion

Inhibition of endothelin binding to both the ET_A and ET_B receptors was determined in an *in vitro* ^{125}I-labeled ET-1 competition assay (Table 1). A simple primary amide had an IC_{50} of 838 nM for ET_A and no detectable activity against ET_B. Phenyl substitution of the amide caused a 5-fold increase in affinity. However, the *N*-methylanilino analog **5c** was more than 10-fold less potent. This was probably due more to steric factors than to the absence of a proton since the corresponding ester maintained potent activity.[36] Lengthening the distance between the amide nitrogen and the aromatic ring by one carbon unit resulted in a moderate decrease of ET_A activity. Introduction of the 3-methoxyl substituent was preferred, and a comparison between **5e** and **9** showed that the amide carbonyl group was required for potent ET_A activity and selectivity.

Substituents at the *ortho*, *meta*, and *para* positions of the phenyl ring were investigated. For methoxy and methyl substitution, all three positions showed improvement in activity over the parent compound **5b** with the *para* position being the most effective. Carboxylic acid

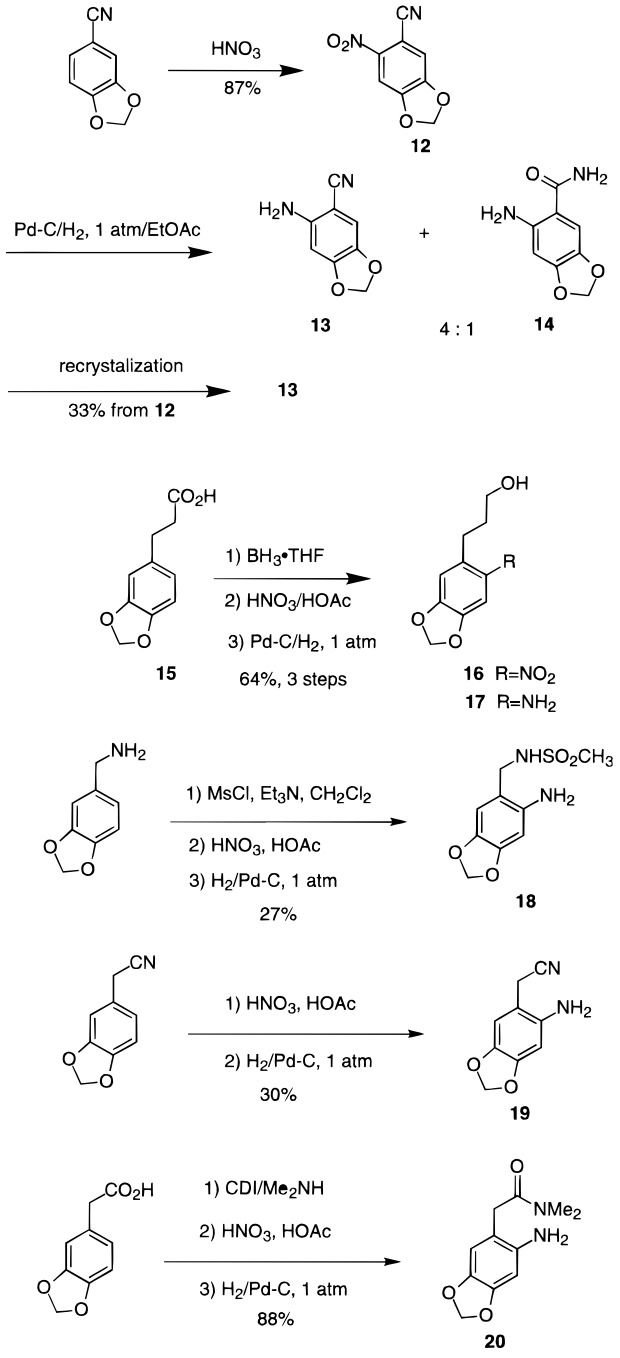
Scheme 3. Additional Synthetic Methodologies



substitution in the *meta* position (**5i**) was preferred over the parent, but not *ortho* substitution (**5h**). Realizing the effectiveness of *para* substitution, we then synthesized and evaluated a series of compounds with *para* alkyl or phenyl substitution. The data indicate that the *para* position can tolerate alkyl groups of considerable size, from a methyl to a *tert*-butyl group. While all of them showed better activity than the parent compound, the methyl substituent remained the most active of the set. However, when the alkyl group was too long, such as *n*-butyl (**5p**), the activity was compromised. A phenyl group (**5s**) was not well tolerated at the *para* position. Phenyl and *n*-butyl groups at the *para* position also lowered ET_A selectivity.

Since it was known that a halogen substitution at the 4-position of the isoxazole improves the activity 3−5-

Scheme 4. Syntheses of Anilines

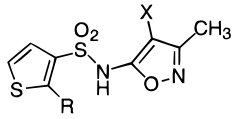


fold,[32,33] we wanted to investigate this with the more active compounds we had in hand. As expected, the methylisoxazole **5t** was 3-fold less active than **5l** and **5u**, while the activity of the chloro- and bromoisoxazolyl sulfonamides were almost identical. We subsequently report mainly chloroisoxazolyl sulfonamides since this series have preferred physical and pharmacological properties.

Heterocycle-substituted amides are reported in Table 2. Except for the pyridine derivative **5x**, the heterocycle-substituted compounds were less active than the all carbon aromatic system and were not extensively investigated due to the additional synthetic challenge presented.

