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The Discovery and Structure–Activity Relationships of Nonpeptide, Low Molecular Weight Antagonists Selective for the Endothelin ET_B receptor[1]

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Abstract—The systematic modification of the ET_A selective *N*-(5-isoxazolyl)benzene-sulfonamide endothelin antagonists to give ET_B selective antagonists is reported. The reversal in selectivity was brought about by substitution of the 4-position with aryl and substituted aryl groups. Of all the aromatic substituents studied, the *para*-tolyl group gave rise to the most active and selective ET_B antagonist. Larger substituents caused a decrease in both ET_B activity and selectivity. A similar trend was observed by substitution at the 5-position of the *N*-(5-isoxazolyl)-2-thiophenesulfonamide ET_A receptor antagonists. The *para*-tolyl group was again found to be optimal for the ET_B activity and selectivity. The structural features that were found to be favorable for binding to the ET_B receptor, that is, the presence of a linear, conjugated π-system of definite shape and size, have been successfully incorporated into the design of ET_B selective polycyclic aromatic sulfonamides antagonists.

Introduction

Research on the endothelins (ET-1, ET-2, and ET-3), a family of 21-residue peptides with two disulfide bonds, has progressed rapidly since their discovery in 1988.[2] Apart from being one of the most potent vasoconstrictors known,[2] these peptides have wide-ranging biological effects including vasodilation, inotropism, modulation of nervous functions, and mitogenesis.[3,4] It is, therefore, not surprising to find that the endothelins are implicated in the pathophysiology of a number of diseases, including acute renal failure, acute hypertensive crisis, immunosuppressant-induced renal failure and pulmonary hypertension.[3]

The biological effect of endothelin is mediated by cell surface receptors. Currently two subtypes, ET_A and ET_B, have been identified through pharmacological characterization and cloning of the individual receptors.[5,6] Evidence for the presence of a third subtype of the endothelin receptor in non-mammalian tissues, tentatively named ET_C, has also been implicated.[7] ET_A receptors have a high affinity for ET-1 whereas ET_B receptors have high affinities for all the endothelin peptides and the structurally related snake venom peptide, sarafotoxin 6c.[8] Endothelin receptors are widely distributed in the body and there is strong evidence that the tissue distribution of these receptors is highly species dependent. The presence of receptor subtypes and their specific distribution in different tissues offer a unique opportunity for the therapeutic intervention of disease states by endothelin antagonists. Although many

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endothelin antagonists are known, they are either ET_A selective such as BQ-123,[9] FR 139317,[10] BMS-182874[11] and a closely related compound, 5-(dimethylamino)-*N*-(5-chloro-3-methoxy-2-pyrazinyl)-1-naphthalenesulfonamide,[12] PD 156707,[13] A-127722,[14] LU 135252 (also known as LU 127043),[15] TBC-11251[16] or nonselective such as Ro 46-2005,[17] Bosentan (Ro 47-0203),[18] SB 209670,[19] PD 142893,[20] and L-749,329.[21] ET_B selective antagonists, on the other hand, are relatively rare. The only known examples are IRL-1038,[22] BQ-788[23] and RES-701-1[24] which are all peptidic in nature and the recently discovered nonpeptide compound Ro 46-8443.[25] We[26] and others[27] have reported that the *N*-(isoxazolyl)benzenesulfonamides such as sulfisoxazole (**1**, Figure 1) possess endothelin antagonist activity through a pharmacophore directed screening approach. Our optimization of this series of compounds led to a class of potent ET_A selective 2,5-disubstituted benzenesulfonamide antagonists such as TBC-10662 (**2**).[28] In the course of this work, we observed that the *para*-substituted benzenesulfonamides tend to be ET_B selective even though the *ortho*-isomers have a slight preference for the ET_A receptor. In this paper, we describe the detailed study of the factors favoring ET_B selectivity which led to the discovery of several classes of sulfonamides including the 4-biphenylsulfonamides and the 5-aryl-2-thiophenesulfonamides as potent, nonpeptide ET_B selective antagonists.

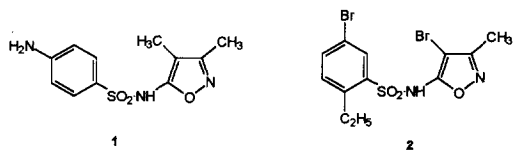
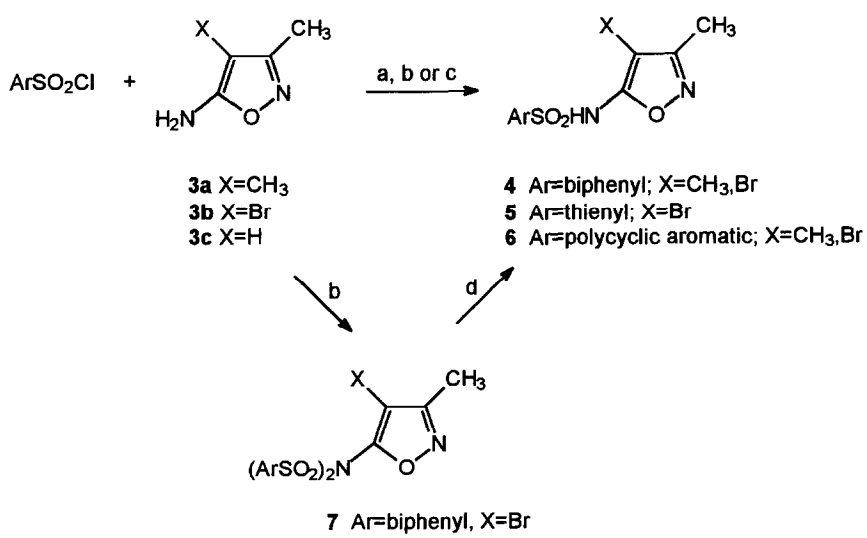


Figure 1.

