Discovery of TBC11251, a Potent, Long Acting, Orally Active Endothelin Receptor-A Selective Antagonist[1]

Chengde Wu,*[,†,‡] Ming F. Chan,[†] Fiona Stavros,[†,‡] B. Raju,[†,‡] Ilya Okun,[†] Seymour Mong,[†] Karin M. Keller,[§] Tommy Brock,[§] Timothy P. Kogan,[§] and Richard A. F. Dixon[§]

ImmunoPharmaceutics Inc. (a subsidiary of Texas Biotechnology Corporation), 11011 Via Frontera, San Diego, California 92127, and Texas Biotechnology Corporation, 7000 Fannin, Suite 1920, Houston, Texas 77030

Received January 6, 1997[⊗]

Previously we reported the discovery of amidothiophenesulfonamides as endothelin receptor-A antagonists with high potency and selectivity. Replacement of an amide group in this class of compounds with an acetyl group maintained the *in vitro* binding affinity and *in vivo* activity while providing a compound with oral bioavailability and longer duration of action. The optimal compound discovered during these studies, **15q** (TBC11251), binds competitively to human ET_A receptors with a K_i of 0.43 ± 0.03 nM and an IC_{50} of 1.4 nM (IC_{50} for ET_B = 9800 nM). This compound inhibits ET-1-induced stimulation of phosphoinositide turnover with a K_i of 0.686 nM and a pA_2 of 8.0. The compound has a serum half-life in the rat and the dog of 6−7 h and 60−100% oral bioavailability. This compound is one of the most selective ET_A antagonists reported and therefore is suitable for additional pharmacological and clinical investigation of the role of ET_A receptors in diseases.

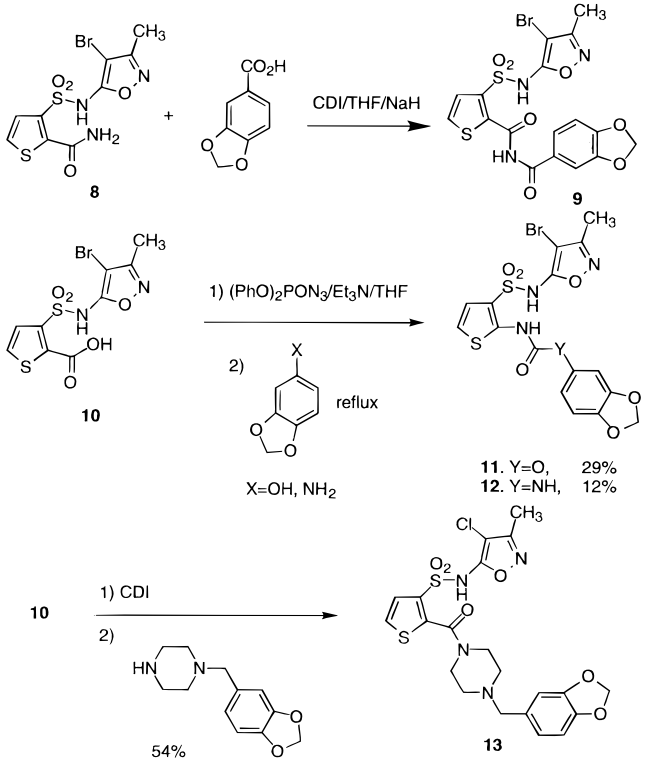
Introduction

In the previous paper, we have reported on a series of 3-thiophenesulfonamides with an arylamide at the 2-position as endothelin receptor antagonists. Some of the most interesting amides are summarized in Figure 1. Although these amidosulfonamides have high binding affinities for ET_A in radioligand binding assays (10−20 nM for **1**−**3**; 2−4 nM for **4**−**7**), the most potent compounds lack oral bioavailability and have short *in vivo* half-lives, perhaps as a result of proteolytic cleavage of the amide bond. In order to develop compounds that maintain the desired *in vitro* and *in vivo* activity of this series but demonstrate improved oral bioavailability and better metabolic properties, we investigated replacing the proteolytically susceptible amide bond in compound **1** with more durable linkers. In this paper, we report the synthesis and structure−activity relationships of various linkers to replace the amide of **1**, which led to the discovery of **15q**, a compound which has good potency, a long duration of action, and good oral bioavailability.

Synthesis

Scheme 1 shows the synthesis of compounds with imide, urethane, urea, and extended amide linkages. Piperyllic acid was treated with carbonyldiimidazole and then with the dianion generated by deprotonating **8** with 2 equiv of sodium hydride to yield the imide **9**. The thienyl carboxylic acid **10** was first subjected to Curtius rearrangement using diphenyl phosphorazidate, and the resulting isocyanate was treated with either 5-hydroxybenzo[*d*][1,3]dioxole to give the urethane **11** or treated with 5-aminobenzo[*d*][1,3]dioxole to give the urea **12**. Alternatively, **10** was coupled with 5-(1-piperadinylmethyl)benzo[*d*][1,3]dioxole using carbonyldiimidazole as the coupling reagent to yield the amide **13**.

Scheme 1. Syntheses of Sulfonamides with Imide, Urethane, Urea, and Amide Linker Groups



The general methodology for synthesizing sulfonamides with a ketone linkage is shown in Scheme 2. Acid **10** was first coupled with *N*,*O*-dimethylhydroxylamine using carbonyldiimidazole as the coupling reagent to give the Weinreb amide **14**.[2] Compound **14** was then treated with aryl or benzylic Grignard reagents followed by acidic workup to give the ketones **15**. This general method also provided for access to the ketone interme-

* Address for correspondence: Texas Biotechnology Corp., 7000 Fannin, Suite 1920, Houston, TX 77030. Phone: (713) 796-8822. Fax: (713) 796-8232. E-mail: cwu@tbc.com.
† ImmunoPharmaceutics, Inc.
‡ Present address: Texas Biotechnology Corp., 7000 Fannin, Suite 1920, Houston, TX 77030.
§ Texas Biotechnology Corp.
⊗ Abstract published in *Advance ACS Abstracts,* May 1, 1997.