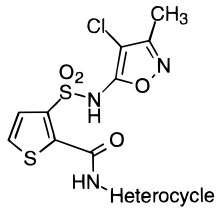
Although some of the *para*-substituted phenylaminocarbonyl thiophenesulfonamides are quite potent in *in vitro* assays, they did not demonstrate good activity

Table 1. Effect of Thiophene Substitution on [^{125}I]Endothelin-1 Binding



			IC$_{50}$, nM (*n*)	
entry	R	X	ET$_A$	ET$_B$
5a	$CONH_2$	Br	838 ± 229 (2)	>100000 (2)
5b	$CONHC_6H_5$	Br	166 (1)	77600 (1)
5c	$CON(CH_3)C_6H_5$	Br	1910 (1)	>100000 (1)
5d	$CONHCH_2C_6H_5$	Br	229 (1)	97700 (1)
5e	$CONH(C_6H_4$-*m*-$OCH_3)$	Br	85 (1)	63100 (1)
5f	$CONH(C_6H_4$-*o*-$OCH_3)$	Br	132 (1)	61700 (1)
5g	$CONH(C_6H_4$-*p*-$OCH_3)$	Br	24 (1)	30200 (1)
5h	$CONH(C_6H_4$-*o*-$CO_2H)$	Br	214 ± 1 (2)	19500 ± 3300 (2)
5i	$CONH(C_6H_4$-*m*-$CO_2H)$	Br	62 ± 10 (3)	117000 (3)
5j	$CONH(C_6H_4$-*o*-$CH_3)$	Br	27 ± 2 (2)	>100000 (2)
5k	$CONH(C_6H_4$-*m*-$CH_3)$	Br	104 ± 6 (2)	67400 ± 12000 (2)
5l	$CONH(C_6H_4$-*p*-$CH_3)$	Br	12 ± 9 (4)	9120 ± 300 (4)
5m	$CONH(C_6H_4$-*m*-OH)	Br	146 ± 24 (3)	67500 ± 2500 (3)
5n	$CONH(C_6H_4$-*p*-$CH_2CH_3)$	Br	17 (1)	7410 (1)
5o	$CONH(C_6H_4$-*p*-$CH(CH_3)_2)$	Br	20 (1)	4370 (1)
5p	$CONH(C_6H_4$-*p*-$CH_2(CH_2)_2CH_3)$	Br	200 (1)	6170 (1)
5q	$CONH(C_6H_4$-*p*-$CH(CH_3)CH_2CH_3)$	Br	54 (1)	5890 (1)
5r	$CONH(C_6H_4$-*p*-$C(CH_3)_3)$	Br	47 (1)	6920 (1)
5s	$CONH(C_6H_4$-*p*-$C_6H_5)$	Br	977 (1)	8710 (1)
5t	$CONH(C_6H_4$-*p*-$CH_3)$	CH_3	32 (1)	67600 (1)
5u	$CONH(C_6H_4$-*p*-$CH_3)$	Cl	15 ± 5 (2)	14100 ± 0 (2)
9	$CH_2NH(C_6H_4$-*m*-$OCH_3)$	Br	933 (1)	7590 (1)

Table 2. Effect of Heterocyclic Amides on [^{125}I]Endothelin-1 Binding



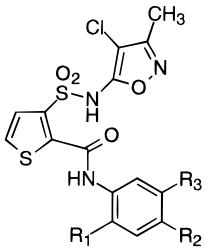
			IC$_{50}$, nM (*n*)	
entry	heterocycle	X	ET$_A$	ET$_B$
5v	5-CH_3-3-isoxazolyl	Cl	57 ± 15 (2)	92100 ± 25400 (2)
5w	5-CH_3-1,3,4-thiadiazol-2-yl	Cl	1290 ± 470 (2)	>100000 (2)
11	6-OH-3-pyridazyl	Br	65 ± 3 (2)	81400 ± 5300 (2)
5x	5-CH_3-2-pyridyl	Cl	13 ± 1 (2)	44700 ± 1400 (2)

in vivo in a DOCA salt model[19] of hypertension or ET-1 challenge model.[25] While this may be due to a number of factors, we considered the possibility that the amide bond was rapidly cleaved in serum. We therefore decided to investigate the effect of an *ortho* substituent in addition to a *para* substitution to see if this increase in steric hindrance around the amide would slow down cleavage of the amide linkage. Initially we investigated effects on potency of this class of sulfonamides.

We evaluated several 2,4-disubstituted anilino amides (Table 3) derived from commercially available anilines. Although a 2-methoxy group (**5y**) did not show much improvement over the corresponding *para* monosubstituted analog (**5g**), 2-methyl (**5aa**) and 2-hydroxyl group (**5z**) showed marked increase of activity over their corresponding *para* monosubstituted analogs (**5t**, **5g**).