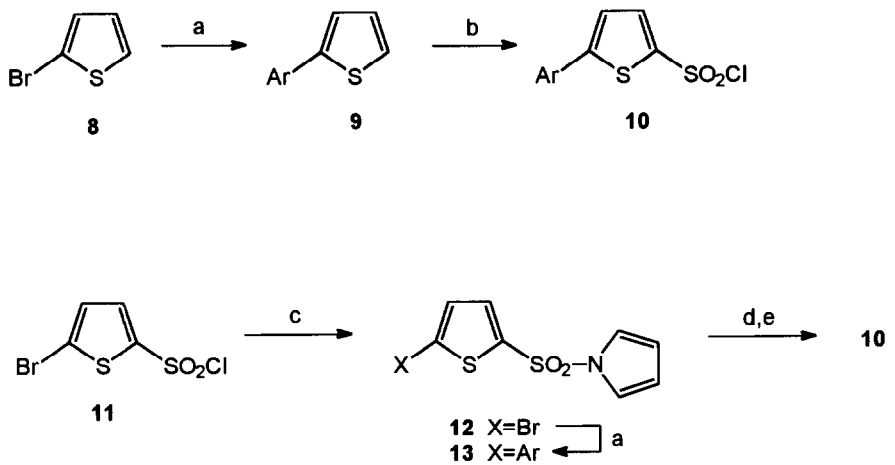
Chemistry

The 5-isoxazolylsulfonamides were synthesized from the corresponding sulfonyl chlorides and 5-aminoisoxazole using pyridine as a solvent (Scheme 1, condition a; compounds **4a–b, 4g, 5a, 5e–l, 6a**). The addition of *N,N*-dimethylaminopyridine as a catalyst was found to be beneficial (Scheme 1, condition b; compounds **4c, 6b, 6d–e**). In the case of **4f**, the sulfonimide **7** resulting from the reaction of two molecules of the sulfonyl chloride with one molecule of aminoisoxazole was formed as the sole product. The sulfonimide **7** was easily hydrolyzed to the corresponding sulfonamide by heating with sodium hydroxide in aqueous THF. For the less reactive 5-amino-4-bromoisoxazole **3b**, deprotonation with sodium hydride prior to coupling with sulfonyl chlorides was necessary to ensure good yields (Scheme 1, condition c; compounds **4d–e, 5a–k, 5q–u, 5w–y, 6c**, and **6f**).[16]

The sulfonyl chlorides used in the preparation of the sulfonamides discussed here were either commercially available or were synthesized from the corresponding sulfonic acid or sulfonate salt with $PCl_5/POCl_3$. 5-Aryl-2-thiophenesulfonyl chlorides (**10**) were prepared by chlorosulfonation of the parent hydrocarbon (**9**) with chlorosulfonic acid, followed by reaction with $PCl_5/POCl_3$. When the parent hydrocarbon was not commercially available, it could be synthesized from the Suzuki coupling[29] of 2-bromothiophene (**8**) with an appropriate aryl boronic acid. Alternatively, the Suzuki coupling could be performed on *N*-(5-bromo-2-thio-



Scheme 1. General synthesis of *N*-isoxazolylsulfonamides. (a) Pyridine; (b) pyridine, *N,N*-dimethylaminopyridine (cat.); (c) NaH. THF, 0–25°C; (d) NaOH, THF/H_2O, 45°C.



Scheme 2. Synthesis of 5-Aryl-2-thiophenesulfonyl chlorides. (a) $ArB(OH)_2$, EtOH, C_6H_6, 2M Na_2CO_3, $Pd(Ph_3P)_4$ (cat.), 80°C; (b) $ClSO_3H$, PCl_5, $POCl_3$; (c) pyrrole, NaH, THF, 25°C; (d) NaOH, MeOH, reflux; (e) PCl_5, $POCl_3$, 25°C.

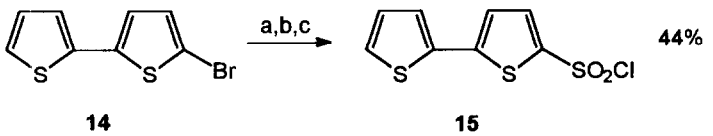
phenesulfonyl)pyrrole (**12**), a protected form of the bromosulfonyl chloride **11**, to give the coupling product **13**. The sulfonyl chloride was then regenerated by hydrolysis of **13** to the sulfonate salt with refluxing aqueous NaOH and subsequent conversion of the sulfonate salt to the corresponding sulfonyl chloride by means of $POCl_3/PCl_5$ (Scheme 2). (2,2′-Bithiophene)-5-sulfonyl chloride (**15**) used in the preparation of **5r** was synthesized by the lithium–bromine exchange of 5-bromo-(2,2′-bithiophene)[30] (**14**) with *t*-BuLi followed by the reaction of the anion with SO_2 and *N*-chlorosuccinimide[31] (Scheme 3).

4-Biphenylsulfonamides without a bromine substituent in the isoxazole ring were synthesized by the Suzuki coupling[29] of an *N*-(5-isoxazolyl)-4-bromophenylsulfonamide with a suitable aryl boronic acid. For *N*-(4-bromo-5-isoxazolyl)sulfomamides **4h–m**, competing reaction of the 4-bromo substituents on the isoxazole necessitates that the Suzuki coupling be performed with the *N*-(3-unsubstituted-5-isoxazolyl) compound **16**. The bromine atom was subsequently introduced by reaction with *N*-bromosuccinimide (Scheme 4).[16,32]

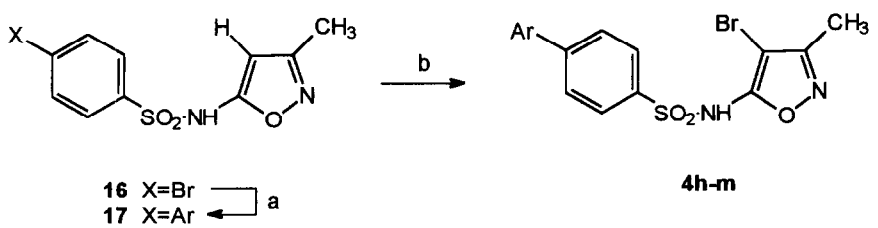
Purification of the sulfonamides was generally achieved by crystallization or by column chromatography on silica gel using $MeOH/CHCl_3$ as eluent followed by recrystallization. For noncrystalline sulfonamides, preparative HPLC was the method of choice.

Pharmacology

Radioreceptor binding studies were carried out at 4 °C with either TE 671 (ATCC # CRL 8803) cell membrane,



Scheme 3. (a) *t*-BuLi, Et_2O/pentane, −78°C, 20 min; (b) SO_2, −78°C; (c) NCS/THF, −78°C to room temp.