S0022-2623(97)00006-X CCC: $14.00

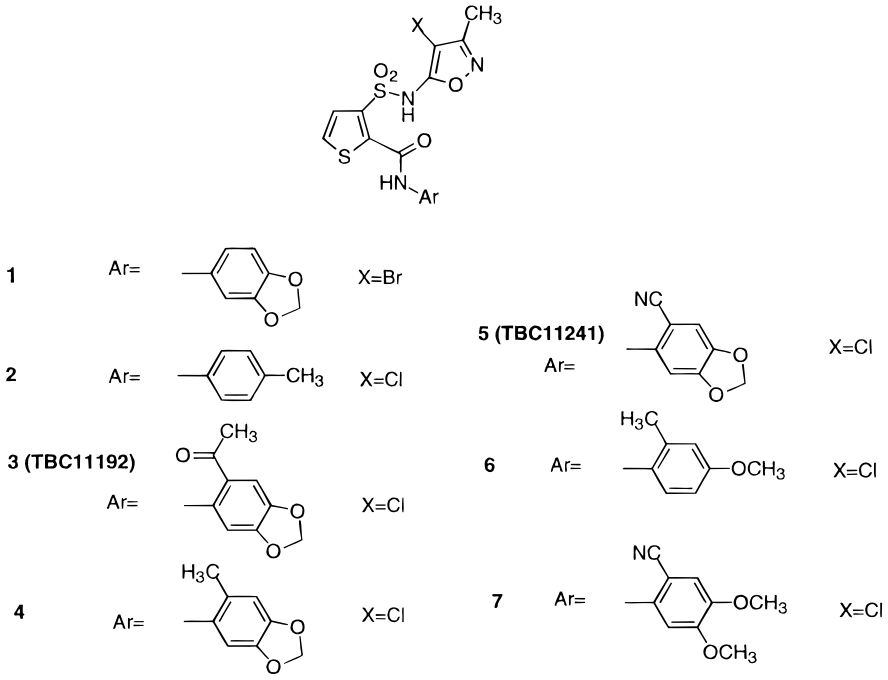
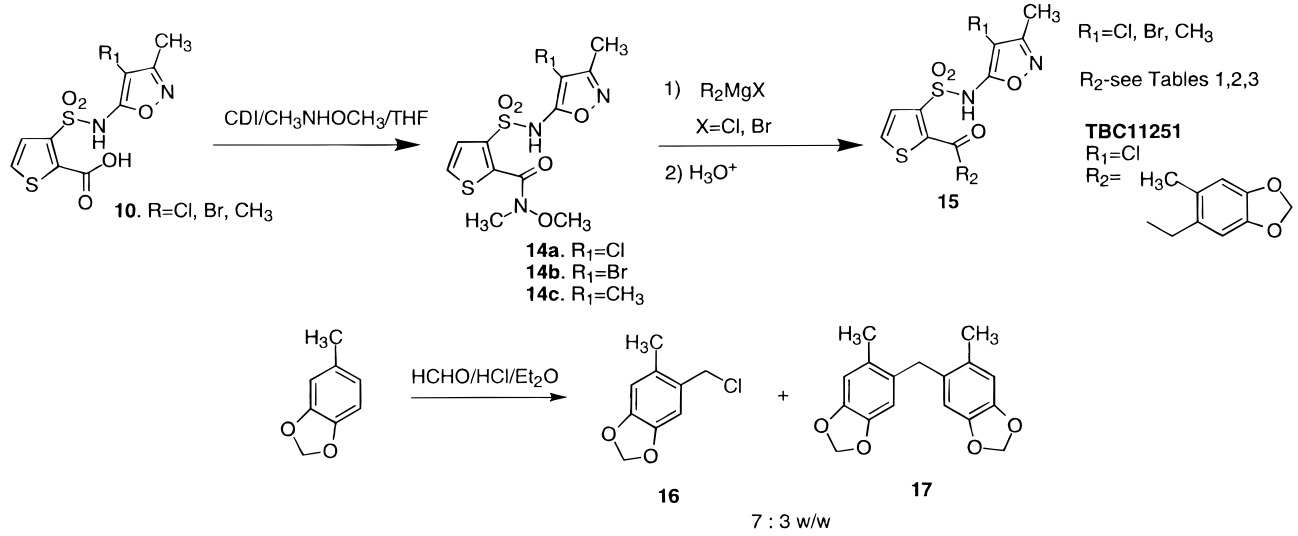


Figure 1. High-affinity amidothiophenesulfonamides ET_A receptor antagonists.

Scheme 2. General Synthesis of Ketosulfonamides



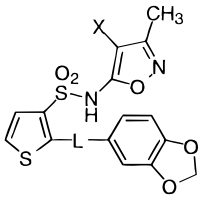
diate **15d** (Table 1) as well as **15q** (Table 3). 5-Methylbenzo[*d*][1,3]dioxole was treated with aqueous formaldehyde and concentrated hydrochloric acid in ethyl ether to give the desired benzyl chloride **16** and condensation product **17**. This mixture of **16** and **17** was used to form the Grignard reagent of **16** without separation.

In Scheme 3 an outline of the synthesis of several derivatives of ketone **15d** is given. Reduction using sodium borohydride gave alcohol **18**. Treatment of **15d** with hydroxylamine gave oxime **19**. Reductive amination using dimethylamine and sodium cyanoborohydride produced amine **20**. Ketalization under azeotropic conditions using ethylene glycol gave ketal **21**. Addition of methylmagnesium bromide to ketone **15d** gave alcohol **22**.

The synthesis of cyano ketones **28** and **29** is shown in Scheme 4.

5-Benzo[*d*][1,3]dioxolylacetic acid was esterified in the presence of 2,2-dimethoxypropane in acidic methanol.[3] The resulting ester **23** was brominated to give **24** which was further treated with cuprous cyanide in hot DMF to give the benzonitrile **25**.[4] The methyl ester of **25** was then exchanged to a *tert*-butyl ester by saponification, treatment with thionyl chloride, and reaction of the resulting acid chloride with *tert*-butyl alcohol to give **26**. The benzonitrile **26** was then deprotonated using sodium hydride and reacted with the acylimidazolide generated *in situ* by reacting acid **10** and carbonyldiimidazole. The intermediate *tert*-butyl ester **27** was concomittantly hydrolyzed and decarboxylated in warm acid to give the desired cyano ketone **28**. The methyl to *tert*-butyl ester exchange (**25**−**26**) was necessary because of difficulties in hydrolyzing the methyl ester in the coupled product: alkaline hydrolysis caused a retro-aldol reaction to give back acid **10**. Compound **29** was synthesized via carbonyldiimidazole-mediated coupling of acid **10** and the anion generated by reacting 5-(cyanomethyl)benzo[*d*][1,3]dioxole with sodium hydride.