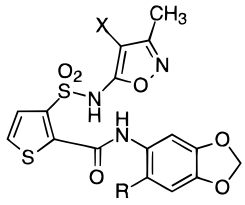
We then studied functional groups at the 2-position with the 4- and 5-positions substituted with methoxy

Table 3. Effect of Di- and Trisubstituted Anilino Amides on [^{125}I]Endothelin-1 Binding



				IC_{50}, nM (*n*)	
entry	R_1	R_2	R_3	ET_A	ET_B
5y	OCH_3	OCH_3	H	15 ± 1 (2)	51900 ± 15800 (2)
5z	OH	CH_3	H	6.6 ± 1 (2)	8240 ± 1860 (2)
5aa	CH_3	OCH_3	H	2.6 ± 0.5 (2)	14000 ± 2050 (2)
5bb	CO_2CH_3	OCH_3	OCH_3	549 ± 226 (2)	>100000 (2)
7	CO_2H	OCH_3	OCH_3	1430 ± 390 (2)	>100000 (2)
8	$CONH_2$	OCH_3	OCH_3	10.8 ± 0.4 (2)	61000 ± 31000 (2)
5cc	CN	OCH_3	OCH_3	3.9 ± 0.6 (3)	12200 ± 1400 (3)

Table 4. Effect of Substituted Benzo[*d*][1,3]dioxoles on [^{125}I]Endothelin-1 Binding



			IC_{50}, nM (*n*)	
entry	R	X	ET_A	ET_B
5dd	H	Br	19 ± 5 (5)	10100 ± 1500 (5)
5ee	CH_3	Cl	3.0 ± 0.7 (4)	35500 ± 112300 (4)
19a	$(CH_2)_2OH$	Cl	5.6 ± 0.8 (2)	16800 ± 850 (2)
19b	$(CH_2)_3OH$	Cl	6.0 ± 3.8 (3)	9080 ± 1870 (3)
5ff	$O(CH_2)_2OH$	Cl	5.1 ± 1.3 (3)	14400 ± 2500 (3)
5gg	$(CH_2)_2OAc$	Cl	4.5 ± 0 (2)	25700 ± 850 (2)
5hh	$O(CH_2)_2OAc$	Cl	6.1 ± 0.7 (2)	15800 ± 2300 (2)
5ii	$CH_2CON(CH_3)_2$	Cl	9.3 ± 2.3 (2)	13800 ± 0 (2)
5jj	$CH_2NHSO_2CH_3$	Cl	6.8 ± 0.4 (2)	19800 ± 900 (2)
5kk	$COCH_3$	Cl	10 ± 2 (5)	32600 ± 1800 (5)
5mm	$COCH_3$	CH_3	31 ± 6 (2)	>100000 (2)
5nn	CN	Cl	3.4 ± 0.4 (4)	40400 ± 6400 (5)
5pp	CH_2CN	Cl	3.8 ± 0.3 (2)	25700 ± 850 (2)

groups. A cyano substituent (**5cc**) was particularly active, a primary amido group (**8**) was about 3-fold less active, a methyl ester (**5bb**) was 100-fold less active, and a carboxylic acid (**7**) caused the loss of essentially all activity.

Although these dimethoxy sulfonamides did show some *in vivo* activity, their serum half-lives in rats were very short. This was possibly due at least partially to P_{450} enzymatic demethylation reactions. We became interested in replacing the dimethoxyphenyl group with a benzo[*d*][1,3]dioxole group because of the wealth of published information from various laboratories showing its importance in a variety of endothelin antagonist scaffolds.[20−24] In this benzo[*d*][1,3]dioxole series, we continued to investigate the effect of different substitutents at the 2-position (Table 4). The 2-position tolerated groups such as hydroxylalkyl, acetate, amide, sulfonamide, ketone, and cyano with a tether of reasonable length, with methyl and cyano groups being the best substituents. The cyano compound **5nn** (ET_A IC_{50} = 3.4 ± 0.4 nM, with selectivity of >10000, phosphoinositide hydrolysis K_i = 1.4 nM, pA_2 = 7.9) had 20% inhibition of ET-1-induced pressor response in conscious, autonomically blocked rats (n = 4, 15 mg/kg iv bolus at 30 min). Compound **5nn** (TBC11241) was also active (5 mg/kg iv) in an acute hypoxia-induced pulmonary hypertension rat model[38,39] and had a half-life of 2.5 h in the rats (60 mg/kg). The ketone compound **5kk** (TBC11192, ET_A IC_{50} = 10.1 ± 1.7 nM, selectivity >3000, phosphoinositide hydrolysis K_i = 0.59 nM, pA_2 = 7.87) had 18% inhibition of ET-1-induced pressor response in conscious, autonomically blocked rats (n = 4, 15 mg/kg iv bolus, at 30 min) with a serum half-life of 2.5−3 h (60 mg/kg).