Scheme 4. Syhthesis of *N*-(4-bromo-3-methyl-5-isoxazoyl)biphenylsulfonamides. (a) $ArB(OH)_2$, EtOH, C_6H_6, 2M Na_2CO_3, $Pd(Ph_3P)_4$ (cat.), 80°C; (b) NBS, CH_2Cl_2, 0°C.

containing human ET_A receptors or stably transfected COS 7 cell membrane, containing human ET_B receptors. Phosphoinositide hydrolysis experiments were carried out with transfected COS 7 cells at 37 °C. Details of these experiments have been described in previous publications.[16,26,33]

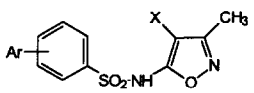
Results and Discussion

4-Biphenylsulfonamides. During the course of studying the benzenesulfonamide series of endothelin receptor antagonists, the effects of *ortho*-, *meta*-, and *para*-substitution were examined.[28] In general, the *para*-substituted compounds displayed higher *selectivity* to the ET_B receptor than the corresponding *ortho*- or *meta*-substituted derivatives. This ET_B selectivity seemed to result from a decreasing affinity for the ET_A and an increasing affinity for the ET_B receptor as one goes from the *ortho*- to the *para*-isomer. The size of the substituents, rather than their electronic nature, was determined to be critical in enhancing the ET_B affinity and selectivity. Whilst the effect was modest for small substituents such as the halogens, it became more pronounced for larger substituents such as phenyl. The dramatic effects of the substitution position on the ET_B affinity and selectivity was demonstrated in a study of the isomeric biphenylsulfonamides (Table 1). Whereas the 2-biphenylsulfonamides (**4a** and **4d**) were highly active and selective for the ET_A receptor, the 3-isomers (**4b** and **4e**) were nonselective and the 4-isomers (**4c** and **4f**) actually ET_B selective. However, further increase in the size of the *para* substituent to the phenylethynyl group in **4g** resulted in a significant erosion of both activity and selectivity, indicating that the receptor can only accommodate groups intermediate in size between **4c** and **4g** (vide infra). The previously observed activity enhancement effect of a 4-bromo substituent in the isoxazole ring was also observed in these biphenylsulfonamides.[32] Thus, the *N*-(4-bromo-3-methyl-5-isoxazolyl)-biphenylsulfonamides **4d–f** were found to be four to seven times more active than the corresponding *N*-(3,4-dimethyl-5-isoxazolyl)biphenylsulfonamides **4a–c**.

In our previous study of the benzenesulfonamide endothelin antagonists, we observed that the ET_B activity and selectivity decreased with di- and tri-substitution at various positions of the benzene ring.[28] This, coupled with the fact that *ortho* and *meta* substitution also dramatically decreased the ET_B selectivity, led us to focus our attention on the distal rather than proximal benzene ring of the 4-biphenylsulfonamides for optimizing the ET_B activity. Activities of the 2′-methoxy (**4j**), 3′-methoxy (**4k**) and 4′-methoxy (**4l**) series of compounds indicated that the 4′-substituted 4-biphenylsulfonamides has the highest affinity for the ET_B receptor. Of the 4′-substituted-4-biphenylsulfonamides studied (**4h–i, 4l**), 4-(4-tolyl)benzenesulfonamide (**4h**) showed the highest ET_B binding affinity (17 nM) and selectivity ($ET_A/ET_B = 290$). A 3′,4′-disubstituted biphenylsulfonamide (**4m**) was also synthesized but its activity was only modest (Table 2).

5-Aryl-2-thiophenesulfonamides. Our initial success with the 4-biphenylsulfonamide series of ET_B selective antagonists prompted us to study other aromatic sulfonamides. We focused our attention on the 5-aryl-2-thiophenesulfonamides due to the isosteric nature of benzene and thiophene[34] and the fact that some thiophenesulfonamides were found to be potent ET_A receptor antagonists.[16,35–39] The parent compound of the series, 5-phenyl-2-thiophenesulfonamide (**5a**), was synthesized and found to be moderately ET_B selective. We were particularly encouraged by the activity (0.42 μM) and selectivity (8.4) of **5a**, which were similar to those of the corresponding biphenylsulfonamide **4f**. As observed in the 4-biphenylsulfonamide series, the position of the substituent(s) in the benzene ring could have a dramatic

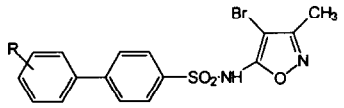
Table 1. Isomeric biphenyl sulfonamide endothelin antagonists



Compd	Ar	X	ET_A binding IC_{50}, μM (n)	ET_B binding IC_{50}, μM (n)	ET_B selectivity[a]
4a	2-Ph	CH_3	0.0083 ± 0.0013 (2)	55 (1)	0.00015
4b	3-Ph	CH_3	0.58 ± 0.05 (2)	4.8 ± 1.4 (3)	1.2
4c	4-Ph	CH_3	9.9 ± 2.0 (4)	0.63 ± 0.3 (3)	16
4d	2-Ph	Br	0.0014 ± 0.0005 (2)	7.9 ± 1.5 (3)	0.00018
4e	3-Ph	Br	0.12 ± 0.01 (2)	1.1 ± 0.3 (3)	0.11
4f	4-Ph	Br	3.4 ± 1.4 (5)	0.14 ± 0.05 (4)	24
4g	4-(Ph-C≡C-)	CH_3	73 ± 45 (3)	42 ± 17 (3)	1.7

[a]ET_B selectivity = $(IC_{50}\ ET_A)/(IC_{50}\ ET_B)$.