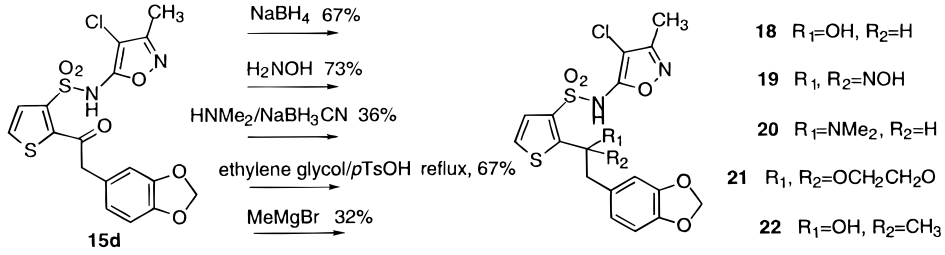
Table 1. Effect of Linker Groups on [^{125}I]Endothelin-1 Binding



			$IC_{50,}$[a] nM (*n*)	
entry	L	X	ET_A	ET_B
1	CONH	Br	19 ± 5 (5)	10100 ± 1500 (5)
2			15 ± 5 (2)	14100 ± 0 (2)
3			10 ± 2 (5)	32600 ± 1800 (5)
4			3.0 ± 0.7 (4)	355500 ± 112300 (4)
5			3.4 ± 0.4 (4)	40400 ± 6400 (5)
6			2.6 ± 0.5 (2)	14000 ± 2050 (2)
7			3.9 ± 0.6 (3)	12200 ± 1400 (3)
9	CONHCO	Br	887 ± 401 (2)	37200 ± 7800 (2)
11	$NHCO_2$	Br	88 ± 64 (3)	5900 ± 700 (3)
12	NHCONH	Br	574 ± 298 (3)	1300 ± 300 (3)
13	$CON(CH_2CH_2)_2NCH_2$	Cl	19600 ± 5400 (2)	>100000 (2)
15a	CO	Br	2000 ± 200 (2)	15400 ± 3300 (2)
15b	$COCH_2$	CH_3	27 ± 4 (2)	27200 ± 400 (2)
15c	$COCH_2$	Br	14 ± 10 (4)	5500 ± 500 (4)
15d	$COCH_2$	Cl	18 ± 6 (6)	7000 ± 960 (6)
18	$CH(OH)CH_2$	Cl	56 ± 16 (4)	15700 ± 2700 (4)
19	$C(NOH)CH_2$	Cl	1100 ± 50 (2)	4200 ± 500 (2)
20	$CH(N(CH_3)_2)CH_2$	Cl	21000 ± 1300 (2)	>100000 (2)
21	$C(OCH_2CH_2O)CH_2$	Cl	123 ± 13 (2)	2100 ± 70 (2)
22	$C(OH)(CH_3)CH_2$	Cl	22200 ± 10400 (2)	>100000 (2)
29	COCH(CN)	Cl	2100 ± 100 (2)	27300 ± 3000 (2)

[a] Values are means ± SEM for separate determinations.

Scheme 3. Derivatization of Ketone **15d**



Structure−Activity Relationships

In this paper we report the investigation of a series of linker groups as replacements for the amide group in compound **1** (Figure 1) in order to afford compounds with improved properties as oral endothelin antagonists. The results are summarized in Table 1. When the amide group was replaced by an imide group, the resultant compound **9** was more than 40-fold less active. Replacement of the amide group by a urethane (**11**) or ureido group (**12**) gave compounds that were 4- or 30-fold less active, respectively. Introduction of a long and bulky linker such as a piperazinylcarbonyl group (**13**) was very deleterious to activity, as was the inclusion of a very short linker such as a ketone group (**15a**). Therefore, it was concluded that the preferred linker should contain two carbon atoms, or an equivalent. Replacing the amide group with an acetyl group gave compound **15c**, which showed comparable activity to **1**, as did compounds **15b** and **15d**. We next investigated replacement of the carbonyl group with other functionality. Although a hydroxyl group replacement of the carbonyl (**18**) only reduced the activity by 2-fold and an ethylenedioxy ketal replacement of the carbonyl (**21**) lowered the potency by 6-fold, replacements with oxime (**19**), dimethylamino (**20**), and tertiary alcohol (**22**) all drastically decreased the activity. Substitution on the ketone α-carbon with a cyano group (**29**) was also deleterious. This study coupled with the observation that direct attachment of the aryl group to the thiophene or when the linker was methylene, ethylene, or vinylene, the activity was lost to various degrees[5] argued that an acetyl group was the best identified replacement of the amide group in compound **1**.

Replacement of the benzo[*d*][1,3]dioxole group with 4-methyl (**15f**), 4-methoxy (**15e**), and 3-methoxyphenyl groups (**15g**) gave slightly reduced activity (Table 2) compared to that of **15c** and **15d**. A series of ketones were synthesized to study the effects of substitution patterns of dimethyl groups. The rank order of potency seemed to be 2,4-dimethyl > 2,5-dimethyl > 3,5-dimethyl (Table 2). This exercise prompted us to synthesize **15q** and introduce the favorable 2-methyl substituent in the benzodioxole moiety (Table 3). The results of **15q**, the corresponding dimethylisoxazole **15p**, and the isomeric chloroisoxazolesulfonamide **15r** indicate that the 6-methylbenzo[*d*][1,3]dioxol-5-yl substitution rendered compounds of high potency as evidenced by **15q** with a pA_2 of 8.0 and its equipotent isomer **15r** with

Scheme 4. Syntheses of Cyano Ketosulfonamides

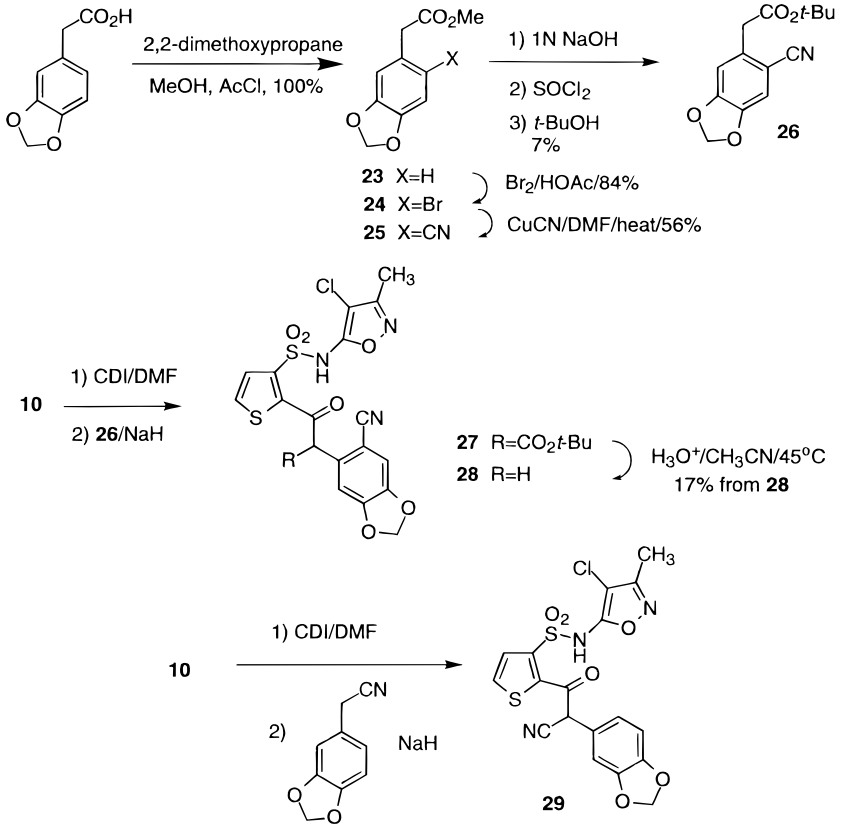
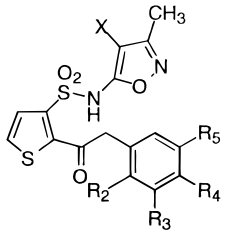