Table 5. Synthetic and Physical Data

entry	synth method	% yield	mp, °C	formula[a]
5a	A	95	168−170	$C_9H_8BrN_3O_4S_2$
5b	F	72	168−170	$C_{15}H_{12}BrN_3O_4S_2$[b]
5c	C	39	51−55	$C_{16}H_{14}BrN_3O_4S_2$·0.2TFA
5d	D	30	186−190	$C_{16}H_{14}BrN_3O_4S_2$[b]
5e	E	23	200−202	$C_{16}H_{14}BrN_3O_5S_2$·0.1TFA
5f	E	26	74−80	$C_{16}H_{14}BrN_3O_5S_2$[b]
5g	E	17	202−205	$C_{16}H_{14}BrN_3O_5S_2$[b]
5h	C	14	185−187	$C_{16}H_{12}BrN_3O_6S_2$·0.15HOAc
5i	C	12	183−185	$C_{16}H_{12}BrN_3O_6S_2$·0.15HOAc
5j	D	10	158 dec	$C_{16}H_{14}BrN_3O_4S_2$[b]
5k	D	10	143−145	$C_{16}H_{14}BrN_3O_4S_2$[b]
5l	D	15	178−180	$C_{16}H_{14}BrN_3O_4S_2$[b]
5m	A	18	42−44	$C_{15}H_{12}BrN_3O_5S_2$
5n	D	31	187−190	$C_{17}H_{16}BrN_3O_4S_2$[b]
5o	D	19	oil	$C_{18}H_{18}BrN_3O_4S_2$[b]
5p	D	18	oil	$C_{19}H_{20}BrN_3O_4S_2$[b]
5q	D	25	205−208	$C_{19}H_{20}BrN_3O_4S_2$[b]
5r	D	28	76−86	$C_{19}H_{20}BrN_3O_4S_2$[b]
5s	D	26	205−212 dec	$C_{21}H_{16}BrN_3O_4S_2$[b]
5t	C	61	185−190	$C_{17}H_{17}N_3O_4S_2$[b]
5u	C	53	177−179	$C_{16}H_{14}ClN_3O_4S_2$[b]
5v	A	8	171−172	$C_{13}H_{11}ClN_4O_5S_2$·0.1TFA
5w	B	15	192−194	$C_{12}H_{10}ClN_5O_4S_3$
5x	B	17	158−160	$C_{15}H_{13}ClN_4O_4S_2$
5y	A	16	162−164	$C_{17}H_{16}ClN_3O_6S_2$·0.1EtOAc
5z	A	42	68−70	$C_{16}H_{14}ClN_3O_5S_2$
5aa	A	66	58−62	$C_{17}H_{16}ClN_3O_5S_2$
5bb	B	13	167−168	$C_{19}H_{18}ClN_3O_8S_2$
5cc	B	36	53−56	$C_{18}H_{15}ClN_4O_6S_2$
5dd	A	15	138−140	$C_{16}H_{12}BrN_3O_6S_2$
5ee	A	45	60−62	$C_{17}H_{14}ClN_3O_6S_2$
5ff	G	86	158−161	$C_{18}H_{16}ClN_3O_8S_2$[b]
5gg	C	12	78−82	$C_{20}H_{18}ClN_3O_8S_2$[b]
5hh	C	21	117−119	$C_{20}H_{18}ClN_3O_9S_2$[b]
5ii	A	19	190−193	$C_{20}H_{19}ClN_4O_7S_2$
5jj	A	13	147−150	$C_{18}H_{17}ClN_4O_8S_3$·0.2TFA
5kk	B	49	191−193	$C_{18}H_{14}ClN_3O_7S_2$[b]
5mm	B	8	228−231	$C_{19}H_{17}N_3O_7S_2$
5nn	B	40	167−168	$C_{17}H_{11}ClN_4O_6S_2$
5pp	A	15	190−193	$C_{18}H_{13}ClN_4O_6S_2$
7	G	87	192−195	$C_{18}H_{16}ClN_3O_8S_2$
8	H	66	189−192	$C_{18}H_{17}ClN_4O_7S_2$
9	J	12	70−73	$C_{16}H_{16}BrN_3O_4S_2$·2NH_3·1.5TFA
11	K	15	56−59	$C_{13}H_{10}BrN_5O_5S_2$·0.7TFA·H_2O
19a	G	84	47−52	$C_{18}H_{16}ClN_3O_7S_2$[b]
19b	A	18	66−69	$C_{19}H_{18}ClN_3O_7S_2$

[a] Analysis for C, H, N are within 0.4% of theory. [b] C, H, N not done due to insufficient sample. High-resolution MS within 0.004% of theory. Sample homogeneity established by two diverse analytical HPLC systems.

Conclusion

We report 3-thiophenesulfonamides as a novel series of endothelin-A receptor antagonists. The benzene ring of the anilino amide moiety was convenient for investigating a wide variety of substitutions. Study of the SAR of the phenyl ring established that the position *para* to the nitrogen was very effective for increasing

activity when substituted with the appropriate group such as methyl, methoxy, or methylenedioxy. In the presence of the required *para* substitution, additional *ortho* substitution was useful to further increase the potency of the compounds. A combination of *ortho* substitution on a benzo[*d*][1,3]dioxole rendered a series of compounds with *in vitro* potency, ET_A selectivity, *in vivo* activity, and a moderate half-life.

Experimental Section

General. Melting points were determined in capillary tubes with a Mel-Temp II apparatus and are uncorrected. Proton NMR (^{1}H NMR) spectra were recorded on a GE QE-300 Plus spectrometer at 300 MHz. Chemical shifts were reported in parts per million as δ units relative to tetramethylsilane or residual solvent as internal standard. IR spectra were recorded on a Mattson GL-2020 Fourier transform infrared spectrophotometer. High-resolution mass spectra were recorded with fast atom bombardment (FAB) ionization by the University of Minnesota Mass Spectrometry Service Laboratory (Minneapolis, MN). Elemental analyses were performed by Desert Analytics (Tucson, AZ) and were within 0.4% of theoretical values unless otherwise indicated. Anhydrous solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI) in Sure-Seal bottles. Unless otherwise stated, reagents and chemicals were of the highest grade from commercial sources and were used without further purification. ET-1 was obtained from Clinalfa Co. (Laufelfingen, Switzerland) and ET-3 from American Peptide Co. (Sunnyvale, CA). [^{125}I]ET-1 was obtained from Amersham (Arlington Heights, IL). Flash chromatography was performed on silica gel 60 (230−400 mesh, E. Merck). Thin layer chromatography was performed with E. Merck silica gel 60 F-254 plates (0.25 mm) and visualized with UV light, phosphomollybdic acid, or iodine vapor. Analytical HPLC was performed on Vydac C18 column (4.6 × 250 mm), preparative HPLC on Dynamax-60A (83-241-c) with acetonitrile:water gradients containing 0.1% trifluoroacetic acid. The detection wave length was 254 nm.