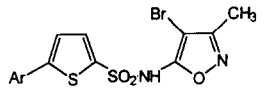
Table 2. Substituent effects on the 4-biphenylsulfonamide ET_B selective antagonists



Compd	R	ET_A binding IC_{50}, μM (n)	ET_B binding IC_{50}, μM (n)	ET_B selectivity[a]
4f	H	3.4 ± 1.4 (5)	0.14 ± 0.05 (4)	24
4h	4-CH_3	4.9 + 2.7 (2)	0.017 ± 0.009 (3)	290
4i	4-CF_3	5.4 ± ± 0.4 (2)	0.083 ± 0.02 (3)	65
4j	2-CH_3O	3.3 ± 2.4 (2)	0.41 ± 0.1 (2)	8
4k	3-CH_3O	4.9 ± 2.4 (2)	0.66 ± 0.18 (2)	7.4
4l	4-CH_3O	15 ± 4 (2)	1.2 ± 0.3 (2)	13
4m	3,4-(OCH_2O)	ND	0.56 ± 0.17 (2)	—

[a]ET_B selectivity = (IC_{50} ET_A)/(IC_{50} ET_B).

Table 3. Substituent effects on the 5-arylthiophene sulfonamide endothelin antagonists



Compd	Ar	ET_A binding IC_{50}, μM (n)	ET_B binding IC_{50}, μM (n)	ET_B selectivity[a]
5a	C_6H_5	3.5 ± 0.5 (3)	0.42 ± 0.13 (3)	8.4
5b	2-$CH_3C_6H_4$	0.43 ± 0.15 (2)	0.31 ± 0.05 (2)	1.4
5c	3-$CH_3C_6H_4$	ND	0.68 (1)	—
5d	4-$CH_3C_6H_4$	7.1 ± 0.3 (3)	0.036 ± 0.005 (3)	200
5e	4-$CF_3C_6H_4$	6.4 ± 0.4 (2)	0.084 ± 0.02 (3)	76
5f	2-$OCH_3C_6H_4$	0.38 ± 0.26 (2)	1.1 ± 0.2 (2)	0.35
5g	3-$OCH_3C_6H_4$	3.3 ± 0.2 (2)	0.78 ± 0.14 (2)	4.2
5h	4-$OCH_3C_6H_4$	8.7 ± 2.3 (2)	0.36 ± 0.03 (2)	21
5i	4-$C_2H_5C_6H_4$	3.2 ± 0.3 (3)	0.086 ± 0.02 (5)	38
5j	4-$C_3H_7C_6H_4$	5.7 ± 0.2 (2)	0.45 ± 0.06 (2)	13
5k	4-(*i*-C_3H_7)C_6H_4	12 ± 1.5 (2)	0.26 ± 0.04 (2)	45
5l	4-(CO_2CH_3)C_6H_4	ND	6.1 ± 1.2 (2)	—
5m	4-(CO_2H)C_6H_4	ND	> 100 (1)	—
5n	3-$NH_2C_6H_4$	0.54 (1)	0.95 ± 0.12 (2)	0.57
5o	2-(CHO)C_6H_4	3.5 ± 1.7 (2)	0.59 ± 0.15 (4)	5.9
5p	3,5-(CF_3)$_2C_6H_3$	0.79 (1)	11.8 ± 0.3 (2)	0.066
5q	5-methyl-2-furyl	ND	1.1 ± 0.3 (2)	—
5r	2-thienyl	5.1 ± 0.8 (2)	0.36 ± 0.3 (2)	14
5s	5-methyl-2-thienyl	ND	0.050 ± 0.016 (2)	—
5t	5-ethyl-2-thienyl	ND	0.10 ± 0.03 (2)	—
5u	3-thienyl	4.5 ± 0.4 (2)	0.38 ± 0.01 (2)	12
5v	2-pyridyl	3.8 ± 0.1 (2)	2.7 ± 0.01 (2)	1.4
5w	$C_6H_5CH_2$	6.9 ± 0.2 (2)	0.35 + 0.13 (3)	20
5x	1-naphthyl	ND	0.27 ± 0.03 (2)	—
5y	2-thianaphthyl	ND	0.15 ± 0.02 (2)	—

[a]ET_B selectivity = (IC_{50} ET_A)/(IC_{50} ET_B).

effect on ET_B activity and selectivity. Studies on isomeric 5-tolyl-2-thiophenesulfonamides **5b–d** (Table 3) unequivocally established that the 4′-substituted compound (**5d**) offered the best activity and selectivity for the ET_B receptor. A similar trend was also observed for the 5-(methoxyphenyl)-2-thiophenesulfonamides **5f–h** although the substitution effect in this series was generally less dramatic. Interestingly, the *ortho*-methoxy compound **5f** was actually found to be ET_A selective.

As observed for the biphenylsulfonamide series, the ET_B activity and selectivity of thiophenesulfonamides were

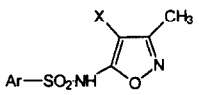
also very sensitive to the size of the 4′-substituents on the phenyl group and dropped off rapidly with substituents larger than methyl. A systematic study revealed that the 4-tolyl compound (**5d**) was more than twice as active and five times more selective than the 4-ethylphenyl compound (**5i**) which in turn was five times more active and three times more selective than the 4-propylphenyl compound (**5j**). Surprisingly, the 4-isopropylphenyl analogue (**5k**) was slightly more active and selective than the 4-propyl analogue (**5j**). The electronic nature of the substituents was found to have played a significant role in the ET_B activity and selectivity which decreased with both electron-donating (4-OMe, **5h**) and electron-withdrawing (4-CO_2Me, **5l**) groups. Substitution with a 4′-carboxyl group led to compound **5m** with no significant ET_B affinity, probably due to the presence of a charged group. However, these results should be interpreted with caution since the larger size of these substituents, as compared to a methyl group, may also decrease the ET_B binding affinity. Substitution with a bulky 5-[3,5-bis(trifluoromethyl)phenyl] group gave rise to an ET_A selective compound (**5p**). The reason for this reversal of selectivity is not apparent and requires further study.