Table 2. Effect of Phenyl Substitution Patterns on [^{125}I]Endothelin-1 Binding

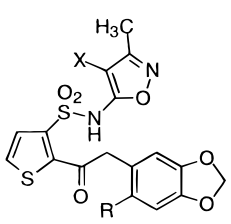


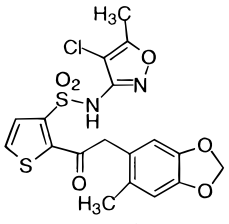
						IC_{50}, nM (*n*)	
entry	R_2	R_3	R_4	R_5	X	ET_A	ET_B
15e	H	H	OCH_3	H	Cl	43 ± 3 (2)	10100 ± 500 (2)
15f	H	H	CH_3	H	Br	20 ± 2 (2)	9100 ± 1900 (2)
15g	H	OCH_3	H	H	Cl	30 ± 18 (3)	19100 ± 1300 (3)
15h	H	H	CH_3	H	CH_3	27 ± 7 (2)	29200 ± 1400 (2)
15i	CH_3	H	CH_3	H	CH_3	2.7 ± 0.1 (2)	17400 ± 10800 (2)
15j	CH_3	H	CH_3	H	Cl	0.97 ± 0.22 (3)	4800 ± 2700 (3)
15k	CH_3	H	CH_3	H	Br	0.76 ± 0.13 (2)	3500 ± 2200 (2)
15m	H	CH_3	H	CH_3	Cl	45 ± 6 (2)	17700 ± 2600 (2)
15n	CH_3	H	H	CH_3	Cl	7.2 ± 0.6 (2)	18100 ± 2100 (2)

a pA_2 of 8.47. Comparisons showed that the methyl substitution at the 6-position increased potency by about 15-fold with an increase in pA_2 of 1.25. The same is true with a cyano group substitution with 1.95 higher pA_2 as suggested by the previous paper.

The general preference of ketones over their corresponding amides in terms of *in vivo* potency, serum half-life and oral bioavailability is demonstrated in Table 4. For the simple benzo[*d*][1,3]dioxole amide **1** or *p*-toluene amide **2**, no *in vivo* activity was observed in an ET-1 challenge rat model; the compounds also had very short half-lives and were not orally bioavailable at detectable levels. In contrast, the corresponding benzo[*d*][1,3]-dioxole ketone **15d** was active in the same model, had a moderate half-life, and was 30% orally bioavailable. Although the 6-position substituted benzo[*d*][1,3]dioxole amides **3** and **5** had acceptable durations of action and 8−27% oral bioavailability, the inhibition of blood pressure potentiation of **3**−**5** was only 8−18%. Amides **6** and **7** had quite high *in vivo* potency, but unfortunately they were metabolized rapidly and scarcely bioavailable. On the other hand, the corresponding ketones **15p**, **15q**, and **28** all had desirable *in vivo* potency, and **15p** and **15q** also had a long duration of action and were highly orally bioavailable. Compound **15r** and **28** were not further developed due to toxicity

Table 3. Effect of Isoxazole and Benzo[*d*][1,3]dioxole 6-Position Substitution on [^{125}I]Endothelin-1 Binding





15r

			IC_{50}, nM (*n*)		
entry	X	R	ET_A	ET_B	phosphoinositol hydrolysis pA_2
15p	CH_3	CH_3	3.3 ± 0.2 (2)	34300 ± 600 (2)	NT[a]
15q	Cl	CH_3	1.4 ± 0.5 (5)	9800 ± 4400 (5)	8.0
15r			0.63 ± 0.1 (2)	3900 ± 60 (2)	8.47
15d	Cl	H	21 ± 8 (5)	7200 ± 1200 (5)	6.75
28	Cl	CN	1.3 ± 0.3 (3)	723 ± 209 (3)	8.

[a] NT: not determined.

Table 4. Percent Inhibition in ET-1 Challenge, Serum Half-Life, and Oral Bioavailability in the Rat of High-Affinity Amide and Ketone ET_A Receptor Antagonists

entry	% inhibition of ET-1 challenge, 15 mg/kg (*n*)	$t_{1/2}$ (h)	oral bioavailability (%)
amides			
1	not active	<0.5	0
2	not active	<0.5	0
3 (TBC11192)	18 (4)	2.5−3.0	27
4	14 (3)	NT[a]	NT
5 (TBC11241)	8 (3)	2.5	8
6	33 (3)	<0.15	NT
7	50 (3)	<0.15	2
ketones			
15d	41 (6) 60 mg/kg	1.5	30
15p	33 (3)	7	100
15q (TBC11251)	41 (6)	6.7	60
28	52 (3)	NT	NT

[a] NT: not determined.

concerns and instability in alkaline aqueous media, respectively. It was concluded that **15q** was suitable for further pharmacological and clinical studies.

Pharmacokinetics and *in Vivo* Data

The pharmacokinetic data for **15q** is expressed as mean plasma concentration plotted against hours after dosing for rats (Figure 2) and dogs (Figure 3). Following bolus intravenous administration of **15q** to both rats and dogs, plasma levels of **15q** declined rapidly over the first 30−60 min and then more slowly over the remaining 24 h observation period. The $t_{1/2}$ for the terminal elimination phase was 5.9−7.5 h for the rat and 4−4.8 h for the dog. Orally administered **15q** was rapidly absorbed in both the rat and the dog with a $t_{1/2}$(abs) of 0.7 and 0.3 h, respectively. Peak plasma concentrations occurred between 2 and 3 h postdosing in the rat and between 45 and 90 min in the dog. Following oral administration the $t_{1/2,\text{elimination}}$ ranged from 2 to 5 h in rats and from 4 to 7 h in dogs. Oral bioavailability, calculated from comparison of administered dose and AUC, indicated that the material was 50−60% bioavailable in the rat and 90−100% bioavailable in the dog.