5-Amino-4-chloro-3-methylisoxazole (2a). To a solution of 5-amino-3-methylisoxazole (**1**) (5.0 g, 51.0 mmol) in methylene chloride (40 mL) at 0 °C was slowly added *N*-chlorosuccinimide (6.8 g, 51.0 mmol). The reaction mixture was stirred for 1 h at 0 °C and an additional 2 h at room temperature. The crude mixture was washed with 1 N NaOH, and the organic layer was dried over $MgSO_4$ and concentrated by evaporation. The residue was shaken with hexanes (50 mL), and the resulting white precipitate was filtered to give **2a** (5.85 g, 87% yield) as a white solid: mp 65−68 °C; ^{1}H NMR ($CDCl_3$) δ 4.54 (br s, 2H), 2.17 (s, 3H).

3-[(4-Chloro-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxylic Acid (4a). To a solution of **2a** (1.82 g, 17.7 mmol) in anhydrous THF (60 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 1.50 g, 37.4 mmol). The resulting mixture was stirred for 10 min before the slow addition of 2-(methoxycarbonyl)thiophene-3-sulfonyl chloride (3.0 g, 12.5 mmol). The reaction was stirred at room temperature for 4 h. To work up, the mixture was diluted with hexanes (60 mL) and the resulting precipitate was filtered and washed with hexanes. The precipitate was then dissolved in 1 N NaOH and stirred at room temperature for 1 h. After acidifying to pH ~2 with 2 N HCl, the resulting precipitate was filtered, washed with water, and dried under vacuum. Compound **4a** was obtained (1.82 g, 45% yield) as a yellow powder: mp 166−170 °C; ^{1}H NMR (DMSO-d_6) δ 7.91 (d, J = 5.1 Hz, 1H), 7.42 (d, J = 5.1 Hz, 1H), 2.13 (s, 3H).

Method A. *N*^{2}-(2,4-Dimethoxyphenyl)-3-[(4-chloro-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamide (5y). To a solution of acid **4a** (1.0 g, 3.1 mmol)) in anhydrous DMF (10 mL) at room temperature was added carbonyldiimidazole (533 mg, 3.41 mmol). The mixture was stirred at room temperature for 15 min before the addition of 2,4-dimethoxyaniline (1.22 g, 7.75 mmol). The mixture was stirred for 5 h. The crude reaction mixture was partitioned between 1 N HCl and EtOAc. The organic layer was washed with 1 N HCl twice before it was dried ($MgSO_4$) and concentrated. The residue was recrystallized (acetonitrile/water) to give **5y** (224 mg, 16% yield) as a yellow solid: mp 162−164 °C; ^{1}H NMR (DMSO-d_6) δ 10.19 (br s, 1H), 7.82 (d, J = 8.7 Hz, 1H), 7.74 (d, J = 5.3 Hz, 1H), 7.35 (d, J = 5.3 Hz, 1H), 6.62 (d, J = 2.4 Hz, 1H), 6.52 (dd, J = 8.7, 2.4 Hz, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 2.00 (s, 3H).

Method B. *N*^{2}-(6-Acetylbenzo[*d*][1,3]dioxol-5-yl)-3-[(4-chloro-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamide (5kk). Carbonyldiimidazole (553 mg, 3.41 mmol) was added to a solution of acid **4a** (1.0 g, 3.1 mmol) in anhydrous DMF (10 mL). The mixture was stirred at room temperature for 15 min to give mixture I. Sodium hydride (60% dispersion in mineral oil, 521 mg, 13.02 mmol) was added to a solution of 6-acetyl-5-aminobenzo[*d*][1,3]dioxole (1.13 g, 6.2 mmol) in anhydrous DMF (10 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min to give mixture II. Mixture I was slowly transferred via cannula into mixture II at 0 °C and was stirred at 0 °C for 4 h. The reaction mixture was poured into 2 N HCl (200 mL) and the resulting precipitate was collected by filtration. The solid was washed with water (2 × 10 mL) and ethyl ether (2 × 10 mL) to give **5kk** (730 mg, 49% yield) as a dull yellow powder: mp 191−193 °C; ^{1}H NMR ($CDCl_3$) δ 13.34 (br s, 1H), 8.44 (s, 1H), 7.50 (s, 2H), 7.36 (s, 1H), 6.11 (s, 2H), 2.65 (s, 3H), 2.24 (s, 3H).

Method C. *N*^{2}-(4-Methylphenyl)-3-[(3,4-dimethyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamide (5t). Phosphonitrilic chloride trimer dissolved in THF (5 mL) was added to a suspension of acid **4c** (2.0 g, 6.6 mmol) in THF (5 mL) and Et_3N at 0 °C. The cold bath was removed and the reaction mixture stirred at room temperature for 2 h. The mixture was diluted with water (150 mL) and acidified to pH 2 using concentrated HCl. The mixture was then extracted with methylene chloride (2 × 100 mL), and the combined organic layers were washed with 2 N HCl (3 × 100 mL), dried over $MgSO_4$, and concentrated. The residue was dissolved in ether and allowed to stand at room temperature to give a precipitate which was filtered and washed with cold ether to give **5t** (1.6 g, 61% yield) as a solid: mp 185−190 °C; ^{1}H NMR (DMSO-d_6) δ 10.62 (br s, 1H), 7.86 (d, J = 5.2 Hz, 1H), 7.51 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 5.1 Hz, 1H), 7.16 (d, J = 8.1 Hz, 2H), 2.28 (s, 3H), 2.04 (s, 3H), 1.65 (s, 3H).