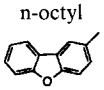
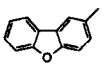
A series of 2-thiophenesulfonamides substituted at the 5-position with various heterocyclic aromatic groups was also synthesized and studied (Table 3). The 2-thienyl (**5r**) and 3-thienyl (**5u**) compounds were found to have similar ET_B activity and selectivity, which in turn were similar to those of the 5-phenyl analogue (**5a**). This is probably a result of the isosteric nature of the benzene and thiophene systems. The effects of substituted thienyl groups were also studied. Again, the size of the substituent on the distal hetero aromatic ring was found to be extremely important for both the activity and selectivity. The 5-(5-methyl-2-thienyl) analogue (**5s**) was substantially more active than both the 5-(2-thienyl) (**5r**) and the 5-(5-ethyl-2-thienyl) (**5t**) analogues, consistent with the trend observed for the 4-biphenylsulfonamide and the 5-phenyl-2-thiophenesulfonamide series. However, substitution with a 5-(5-methyl-2-furyl) group gave a compound (**5q**) that is substantially less active than the corresponding phenyl (**5d**) and thienyl (**5s**) analogues. Whether this effect is a result of the small size of the furan ring or its electron-rich nature remains to be determined. Curiously, the 5-(2-pyridyl)-2-thiophenesulfonamide **5v** was almost six times less active and selective than the 5-phenyl analogue **5a** even though the phenyl and pyridyl groups are very similar in size. In this case, the basic nature of the pyridine ring may have an adverse affect on the binding to the ET_B receptor. A similar situation was also found in **5n** in which a basic amino group was attached to the distal aromatic ring. Unlike the other *meta*-substituted analogues **5c** and **5g**, **5n** was slightly ET_A selective and showed lower ET_B binding affinity. The introduction of other aryl substituents such as benzyl (**5w**), 1-naphthyl (**5x**) and 2-thianaphthyl (**5y**) into the 5-position of the 2-thiophenesulfonamide seemed to have relatively little impact on the ET_B activity of the resulting compounds.

From the above observations, we hypothesize that a linear polycyclic aromatic group that is capable of forming a linear structure with the SO_2 group in *N*-isoxazolylsulfonamides is required for effective binding to the ET_B receptor. The size of the aromatic group is also important. The optimal size would probably be similar to the 4-tolylphenyl group in **4h** in the biphenylsulfonamide series and the 5-tolyl-2-thienyl group in **5d** in the thiophenesulfonamide series. In order to test this hypothesis, several sulfonamides that satisfy some or all of these structural requirements were synthesized and tested (Table 4). The results indicated that compounds that only partially satisfy these requirements were poor ET_B antagonists and were either only modestly ET_B selective or ET_A selective. For example, the nonaromatic 1-octanesulfonamide **6a** was found to bind only weakly to the ET_B receptor and was essentially nonselective even though it has a linear hydrophobic group. Furthermore, the 4-dibenzofuransulfonamide **6d** in which the aromatic group has an angular arrangement with the sulfonamide and hence resembles a 2-biphenyl sulfonamide was actually ET_A selective. In contrast, the 2-dibenzofuransulfonamides **6b** and **6c**, which satisfy all the requirements, were found to have good ET_B affinity and selectivity as predicted by the hypothesis. The best ET_B activity and selectivity among the polycyclic aromatic sulfonamide were found with the 3-phenanthrenesulfonamides **6e** and **6f**. These compounds again have an aromatic group linear with the sulfonamide function, lending further support for the hypothesis.

Recently, the discovery of several selective ET_B receptor antagonists was reported. Of particular interest are BQ-788,[23] a modified tripeptide analogue; the hexadecapeptide RES 701-1[24] and the nonpeptide pyrimidinyl sulfonamide Ro 46-8443.[25] In our hands, RES-701-1 acquired from a commercial source showed a much lower IC_{50} value for the ET_B receptor (0.54 μM) than reported. This discrepancy has been previously reported[25,40,41] and is attributed to the different three-dimensional folding patterns of natural and synthetic RES-701-1.[41] The binding affinity for BQ-788 determined in our system (1.2 nM) is similar to that reported although the ET_B selectivity is slightly lower (200 instead of 1000). Thus, BQ-788 is about 14 times more active than **4h** and 30 times more active than **5d**. This difference in receptor binding affinity was also reflected in the phosphoinositide hydrolysis assay in which BQ-788 (IC_{50} = 0.1 μM) was found to be about 20 times more potent than **4h** (IC_{50} = 2.0 μM) and about 30 times more potent than **5d** (IC_{50} = 2.9 μM) (Table 5). The ET_B

Table 4. Other ET_B selective sulfonamide antagonists



Compd	Ar	X	ET_A binding IC_{50}, μM (n)	ET_B binding IC_{50}, μM (n)	ET_B selectivity[a]
6a	n-octyl	CH_3	4.8 ± 1.0 (2)	2.6 ± 0.3 (2)	1.8
6b		CH_3	6.1 ± 1.1 (2)	0.81 ± 0.13 (2)	7.5
6c		Br	1.05 ± 0.5 (2)	0.23 ± 0.05 (2)	9
6d		CH_3	0.66 (1)	8.1 ± 0.4 (2)	0.081
6e		CH_3	3.4 ± 1.0 (2)	0.32 ± 0.06 (2)	11
6f		Br	1.7 ± 0.3 (2)	0.068 ± 0.027 (3)	25

[a]ET_B selectivity = (IC_{50} ET_A)/(IC_{50} ET_B).

selectivity of these compounds (290 and 200, respectively) is comparable to that of BQ-788. A direct comparison of Ro 46-8443 with **4h** and **5d** has not been performed due to the former's unavailablity. However, from the work of Breu et al.,[25] Ro 46-8443 has similar ET_B binding affinity to BQ-788 in their assay system. Thus, it may appear that Ro 46-8443 should be at least an order of magnitude more active than **4h** in binding to the ET_B receptor. The selectivity of these two compounds should be similar. However, these comparisons should be treated with caution since the IC_{50} values were obtained from different biological assay systems.

Conclusion

Systematic optimization of the ET_A selective benzenesulfonamides endothelin antagonists led to the discovery of 4-biphenyl- and 5-aryl-2-thienylsulfonamide endothelin antagonists selective for the human ET_B receptor. These compounds, exemplified by TBC-10950 (**4h**) and TBC-10894 (**5d**), have lower molecular weights (< 500) than known ET_B selective antagonists and are nonpeptidic in nature. The structural features that enhance ET_B affinity, i.e. the presence of a linear, conjugated π-system of definite shape and size, have been successfully incorporated into the design of polycyclic aromatic sulfonamides to give ET_B selective antagonists such as **6f**. These ET_B selective receptor antagonists could be useful as potential therapeutic agents and as pharmacological tools for elucidating the pathophysiological role of the ET_B receptors in human diseases.