Compound **15q** has demonstrated efficacy in an *in vivo* model of acute hypoxia-induced pulmonary hypertension in rats by both iv and oral routes of administration with an EC_{50} of 0.5 and 1 mg/kg, respectively.[6]

Table 5. Synthetic and Physical Data

entry	synth method	% yield	mp, °C	formula[a]
1	B	15	138−140	$C_{16}H_{12}BrN_3O_6S_2$
9	C	4	90−93	$C_{17}H_{12}BrN_3O_7S_2$
11	D	29	39−43	$C_{16}H_{12}BrN_3O_7S_2 \cdot 0.3TFA$
12	D	12	62−65	$C_{16}H_{13}BrN_4O_6S_2 \cdot 0.1TFA$
13	B	54	221−223	$C_{21}H_{21}ClN_4O_6S_2$
15a	A	12	47−49	$C_{16}H_{11}BrN_2O_6S_2 \cdot 0.3EtOAc$
15b	A	65	152−157	$C_{18}H_{16}N_2O_6S_2$[b]
15c	A	40	oil	$C_{17}H_{13}BrN_2O_6S_2$[b]
15d	A	50	35−38	$C_{17}H_{13}ClN_2O_6S_2$
15e	A	2	35−38	$C_{17}H_{15}ClN_2O_5S_2 \cdot 0.2EtOAc$
15f	A	78	146−150	$C_{17}H_{15}BrN_2O_4S_2$[b]
15g	A	28	oil	$C_{17}H_{15}ClN_2O_5S_2$
15h	A	65	95−100	$C_{18}H_{18}N_2O_4S_2$[b]
15i	A	34	oil	$C_{19}H_{20}N_2O_4S_2$[b]
15j	A	52	48−54	$C_{18}H_{17}ClN_2O_4S_2$[b]
15k	A	28	58−63	$C_{18}H_{17}BrN_2O_4S_2$[b]
15m	A	57	45−50	$C_{18}H_{17}ClN_2O_4S_2$[b]
15n	A	33	72−76	$C_{18}H_{17}ClN_2O_4S_2$[b]
15p	A	14	45−50	$C_{19}H_{18}N_2O_6S_2$
15q	A	71	42−45	$C_{18}H_{15}ClN_2O_6S_2$
15r	A	16	46−50	$C_{18}H_{15}ClN_2O_6S_2$
18	E	67	38−41	$C_{17}H_{15}ClN_2O_6S_2 \cdot 0.6EtOAc$
19	F	73	142−145	$C_{17}H_{14}ClN_3O_6S_2 \cdot 0.2EtOAc$
20	G	36	56−59	$C_{19}H_{20}ClN_3O_5S_2 \cdot 1.6TFA$
21	H	67	60−63	$C_{19}H_{17}ClN_2O_7S_2$
22	J	32	39−41	$C_{18}H_{17}ClN_2O_6S_2$
28	K	17	105−108	$C_{18}H_{12}ClN_3O_6S_2 \cdot 0.2CH_3CN$
29	C	19	142−145	$C_{18}H_{12}ClN_3O_6S_2 \cdot 0.2TFA$

[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. Homogeneity established by two diverse analytical HPLC systems.

Furthermore, **15q** was shown to both prevent and reverse chronic hypoxia-induced pulmonary hypertension following oral administration at a dose of 11 mg/kg/day in the rat.[6]

Conclusion

In extension of the study of amidesulfonamides as ET_A selective endothelin receptor antagonists and to improve the profile of the compounds particularly regarding oral bioavailability and half-life, we investigated replacing the amide bond with various linker groups. This effort resulted in the identification of an acetyl group as the optimal spacer. Incorporation of data from the previous paper regarding the preference for a 2-methyl substituent on the benzodioxole group

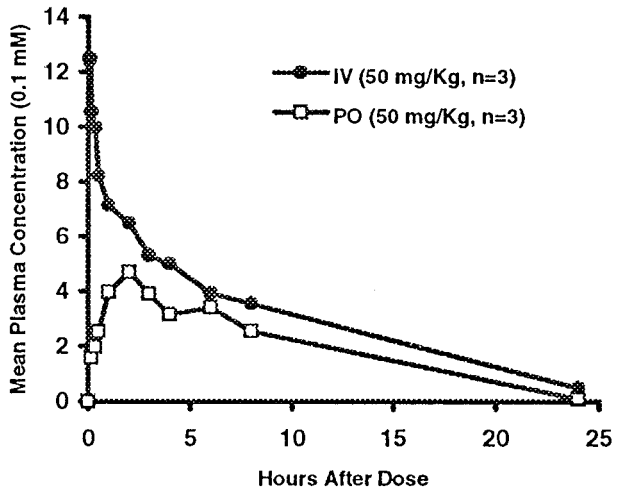


Figure 2. Pharmacokinetics of **15q** in rats.

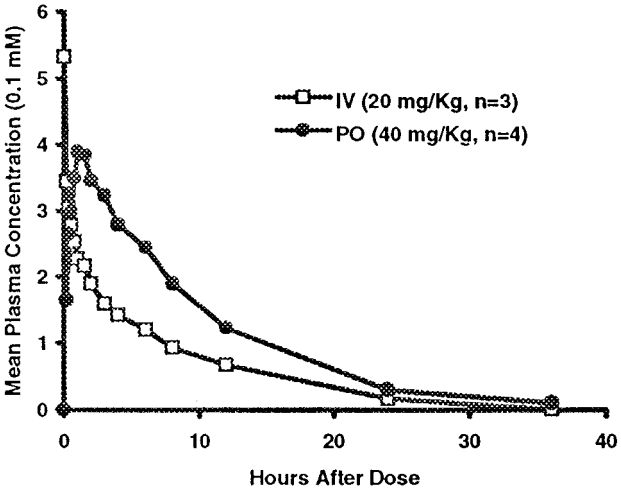


Figure 3. Pharmacokinetics of **15q** in beagle dogs.

led to the eventual discovery of **15q**, which retains high potency and selectivity for the ET_A receptor while having both oral bioavailability and long half-life. Results of clinical development will be reported in due course.