Method D. *N*^{2}-(4-Ethylphenyl)-3-[(4-bromo-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamide (5n). 4-Ethylaniline (242 mg, 2.0 mmol), (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate (Bop) (442 mg, 1.0 mmol) and diisopropylethylamine (0.15 mL) were added to acid **4b** (368 mg, 1.0 mmol), which had been suspended in methylene chloride (3 mL). The solution was stirred for 14 h at room temperature. This was diluted with methylene chloride (50 mL) and washed with 3 N HCl (3 × 50 mL) followed by 5% sodium carbonate solution (2 × 50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography using EtOAc as eluent. Recrystallization from EtOAc/hexanes gave **5n** (151 mg, 31% yield) as a solid: mp 187−190 °C; ^{1}H NMR (DMSO-d_6) δ 11.78 (br s, 1H), 7.73 (d, J = 5.4 Hz, 1H), 7.63 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 5.4 Hz, 1H), 7.20 (d, J = 8.4 Hz, 2H), 2.58 (q, J = 7.5 Hz, 2H), 1.97 (s, 3H), 1.18 (t, J = 7.5 Hz, 3H).

Method E. *N*^{2}-(4-Methoxyphenyl)-3-[(4-bromo-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamide (5g). Compound **5g** was synthesized in the same fashion as compound **5n** (method D) except that bromotris(pyrrolidino)-phosphonium hexafluorophosphate (PyBrop) was used instead of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (Bop). Compound **5g** was obtained in 17% yield as a solid: mp 202−205 °C; ^{1}H NMR (DMSO-d_6) δ 11.74 (br s, 1H), 7.73 (d, J = 5.1 Hz, 1H), 7.65 (d, J = 9.0 Hz, 2H), 7.39 (d, J = 5.1 Hz, 1H), 6.96 (d, J = 9.0 Hz, 2H), 3.76 (s, 3H), 1.98 (s, 3H).

Method F. *N*^{2}-Phenyl-3-[(4-bromo-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamide (5b). Compound **5b** was synthesized in the same fashion as **5n** (method D) except that 1-ethyl-3′-[3-(dimethylamino)propyl]carbodiimide (EDCI) was used instead of Bop. Compound **5b** was

obtained in 72% yield as a yellow solid: mp 168−179 °C; ^{1}H NMR (DMSO-d_6) δ 11.32 (br s, 1H), 8.72 (br s, 1H), 7.76 (d, J = 4.2 Hz, 1H), 7.65 (d, J = 10.2 Hz, 2H), 7.31−7.36 (m, 3H), 7.08 (t, J = 7.3 Hz, 1H), 2.00 (s, 3H).

Method G. *N*^{2}-(6-Carboxylbenzo[*d*][1,3]dioxol-5-yl)-3-[(4-chloro-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamide (7). Sodium hydroxide solution (1.5 N, 250 mL) was added to **5bb**. The resulting suspension was stirred at room temperature overnight to give a clear solution. The solution was acidified using concentrated hydrochloric acid while cooling. The precipitate was filtered, washed with water (3 × 50 mL), and dried under vacuum to give **7** (347 mg, 87% yield) as a yellow powder: mp 192−195 °C; ^{1}H NMR (DMSO-d_6) δ 11.70 (br s, 1H), 7.96 (s, 1H), 7.86 (d, J = 5.1 Hz, 1H), 7.44 (s, 1H), 7.40 (d, J = 5.1 Hz, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 2.08 (s, 3H). Compounds **5ff** and **19a** were synthesized in the same fashion from **5hh** and **5gg**, respectively.

Method H. *N*^{2}-[3,4-Dimethoxy-6-(aminocarbonyl)phenyl]-3-[(4-chloro-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamide (8). Compound **8** was synthesized in the same fashion as for compound **5y** (method A) except that acid **7** was used instead of acid **4a**. Compound **8** was obtained in 66% yield as a yellow powder: mp 189−192 °C; ^{1}H NMR (DMSO-d_6) δ 12.65 (br s, 1H), 8.12 (br s, 1H), 7.96 (s, 1H), 7.85 (d, J = 5.1 Hz, 1H), 7.57 (br s, 1H), 7.42 (d, J = 5.1 Hz, 1H), 7.41 (s, 1H), 3.81 (s, 6H), 2.11 (s, 3H).

Method J. *N*^{2}-(3-Methoxyphenyl)-3-[(4-bromo-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamide (9). To a solution of **5e** (1.0 g, 2.12 mmol) in anhydrous THF (15 mL) was added BH_3·THF (15 mL, 1 M in THF). The mixture was heated under reflux for 8 h and then cooled to room temperature. The volatiles were removed by evaporation, and methanol was added to the residue. The solution was concentrated again and the residue purified by HPLC to give **9** (113 mg, 12% yield) as a light yellow/gray powder: mp 70−73 °C; ^{1}H NMR (DMSO-d_6) δ 7.48 (d, J = 5.6 Hz, 1H), 7.21 (d, J = 5.6 Hz, 1H), 6.96 (dd, J = 8.1, 8.2 Hz, 1H), 6.16 (d, J = 8.1 Hz, 1H), 6.12 (d, J = 8.2 Hz, 1H), 6.10 (s, 1H), 4.59 (s, 2H), 3.62 (s, 3H), 2.19 (s, 3H).