Table 5. Comparison of selected ET_B receptor antagonists

Compound	Phosphoinositide hydrolysis[a] IC_{50} (μM)	ET_B Selectivity
BQ-788	0.1	200[a] (1000[b])
TBC-10950 (**4h**)	2.0	290
TBC-10894 (**5d**)	2.9	200
TBC-10667 (**6f**)	11.5	25

[a]This work.

Experimental

Chemistry

Melting points were determined in capillary tubes with a Mel-Temp II apparatus and are uncorrected. ^1H NMR spectra were recorded on a GE QE-300 Plus spectrometer at 300 MHz. Chemical shifts are reported in parts per million as δ units relative to tetramethylsilane or residual solvent as internal standard. The multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets and br, broad), coupling constant (*J*) and relative integral are reported in parenthesis following the chemical shift. IR spectra were recorded on a Mattson GL-2020 Fourier transform infrared spectrometer. Mass spectra were recorded with electron impact (EI) or fast atom bombardment (FAB) ionization; either by the University of Minnesota Mass Spectrometry Service Laboratory (Minneapolis, MN) or by SynPep Corporation (Dublin, CA). Elemental analyses were performed by Desert Analytical (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 the highest grade obtainable from commercial sources and were used without further purification. ET-1 was obtained from Clinalfa Co. (Laufelfingen, Switzerland), ET-3 and BQ-788 from American Peptide Co. (Sunnyvale, CA) and RES 701-1 from Peptides International (Louisville, KY). [^{125}I]ET-1 (specific activity 4000 Ci/mmol) 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 Vydac C_{18} column (4.6×250 mm) using 5–95% CH_3CN/H_2O gradient over 30 min with 0.1% trifluoroacetic acid at a flow rate of 1.2 mL/min. Preparative HPLC was performed with a Dynamax-60A column (25×250 mm) using a 5% to 100% CH_3CN/H_2O gradient over 30 min at a flow rate of 30 mL/min. The detection wavelength in both cases was 254 nm. The following abbreviations are used: DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; NBS, *N*-bromosuccinimide; THF, tetrahydrofuran; TFA, trifluoroacetic acid.

Method A

***N*-(3,4-Dimethyl-5-isoxazolyl)-2-biphenylsulfonamide (4a).** 2-Biphenylsulfonyl chloride (463 mg, 2.0 mmol) was added to a solution of 5-amino-3,4-dimethylisoxazole (250 mg, 2.2 mmol) in dry pyridine (2.0 mL). The reaction mixture was stirred at room temperature for 4 h. Pyridine was removed under reduced pressure and the residue was partitioned between water (20 mL) and EtOAc (25 mL). The organic layer was washed with 1N HCl (2×25 mL), brine (25 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvents gave an oily residue which was purified by column chromatography (1% MeOH/$CHCl_3$) to give 337 mg (45%) of a white powder. Recrystallization from $CHCl_3$/hexanes gave white crystals, mp 173–175 °C. IR (KBr): 2800–3200, 2500–2800, 1649, 1466, 1352, 1167, 764, 702, 590 cm^{-1}; ^{1}H NMR ($CDCl_3$) 10.91 (br s, 1H), 8.06 (dd, J=1.0, 7.5 Hz, 1H), 7.63–7.71 (m, 2H), 7.22–7.39 (m, 6H), 2.08 (s, 3H), 1.62 (s, 3H). Anal. calcd for $C_{17}H_{16}N_2O_3S$: C, 62.18; H, 4.91; N, 8.53; S, 9.76. Found: C, 61.87; H, 4.70; N, 8.33; S, 9.72.

The following compounds were prepared using Method A:

***N*-(3,4-Dimethyl-5-isoxazolyl)-3-biphenylsulfonamide (4b).** Gum (HPLC). IR (KBr): 2600–3500, 1655, 1466, 1458, 1422, 1389, 1348, 1167, 1125, 1098 cm^{-1}; ^{1}H NMR ($CDCl_3$): 8.02 (d, J=1,3 Hz, 1H), 7.82 (dd, J=1.0, 7.6 Hz, 1H), 7.77 (dd, J=1.0 7.6 Hz, 1H), 7.38–7.62 (m, 6H), 6.63 (br s, 1H), 2.20 (s, 3H), 1.94 (s, 3H). Anal. calcd for $C_{17}H_{16}N_2O_3S$: C, 62.18; H, 4.91; N, 8.53; S, 9.76. Found: C, 62.42; H, 4.95; N, 8.83; S, 9.60.

4-(Phenylethynyl)-*N*-(3,4-dimethyl-5-isoxazolyl)benzenesulfonamide (4g). Mp 200–202 °C (HPLC). IR (KBr): 3025, 2836, 2782, 1651, 1586, 1497, 1445, 1356, 1196, 1123, 1084, 870, 760 cm^{-1}; ^{1}H NMR (DMSO-d_6): 11.2 (br s, 1H), 7.80 (s, 4H), 7.60–7.63 (m, 2H), 7.45–7.50 (m, 3H), 2.10 (s, 3H), 1.64 (s, 3H). HRMS (EI) calcd for $C_{19}H_{16}N_2O_3S$: 352.0822, found: 352.0878. Anal. calcd for $C_{19}H_{16}N_2O_3S{\cdot}0.1H_2O$: C, 64.43; H, 4.61; N, 7.91. Found: C, 64.26; H, 4.46; N, 7.71.

***N*-(3,4-Dimethyl-5-isoxazolyl)octanesulfonamide (6a).** Colorless oil (HPLC). IR (neat) 3692, 3364, 2930, 2858, 1663, 1460, 1425, 1391, 1337, 1155,745 cm^{-1}; ^{1}H NMR ($CDCl_3$): 3.15–3.25 (m, 2H), 2.20 (s, 3H), 1.96 (s, 3H), 1.93–1.99 (m, 2H), 1.21–1.51 (m, 10H), 0.88 (distorted t, J=ca. 7 Hz, 3H). HRMS(EI) calcd for $C_{13}H_{24}N_2O_3S$ [M^+]: 288.1508, found: 288.1511.