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, phosphomolybdic acid, or iodine vapor. Analytical HPLC was performed on a Vydac C18 column (4.6 × 250 mm); preparative HPLC, on a Dynamax-60A (83-241-c) with acetonitrile:water gradients containing 0.1% trifluoroacetic acid. The detection wavelength was 254 nm.

Method A. 4-Chloro-3-methyl-5-[[2-[(benzo[*d*][1,3]-dioxol-5-yl)acetyl]-3-thienyl]sulfonamido]isoxazole (15d). To a solution of acid **10a** (5.0 g, 15.49 mmol) in anhydrous THF (70 mL) was added 1,1′-carbonyldiimidazole (2.76 g, 17.04 mmol). The mixture was stirred at room temperature for 15 min before the sequential addition of imidazole (2.11 g, 30.98 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (2.31 g, 23.24 mmol). The mixture was stirred at room temperature for 4 h. THF was removed under reduced pressure and the residue partitioned between EtOAc and 1 N hydrochloric acid. The organic layer was dried over $MgSO_4$ and concentrated. The residue was treated with toluene and concentrated again. The residual red oil was dissolved in dry THF (20 mL) and treated at 0 °C with a Grignard reagent prepared from 5-(chloromethyl)benzo[*d*][1,3]dioxole (14.0 g, 82.02 mmol) and magnesium turnings (2.3 g, 95.69 mmol) in THF (100 mL). The mixture was stirred at 0 °C for 1 h and at room temperature for an additional 2 h. The reaction was quenched with a mixture of concentrated hydrochloric acid (20 mL) and methanol (50 mL) while cooling. After 10 min of stirring at 0 °C, the mixture was concentrated. The aqueous residue was partitioned between EtOAc and 1 N hydrochloric acid. The organic layer was dried over $MgSO_4$ and concentrated, and the residue was purified via preparative HPLC to give **15d** (3.0 g, 50% yield) as a yellow solid: mp 35−38 °C; ^{1}H NMR (DMSO-d_6) δ 7.89 (d, J = 5.1 Hz, 1H), 7.43 (d, J = 5.1 Hz, 1H), 6.84 (d, J = 7.8 Hz, 1H), 6.80 (s, 1H), 6.70 (d, J = 7.8 Hz, 1H), 5.98 (s, 2H), 4.44 (s, 2H), 2.09 (s, 3H).

Method B. *N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-{[4-(benzo[*d*][1,3]dioxol-5-ylmethyl)piperazin-1-yl]carbonyl}-thiophene-3-sulfonamide (13). To a solution of acid **10a** (1.0 g, 3.10 mmol) in anhydrous THF (20 mL) was added 1,1′-carbonyldiimidazole (603 mg, 3.72 mmol). The mixture was stirred at room temperature for 10 min before the addition of 1-piperonylpiperazine (2.0 g, 9.30 mmol). The mixture was stirred at room temperature for 4 h. THF was removed by evaporation, and the residue was partitioned between 1 N NaOH and a 1:1 mixture of EtOAc and ethyl ether. The aqueous layer was treated with saturated NH_4Cl to pH 7−8 and was then extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated, and the residue was purified via HPLC to give **13** (852 mg, 54% yield) as a white powder: mp 221−223 °C; ^{1}H NMR (DMSO-d_6) δ 9.61 (br s, 1H), 7.61 (d, J = 5.1 Hz, 1H), 7.18 (d, J = 5.1 Hz, 1H), 7.06 (s, 1H), 6.99 (m, 2H), 5.73 (s, 2H), 4.25−4.54 (m, 3H), 2.93-3.72 (m, 7H), 1.99 (s, 3H).

Method C. *N*-(4-Bromo-3-methyl-5-isoxazolyl)-2-[[(benzo[*d*][1,3]dioxol-5-ylcarbonyl)amino]carbonyl]thiophene-3-sulfonamide (9). 1,1′-Carbonyldiimidazole (213 mg, 1.31 mmol) was added to a solution of piperonylic acid (182 mg, 1.09 mmol) in dry THF (10 mL). The resulting mixture was stirred for 15 min. Compound **8** (400 mg, 1.09 mmol) and NaH (175 mg, 60% dispersion in mineral oil, 4.37 mmol) were added sequentially. The mixture was stirred at room temperature for 8 h. Water was added to destroy the excess NaH. The volatiles were then evaporated, and the residue was partitioned between EtOAc and 1 N hydrochloric acid. The organic layer was dried over $MgSO_4$ and concentrated. The residue was recrystallized from EtOAc to give **9** (20 mg, 3.6% yield) as a yellow powder: mp 90−93 °C; ^{1}H NMR (DMSO-d_6) δ 12.50 (br s, 1H), 7.86 (d, J = 5.3 Hz, 1H), 7.80 (dd, J = 8.4, 1.5 Hz, 1H), 7.68 (d, J = 1.5 Hz, 1H), 7.40 (d, J = 5.3 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 6.15 (s, 2H), 1.95 (s, 3H).

Compound **29** was synthesized by the same method as **9** except that acid **10a** was used instead of piperonylic acid, and 5-(cyanomethyl)benzo[*d*][1,3]dioxole was used instead of **8**: yellow powder; mp 142−145 °C; ^{1}H NMR (DMSO-d_6) δ 7.89 (d, J = 5.4 Hz, 1H), 7.43 (d, J = 5.4 Hz, 1H), 7.30 (d, J = 1.2 Hz, 1H), 7.12 (dd, J = 8.4, 1.2 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 6.05 (s, 2H), 2.11 (s, 3H).

Method D. *N*-(4-Bromo-3-methyl-5-isoxazolyl)-2-[[(benzo[*d*][1,3]dioxol-5-yloxy)carbonyl]amino]thiophene-3-sulfonamide (11). Triethylamine (2.28 mL, 16.35 mmol) and diphenyl phosphorazidate (773 mg, 2.72 mmol) were sequentially added to a solution of acid **10b** (1.0 g, 2.72 mmol) in anhydrous THF (40 mL). The mixture was stirred for 8 h. Sesamol (1.54 g, 10.9 mmol) was added, and the mixture was heated under reflux for 2 h and then allowed to cool to room temperature. The solvent was removed by evaporation, and the residue was partitioned between EtOAc and 1 N hydrochloric acid. The organic layer was concentrated and purified by HPLC to give **11** (400 mg, 29% yield) as a beige powder: mp 39−43 °C; ^{1}H NMR (DMSO-d_6) δ 9.94 (br s, 1H), 7.14 (d, J = 5.7 Hz, 1H), 6.94−7.03 (m, 3H), 6.74 (dd, J = 8.3, 2.1 Hz, 1H), 6.08 (s, 2H), 2.10 (s, 3H).