Method K. *N*^{2}-(6-Hydroxy-3-pyridazyl)-3-[(4-bromo-3-methyl-5-isoxazolyl)sulfamoyl]-2-thiophenecarboxamide (11). To a solution of **5a** (572 mg, 1.56 mmol) in anhydrous DMF (10 mL) were sequentially added 3-chloro-6-methoxypyridazine (475 mg, 3.12 mmol) and sodium hydride (60% dispersion in mineral oil, 194 mg, 4.84 mmol). The resulting mixture was heated at 100 °C under a nitrogen atmosphere for 4 h. The reaction mixture was allowed to cooled to room temperature, poured into 1 N HCl acid, and extracted with EtOAc. The organic layer was washed with 1 N HCl twice, dried ($MgSO_4$), and then concentrated. The residue was purified by HPLC to give **11** (111 mg, 15% yield) as a brown powder: mp 56−59 °C; ^{1}H NMR (DMSO-d_6) δ 12.75 (br s, 1H), 8.45 (d, J = 9.3 Hz, 1H), 7.94 (d, J = 9.3 Hz, 1H), 7.87 (d, J = 5.1 Hz, 1H), 7.40 (d, J = 5.1 Hz, 1H), 1.98 (s, 3H).

5-Amino-6-cyanobenzo[*d*][1,3]dioxole (13). Piperonylonitrile (10 g) was added to nitric acid (70%, 40 mL) at 0 °C over 30 min. After 30 min at 0 °C and 30 min at 40 °C, ice was added to the reaction mixture. The resulting precipitate was filtered to give **12** (11.4 g, 87% yield) as a bright yellow powder. To a solution of **12** (11.4 g) in EtOAc (400 mL) was added 10% Pd/C (540 mg). The flask was purged with hydrogen gas three times from a balloon, and the reaction mixture was stirred overnight. The solid was filtered off, and the filtrate was concentrated. The resulting yellow solid mixture of **13** and **14** (1:4) was recrystallized from methanol/water to give **13** (3.67 g, 33% yield) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 6.74 (s, 1H), 6.26 (s, 1H), 5.94 (s, 2H), 4.26 (br s, 2H).

5-Amino-6-(3-hydroxy-1-propyl)benzo[*d*][1,3]dioxole (17). To a solution of 3-(benzo[*d*][1,3]dioxol-5-yl)propanoic acid (5 g, 25.75 mmol) in anhydrous THF (20 mL) at 0 °C was added BH_3·THF (51.5 mL, 1.0 M in THF, 51.5 mmol). The mixture was heated under reflux for 1 h. The solvent was evaporated, the residue was treated with methanol (20 mL), and the solution was concentrated again. This process was repeated six times to give an oil (4.7 g). This oil was then nitrated, and the nitro compound was catalytically hydrogenated in the same manner as for **13** to give **17** (3.2 g, 64% yield): ^{1}H NMR ($CDCl_3$) δ 6.56 (s, 1H), 6.32 (s, 1H), 5.85 (s, 2H), 3.60 (t, 2H), 5.70 (t, 2H), 1.81 (m, 2H).

5-Amino-6-(methanesulfamidomethyl)benzo[*d*][1,3]-dioxole (18). To a solution of piperonylamine (6.07 g, 38.95 mmol) and triethylamine (5.37 g, 53.12 mmol) in dichloromethane (100 mL) at 0 °C was added methanesulfonyl chloride (4.14 g, 35.41 mmol). The reaction was stirred at 0 °C for 1 h before the mixture was diluted with dichloromethane (100 mL) and washed with 1 N HCl (2 × 100 mL). The organic layer was dried over $MgSO_4$ and concentrated to a gray solid (8.4 g). This solid was then nitrated, and the resulting nitro compound was catalytically hydrogenated in the same manner as for **13** to give **18** (2.3 g, 27% yield): ^{1}H NMR ($CDCl_3$) δ 6.61 (s, 1H), 6.31 (s, 1H), 5.85 (s, 2H), 5.16 (br s, 1H), 4.16 (d, 2H), 2.92 (s, 3H).

5-Amino-6-(cyanomethyl)benzo[*d*][1,3]dioxole (19). Compound **19** was synthesized in the same manner as for **13** except that the nitration was done in acetic acid instead of in neat nitric acid to give **19** in 30% overall yield: ^{1}H NMR (DMSO-d_6) δ 7.68 (s, 1H), 6.35 (s, 1H), 5.84 (s, 2H), 4.88 (br s, 2H), 3.66 (s, 2H).

***N*,*N*-Dimethyl-6-(5-aminobenzo[*d*][1,3]dioxolyl)-acetamide (20).** Compound **20** was synthesized in the same manner as for **13** from *N*,*N*-dimethyl-5-(benzo[*d*][1,3]dioxolyl)-acetamide, which in turn was made from 5-(benzo[*d*][1,3]-dioxolyl)acetic acid using method A. Compound **20** was obtained in 88% overall yield: ^{1}H NMR ($CDCl_3$) δ 6.54 (s, 1H), 6.30 (s, 1H), 5.84 (s, 2H), 4.30 (br s, 2H), 3.55 (s, 2H), 3.13 (s, 3H), 2.94 (s, 3H).