Method B

***N*-(3,4-Dimethyl-5-isoxazolyl)-4-biphenylsulfonamide (4c).** 4-Biphenylsulfonyl chloride (509 mg, 2.2 mmol) was added to a solution of 5-amino-3,4-dimethylisoxazole (250 mg, 2.2 mmol) and 4-dimethylaminopyridine (5 mg) in dry pyridine (2.0 mL). The reaction mixture was stirred at room temperature for 4 h. Pyridine was removed under reduced pressure and the residue was partitioned between water and EtOAc. The organic layer was washed with 1 N HCl (2×25 mL), brine (25 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvents left an oily residue which yielded 337 mg (45%) of a white solid after purification by column chromatography over silica gel (1% MeOH in $CHCl_3$). Recrystallization from EtOAc/hexanes gave white crystals, mp 154–155 °C. IR (KBr) 3352, 2928, 1660, 1595, 1425, 1397, 1344, 1181, 879, 594 cm^{-1}; ^{1}H NMR ($CDCl_3$): 7.87 (d, J=8 Hz, 2H), 7.71 (d, J=8 Hz, 2H), 7.61 (d, J=8 Hz, 2H), 7.41–7.51 (m, 3H), 6.78–6.86 (br s, 1H), 2.21 (s, 3H), 1.96 (s, 3H). HRMS (EI) calcd for $C_{17}H_{16}N_2O_3S$: 328.0882, found: 328.0887. Anal. calcd for $C_{17}H_{16}N_2O_3S$: C, 62.18; H, 4.91; N, 8.53; S, 9.76. Found: C, 62.18; H, 4.86; N, 8.41; S, 9.52.

The following compounds were prepared using Method B.

***N*-(3,4-Dimethyl-5-isoxazolyl)-2-dibenzofuransulfonamide (6b).** Mp 173–175 °C (dec) ($CHCl_3$/hexanes). IR ($CHCl_3$) 3354, 1661, 1466, 1445, 1427, 1188, 1167, 1125, 880 cm^{-1}; ^{1}H NMR ($CDCl_3$): 8.46 (s, 1H), 7.95 (d,

J=8 Hz, 1H), 7.90 (d, J=8 Hz, 1H), 7.65 (d, J=8 Hz, 1H), 7.60 (d, J=8 Hz, 1H), 7.55 (t, J=8 Hz, 1H), 7.41 (t, J=8 Hz, 1H), 2.19 (s, 3H), 1.94 (s, 3H). Anal. calcd for $C_{17}H_{14}N_2O_4S \cdot 0.5H_2O$: C, 58.11; H, 4.30; N, 7.97; S, 9.12. Found: C, 58.49; H, 4.19; N, 7.94; S, 9.18.

***N*-(3,4-Dimethyl-5-isoxazolyl)-4-dibenzofuransulfonamide (6d).** Mp 158–160 °C ($CHCl_3$/hexanes); IR ($CHCl_3$) 3221, 1655, 1426, 1344, 1146, 754, 602 cm^{-1}; ^1H NMR (DMSO-d_6): 11.50 (br s, 1H), 8.53 (d, J=7.1 Hz, 1H), 8.27 (d, J=7.5 Hz, 1H), 7.86 (d, J=7.1 Hz, 1H), 7.74 (d, J=8.1 Hz, 1H), 7.43–7.72 (m, 3H), 2.05 (s, 3H), 1.58 (s, 3H). Anal. calcd for $C_{17}H_{14}N_2O_4S$: C, 59.64; H, 4.12; N, 8.18; S, 9.36. Found: C, 59.46; H, 3.94; N, 7.93; S, 9.10.

***N*-(3,4-Dimethyl-5-isoxazolyl)-3-phenanthrenesulfonamide (6e).** Mp 185–188 °C ($CHCl_3$/hexanes); IR (KBr) 3210, 1667, 1429, 1344, 1148, 677 cm^{-1}; ^1H NMR (DMSO-d_6): 11.24 (br s, 1H), 9.16 (s, 1H), 8.75 (d, J=7.9 Hz, 1H), 8.25 (d, J=8.5 Hz, 1H), 7.92–8.20 (m, 2H), 7.67–7.80 (m, 3H), 7.71–7.92 (m, 2H), 2.05 (s, 3H), 1.63 (s, 3H). Anal. calcd for $C_{19}H_{16}N_2O_3S \cdot 0.5H_2O$: C, 63.14; H, 4.74; N, 7.75; S, 8.87. Found: C, 63.12; H, 4.55; N, 7.76; S, 8.64.

Method C

***N*-(4-Bromo-3-methyl-5-isoxazolyl)-2-biphenylsulfonamide (4d).** Sodium hydride (60% dispersion in mineral oil, 90 mg, 2.2 mmol) was freed of mineral oil by washing in hexanes three times, suspended in dry THF (1 mL) and cooled to 0 °C. A solution of 5-amino-4-bromo-3-methylisoxazole[32] (177 mg, 1.0 mmol) in dry THF (1 mL) was added with stirring. Once addition was complete, the reaction mixture was warmed to room temperature until gas evolution ceased (ca. 15 min). The solution was recooled to 0 °C, and 2-biphenylsulfonyl chloride (0.283 mL, 2.2 mmol) was added. Stirring was continued at 25 °C for 2 h. Excess sodium hydride was decomposed by careful addition of MeOH (0.4 mL) at 0 °C followed by water (0.5 mL). The solvents were removed under reduced pressure and the residue was dissolved in water (20 mL) and basified by the addition of NaOH (pH 9–10). Neutral impurities were removed by extraction with EtOAc (2×10 mL) and the extract was discarded. The aqueous layer was acidified to pH 2–3 using concentrated HCl and extracted with EtOAc (3×10 mL). The combined organic extract was dried over $MgSO_4$. Removal of the solvent gave *N*-(4-bromo-3-methyl-5-isoxazolyl)-2-biphenylsulfonamide in 71% yield. Purification was achieved by recrystallization from EtOAc/hexanes to give a crystalline solid, mp 145–147 °C. IR ($CHCl_3$) 2500–3200, 1618, 1466, 1443, 1431, 1408, 1371, 1358, 1171, 1084, 761 cm^{-1}; ^1H NMR ($CDCl_3$): 8.18 (dd, J=1.0, 8.0 Hz, 1H), 7.66 (dt, J=1.1, 8.0 Hz, 1H), 7.53 (dt, J=1.0, 8.5 Hz, 1H), 7.41–7.50 (m, 5H), 7.37 (dd, J=1.0, 7.4 Hz, 1H), 2.18 (s, 3H). Anal. calcd for $C_{16}H_{13}BrN_2O_3S$: C, 48.86; H, 3.33; N, 7.12; S, 8.15. Found: C, 49.15; H, 3.20; N, 7.14; S, 8.40.