Compound **12** was synthesized by the same method as **11** except that 5-aminobenzo[*d*][1,3]dioxole was used instead of sesamol: brown/gray powder; 62−65 °C; ^{1}H NMR (DMSO-d_6) δ 10.10 (br s, 1H), 9.41 (br s, 1H), 7.17 (d, J = 1.2 Hz, 1H), 7.00 (d, J = 5.8 Hz, 1H), 6.95 (d, J = 5.8 Hz, 1H), 6.82−6.88 (m, 2H), 5.99 (s, 2H), 2.11 (s, 3H).

Method E. *N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-[1-hydroxy-2-(benzo[*d*][1,3]dioxol-5-yl)ethyl]thiophene-3-sulfonamide (18). Lithium borohydride (36.6 mg, 1.68 mmol) was added slowly to a solution of **15d** (74 mg, 0.17 mmol) in THF (10 mL). The resulting mixture was stirred for 8 h. Saturated NH_4Cl(aq) was added to quench the excess lithium borohydride, and the resulting mixture was concentrated by evaporation. The residue was partitioned between EtOAc and 1 N hydrochloric acid, the organic layer was dried over $MgSO_4$ and concentrated to give **18** (67 mg, 67% yield) as a yellow powder: mp 38−41 °C; ^{1}H NMR (DMSO-d_6) δ 7.54 (d, J = 7.2 Hz, 1H), 7.22 (d, J = 7.2 Hz, 1H), 6.80 (d, J = 8.1 Hz, 1H), 6.78 (s, 1H), 6.64 (s, 1H), 5.96 (s, 2H), 5.41 (m, 1H), 2.90 (m, 1H), 2.60 (m, 1H), 2.12 (s, 3H).

Method F. *N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-[1-(hydroxyimino)-2-(benzo[*d*][1,3]dioxol-5-yl)ethyl]thiophene-3-sulfonamide (19). To a mixture of **15d** (100 mg, 0.23 mmol) and hydroxylamine hydrochloride (160 mg, 2.3 mmol) was added water (5 mL). To this stirred heterogeneous mixture was then added NaOH pellets (320 mg, 8.0 mmol). The mixture was stirred until it became homogeneous, heated at 80 °C for 10 min, allowed to cool to room temperature, and was then poured onto ice mixed with 1.5 mL of concentrated hydrochloric acid in 100 mL of water. The resulting white precipitate was filtered and dried *in vacuo* to give **19** (75 mg, 73% yield) as a yellow powder: mp 142−145 °C; ^{1}H NMR (DMSO-d_6) δ 11.80 (br s, 1H), 7.59 (d, J = 5.4 Hz, 1H), 7.31 (d, J = 5.4 Hz, 1H), 6.75 (d, J = 7.8 Hz, 1H), 6.61 (d, J = 0.9 Hz, 1H), 6.55 (dd, J = 7.8, 0.9 Hz, 1H), 5.94 (s, 2H), 4.03 (s, 2H), 2.14 (s, 3H).

Method G. *N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-[1-(dimethylamino)-(benzo[*d*][1,3]dioxol-5-yl)ethyl]thiophene-3-sulfonamide (20). To a suspension of dimethylamine hydrochloride (420 mg, 5.15 mmol) in methanol (1.7 mL) was added KOH (170 mg, 3.04 mmol). The suspension was stirred until a homogeneous milky mixture formed. Following the addition of **15d** (100 mg, 0.23 mmol), the mixture was stirred at room temperature for 30 min before the addition of sodium cyanoborohydride (240 mg, 3.82 mmol). The resulting mixture was stirred at room temperature for 1 h before it was quenched with acetic acid. The crude mixture was then purified by HPLC to give **20** (38 mg, 36% yield) as a white powder: mp 56−59 °C; ^{1}H NMR (DMSO-d_6) δ 9.53 (br s, 1H), 7.67 (d, J = 5.1 Hz, 1H), 7.28 (d, J = 5.1 Hz, 1H), 6.81 (br s, 1H), 6.70 (m, 2H), 5.93 (s, 2H), 5.64 (m, 1H), 3.50 (m, 1H), 3.20 (m, 1H), 2.85 (s, 6H), 1.99 (s, 3H).

Method H. *N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-[2-(benzo[*d*][1,3]dioxol-5-ylmethyl)-1,3-dioxol-2-yl]thiophene-3-sulfonamide (21). To a 100 mL round bottom flask were sequentially added **15d** (100 mg, 0.23 mmol), *p*-toluenesulfonic acid monohydrate (140 mg, 0.74 mmol), ethylene glycol (10 mL), and benzene (15 mL). The resulting mixture was heated under reflux while water was removed azeotropically for 7 h. The mixture was allowed to cool to room temperature and partitioned between benzene and water. The organic layer was dried over $MgSO_4$ and concentrated to give **21** (74 mg, 67% yield) as a white powder: mp 60−63 °C; ^{1}H NMR (DMSO-d_6) δ 7.91 (br s, 1H), 7.30 (d, J = 5.4 Hz, 1H), 7.13 (d, J = 5.4 Hz, 1H), 6.80 (br s, 1H), 6.70 (m, 2H), 5.92 (s, 2H), 3.89−4.05 (m, 4H), 3.55 (s, 2H), 2.24 (s, 3H).

Method J. *N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-[2-hydroxy-2-(benzo[*d*][1,3]dioxol-5-ylmethyl)-2-propyl]thiophene-3-sulfonamide (22). To a solution of **15d** (100 mg, 0.23 mmol) in anhydrous THF at −78 °C was added methylmagnesium bromide (378 μL, 3 M in Et_2O). The resulting mixture was stirred at −78 °C for 10 min before it was allowed to warm to 0 °C and was quenched with saturated NH_4Cl(aq). The mixture was worked up as usual to give **22** (33 mg, 32% yield) as a pink/orange powder: mp 39−41 °C; ^{1}H NMR ($CDCl_3$) δ 8.85 (br s, 1H), 7.56 (d, J = 3.0 Hz, 1H), 7.21 (d, J = 3.0 Hz, 1H), 6.74 (m, 1H), 6.56 (m, 2H), 5.94 (s, 2H), 3.32 (d, 1H), 3.14 (d, 1H), 2.17 (s, 3H), 1.71 (s, 3H).