Membrane Preparation. A membrane preparation containing human ET_A receptor was prepared from TE 671 (ATCC # HTB 139). Cells, grown to confluence, were harvested using a rubber policeman and centrifuged at 190*g* for 10 min at 4 °C. The pellet was resuspended in 5 mM HEPES (pH 7.4) containing 5 mM EDTA and 100 KIU aprotinin and homogenized using a Tenbroeck homogenizer. The suspension was centrifuged at 57800*g* for 15 min at 4 °C, and the pellet was resuspended in 5 mL of 5 mM HEPES buffer, pH 7.4, containing 10 mM $MnCl_2$ to which 5 mL of a 0.001% deoxyribonuclease Type 1 was added. The suspension was mixed, incubated at 37 °C for 30 min, and then centrifuged at 57800*g* for 15 min at 4 °C. The pellet was then washed twice with 5 mM HEPES buffer containing 5 mM EDTA before finally being resuspended in 30 mM HEPES buffer, pH 7.4, containing aprotinin (100 KIU/mL) to give a final membrane protein concentration of 2 mg/mL. Aliquots of membrane were stored at −70 °C until use. Protein determinations were carried out using the Pierce BCA assay kit with bovine serum albumin (BSA) as a standard.

A membrane preparation containing human ET_B receptors was prepared as described above from COS 7 cells which were transfected with DNA encoding the human ET_B receptor, as previously described.[40,41]

Ligand Binding Studies. Binding studies were performed in a 30 mM HEPES buffer, pH 7.4, containing 150 mM NaCl, 5 mM $MgCl_2$, and 0.05% bacitracin using 3 mg/tube (ET_A) or 0.75 mg/tube (ET_B) membrane. Test compounds were dissolved in DMSO and diluted with the assay buffer to give a final concentration of 0.25% DMSO. Competitive inhibition experiments were performed in triplicate in a final volume of 200 μL containing 4 pM [^{125}I]ET-1 (1.6 nCi). Nonspecific binding was determined in the presence of 100 nM ET-1. Samples were incubated for 16−18 h at 24 °C. One milliliter of PBS was then added and the assay centrifuged at 2000*g* for 25 min at 24 °C. The supernatant was decanted and the membrane bound radioactivity counted on a Genesys gamma counter.

Phosphoinositide Hydrolysis in Cells. TE 671 or transfected COS 7 cells were grown to confluence in six-well plates. Sixteen hours prior to use, the media in each well was replaced with 2 mL of inositol-free RPMI-164 (IF-RPMI) media containing 10% inositol-free FCS and 2 mCi [^{3}H]myoinositol, and this was incubated at 37 °C in the presence of 6% CO_2. The media was aspirated, and the cells were washed twice with PBS. Cells

were preincubated for 10 min in 1 mL of lithium buffer (15 μM HEPES, pH 7.4, 145 μM NaCl, 5.4 μM KCl, 1.8 μM $CaCl_2$, 0.8 μM $MgSO_4$, 1.0 μM NaH_2PO_4, 11.2 μM glucose, 20 μM LiCl) with or without test compound prior to the addition of 100 μM of ET-1 at different concentrations. Cells were then incubated for an additional 45 min. The buffer was discarded, and the accumulated inositol phosphates were extracted with ice cold methanol and measured according to the method of Berridge. The total cell protein in each well was measured using the Pierce BCA assay after solubilizing the cells in 0.1 M NaOH.

Conscious, Autonomically Blocked Rat Pressor Assays (ET-1 Challenge Assay). Male Sprague−Dawley rats (250−350 g) were anesthetized with a short-acting barbiturate (methohexital: 50 mg/kg ip). Cannula were inserted into the femoral artery and vein exteriorized. The catheter in the femoral artery was connected to a P23XL Spectromed pressure transducer attached to a PO-NE-MAH Digital Acquisition and Analysis system. Animals were placed in a Brainridge restrainer and allowed to recover for 60 min prior to the start of the experiment. Autonomic blockade was established by intravenous administration of atropine methyl nitrate (3 mg/kg) and propranalol (2 mg/kg). Blood pressure was allowed to stabilize for 30 min prior to administration of vehicle (1 M Tris base or sodium bicarbonate). Thirty minutes later animals were challenged by intravenous bolus administration of ET-1 (1 mg/kg, control). Ninety minutes later, when the mean arterial pressure (MAP) had returned to baseline, antagonist was administered by intravenous bolus injection followed 30 min later by a second ET-1 challenge. The degree in inhibition was calculated as

$$100 \times (\text{control}_{\text{max. pressor response}} - \text{antagonist}_{\text{max. pressor response}})/\text{control}_{\text{max. pressor response}}$$

Pharmacokinetics in Rats. All surgical procedures were performed under aseptic conditions. Adult Sprague−Dawley rats were anesthetized with a ketamine-based anesthetic (containing ketamine, xylazine, and promace), and the jugular vein was cannulated. The cannula were channeled under the skin, exteriorized between the scapulae, and protected by a spring tether (BioResearch, Montreal, Quebec). Rats were allowed to recover for 48 h prior to use. The compound, 60 mg/kg, was administered at a dose of 1 mL/kg iv or 10 mL/kg po, and serial blood samples (100 μL) were withdrawn from the caudal vein over the next 24 h. Blood samples were immediately centrifuged and the plasma frozen and stored at −20 °C until assay. Samples were assayed by HPLC following extraction into acetonitrile.

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Supporting Information Available: ^{1}H NMR, IR, and high-resolution mass spectral data for compounds **5a**−**z**, **5aa**−**pp**, **7**−**11**, and **19a**,**b** (10 pages). Ordering information is given on any current masthead page.

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