Method K. *N*-(4-Chloro-3-methyl-5-isoxazolyl)-2-[(6-cyanobenzo[*d*][1,3]dioxol-5-yl)acetyl]thiophene-3-sulfonamide (28). Compound **28** was synthesized in the same fashion as for **9** (method C) except that acid **10a** was used instead of piperonylic acid, and **26** was used instead of **8**. The crude mixture was poured into a 2:2:1 mixture of acetonitrile/water/concentrated hydrochloric acid, and the resulting mixture was heated at 40 °C for 12 h. Acetonitrile was then removed by evaporation, and the aqueous residue was partitioned between EtOAc and 1 N hydrochloric acid. The organic layer was concentrated and purified by HPLC to give **28** in 17% yield as a light dull yellow powder: mp 105−108 °C; ^{1}H NMR (DMSO-d_6) δ 7.95 (d, J = 5.1 Hz, 1H), 7.47 (d, J = 5.1 Hz, 1H), 7.42 (s, 1H), 7.04 (s, 1H), 6.17 (s, 2H), 4.83 (s, 2H), 2.06 (s, 3H).

5-(Chloromethyl)-6-methylbenzo[*d*][1,3]dioxole (16). To a mixture of ether (100 mL) and concentrated hydrochloric acid (100 mL) at 0 °C were sequentially added 5-methylbenzo[*d*]-[1,3]dioxole (10 mL, 81 mmol) and formaldehyde (30% in water, 20 mL, 267 mmol). The mixture was stirred at 0 °C for 1 h and at room temperature for 4 h. The mixture was diluted with ether (100 mL), and the organic layer was separated, dried ($MgSO_4$), and then concentrated. The solid residue was heated with hexanes (100 mL), and the insolubles were filtered off. The filtrate was concentrated to give a mixture of **16** and **17** (7:3 by weight with 13 g combined yield) as a white solid. **16**: ^{1}H NMR ($CDCl_3$) δ 6.80 (s, 1H), 6.68 (s, 1H), 5.92 (s, 2H), 4.55 (s, 2H), 2.34 (s, 3H). **17**: δ 6.68 (s, 2H), 6.40 (s, 2H), 5.90 (s, 4H), 3.70 (s, 2H), 2.18 (s, 6H).

Methyl (6-Bromobenzo[*d*][1,3]dioxol-5-yl)acetate (24). To a solution of **23** (5 g, 25.8 mmol) in acetic acid (15 mL) was added dropwise bromine until a red-brown color persisted. After being stirred at room temperature for 30 min, the reaction mixture was partitioned between water (200 mL) and ether (200 mL). The organic layer was washed with water (3 × 200 mL), dried over $MgSO_4$, and concentrated to give **24** (5.9 g, 84% yield) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.02 (s, 1H), 6.76 (s, 1H), 5.98 (s, 2H), 3.71 (s, 3H), 3.69 (s, 2H).

***tert*-Butyl (6-Cyanobenzo[*d*][1,3]dioxol-5-yl)acetate (26).** To a solution of **25** (5g, 18.32 mmol) in methanol (100 mL) was added 1 N NaOH (50 mL). The reaction mixture was stirred at room temperature for 1.5 h, and methanol was removed by evaporation. The aqueous residue was acidified with concentrated hydrochloric acid to pH ∼1 and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to give a solid. The solid was treated with thionyl chloride (50 mL), and the mixture was heated under reflux for 10 min before the volatiles were removed by evaporation. The residue was dissolved in dichloromethane (15 mL) and added dropwise to a solution of 2-methyl-2-propanol (6.8 g, 91.6 mmol) and triethylamine (9.3 g, 91.6 mmol) in dichloromethane (100 mL) at 0 °C. The reaction was stirred at room temperature for 2 h. The mixture was washed with water (3 × 150 mL), and the organic layer was dried over $MgSO_4$ and concentrated to give **26** (335 mg, 7% yield) as a solid.

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 then the pellet 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.[7,8]

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 2 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 4 °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 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 (Challenge). The degree in inhibition was calculated as

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

Pharmacokinetic Assays. All surgical procedures were performed under aseptic conditions.

Rats. Adult Sprague−Dawley rats were anesthetized with a ketamine-based anaesthetic (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, 20−50 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.

Dogs. Under aseptic conditions three male beagle dogs (8−10 kg) underwent chronic cannulation. Catheters were introduced into the thoracic aorta via the carotid artery and into the superior vena cava via the right jugular veins. Catheters were exteriorized between the scapulae, capped, and secured under a mesh jacket. Animals were allowed to recover for 7 days prior to receiving **15q** either intravenously, 1 mL/kg via the right saphenous vein, or by oral gavage (2 mL/kg followed by an aqueous flush). Animals receiving drug by gavage were fasted overnight prior to drug administration. Seven days later animals receiving **15q** intravenously were dosed with **15q** by oral gavage and vice versa. In all cases serial blood samples were collected via the jugular cannula catheter over the next 24 h following dosing. Samples were stored frozen until assayed using an HPLC based assay.

Supporting Information Available: ^{1}H NMR, IR, and high-resolution mass spectral data for compounds **1**, **9**, **11**−**13**, **15a**−**r**, **18**−**22**, **28** and **29** (6 pages). Ordering information is given on any current masthead page.

References

(1) Presented in part: Wu, C.; Raju, B.; Okun, I.; Stavros, F.; Chan, M. F. 2-Benzylcarbonylthiophene-3-sulfonamides Are Orally Active ET_A Selective Endothelin Antagonists. The 211th ACS National Meeting, New Orleans, LA, March 24−28, 1996, Abstract MEDI 148.
(2) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815−1818.
(3) Rachele, J. R. *J. Org. Chem.* **1963**, *28*, 2898.
(4) Friedman, L.; Shechter, H. *J. Org. Chem.* **1961**, *26*, 2522−2524.
(5) Raju, B.; Okun, I.; Stavros, F.; Chan, M. F. Amide Bond Surrogates: a Study in Thiophenesulfonamides Endothelin Antagonists. The 211th ACS National Meeting, New Orleans, LA, March 24−28, 1996, Abstract MEDI 147.
(6) Chen, S. J.; Brock, T.; Stavros, F.; Okun, I.; Wu, C.; Chan, F.; Mong, S.; Dixon, R. A. F.; Oparil, S.; Chen, Y. F., Experimental Biology '96, Washington, DC, April 14−17, 1996.
(7) Stavros, F. D.; Hasel, C. W. O.; Okun, I.; Baldwin, J.; Freriks, K. COS-7 Cells Stable Transfected to Express the Human ET-B Receptor Provide a Useful Screen for Endothelin Receptor Antagonists. *J. Cardiovasc. Pharmacol.* **1993**, 22 (suppl. 8).
(8) Berridge, J. M.; Downes, C. P.; Hanley, M. R. *Biochem. J.* **1982**, *206*, 587−595.

JM9700068