The following compounds were prepared using Method C:

***N*-(4-Bromo-3-methyl-5-isoxazolyl)-3-biphenylsulfonamide (4e).** Mp 78–82 °C (HPLC). IR (KBr) 3400, 3057, 2955, 1622, 1414, 1354, 1169, 1086 cm^{-1}; ^1H NMR ($CDCl_3$): 8.19 (s, 1H), 7.96 (d, J=8.0 Hz, 1H), 7.89 (d, J=8.0 Hz, 1H), 7.66 (d, J=7.8 Hz, 1H), 7.61 (d, J=7.1 Hz, 2H), 7.44–7.54 (m, 3H), 6.92 (br s, 1H), 2.25 (s, 3H). Anal. calcd for $C_{16}H_{13}BrN_2O_3S$: C, 48.86; H, 3.33. Found: C, 48.44; H, 3.09.

***N*-(4-Bromo-3-methyl-5-isoxazolyl)-2-dibenzofuransulfonamide (6c).** Mp 175–177 °C (EtOAc/hexanes). IR ($CHCl_3$) 3187, 1634, 1445, 1427, 1350, 1339, 1188, 1156, 1082, 750, 715 cm^{-1}; ^1H NMR ($CDCl_3$): 8.61 (s, 1H), 8.09 (dd, J=1, 8 Hz, 1H), 8.02 (dd, J=1, 8 Hz, 1H), 7.69 (d, J=8 Hz, 1H), 7.63 (d, J=8 Hz, 1H), 7.58 (t, J=8 Hz, 1H), 7.43 (t, J=8 Hz, 1H), 7.13 (br s, 1H), 2.20 (s, 3H). Anal. calcd for $C_{16}H_{11}BrN_2O_4S$: C, 47.19; H, 2.72; N, 6.88; S, 7.87. Found: C, 47.30; H, 2.73; N, 6.97; S, 7.97.

***N*-(4-Bromo-3-methyl-5-isoxazolyl)-3-phenanthrenesulfonamide (6f).** Mp 190–192 °C ($CHCl_3$/hexanes); IR (KBr) 3190, 1636, 1345, 1167, 1082, 841, 669 cm^{-1}; ^1H NMR ($CDCl_3$): 9.31 (s, 1H), 8.71 (d, J=8.1 Hz, 1H), 8.02–8.10 (m, 2H), 7.92–7.96 (m, 2H), 7.67–7.80 (m, 3H), 7.10 (br s, 1H), 2.19 (s, 3H). Anal. calcd for $C_{18}H_{13}BrN_2O_3S$: C, 51.81; H, 3.14; N, 6.71; S, 7.68. Found: C, 51.58; H, 3.07; N, 6.43; S, 7.47.

Method D

***N*-(4-Bromo-3-methyl-5-isoxazolyl)-4-biphenylsulfonamide (4f).** 5-Amino-4-bromo-3-methylisoxazole (179 mg, 1.0 mmol) was dissolved in dry pyridine (2 mL). 4-Biphenylsulfonyl chloride (509 mg, 2.2 mmol) was added with stirring at ambient temperature followed by 4-dimethylaminopyridine (5 mg). The reaction was stirred at 50 °C for 16 h. The reaction mixture was cooled, diluted with CH_2Cl_2 (25 mL), washed with 1N HCl (2×50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography (20% EtOAc in hexanes) to give 390 mg (60%) of *N*-(4-biphenylsulfonyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-4-biphenylsulfonamide.

The *N*-(4-biphenylsulfonyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-4-biphenylsulfonamide (150 mg, 0.23 mmol) obtained above was dissolved in aqueous THF (20%

water, 1 mL). Sodium hydroxide (0.120 mg, 3.0 mmol) was added and the solution was warmed to 45 °C to dissolve the solid. Stirring was continued for 20 min. Tetrahydrofuran was removed under reduced pressure. The residue was dissolved in water, cooled to 0 °C and acidified to pH 3–4 with concentrated HCl. The solid precipitate was filtered off and dried in vacuo to give *N*-(4-bromo-3-methyl-5-isoxazolyl)-4-biphenylsulfonamide (94% yield), which was further purified by recrystallization from $CHCl_3$/hexanes, mp 133–135 °C. IR (KBr) 3212, 1632, 1427, 1346, 1171, 1084 cm^{-1}; ^1H NMR ($CDCl_3$): 8.03 (d, J = 8.6 Hz, 2H), 7.75 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 7.40–7.52 (m, 3H), 2.23 (s, 3H). Anal. calcd for $C_{16}H_{13}BrN_2O_3S$: C, 48.86; H, 3.33; N, 7.12; S, 8.15. Found: C, 49.18; H, 3.31; N, 7.21; S, 8.33.

Method E

(a) 4-(4-Tolyl)-*N*-(3-methyl-5-isoxazolyl)benzenesulfonamide. 4-Bromobenzenesulfonyl chloride (10.2 g, 40.0 mmol) was added, in five portions, to a solution of 5-amino-3-methylisoxazole (3.82 g, 40.0 mmol) in dry pyridine (20 mL). The reaction was stirred at room temperature for 3 h and the pyridine was removed under reduced pressure. The residue was dissolved in THF (300 mL) and a 5% NaOH solution (100 mL) was added. Stirring was continued for 1 h at room temperature. The THF was removed under reduced pressure and the residue was acidified to pH 2 with concentrated HCl. The crude mixture was extracted with EtOAc (3×25 mL), washed with brine (25 mL) and dried over $MgSO_4$. Removal of solvents under reduced pressure gave a solid which was recrystallized from hexane/EtOAc to give 4-bromo-*N*-(3-methyl-5-isoxazolyl)-benzenesulfonamide **16** as a light brown solid (9.2 g, 72% yield).

Nitrogen was bubbled through a biphasic mixture of EtOH (15 mL), toluene (15 mL) and 2M Na_2CO_3 solution (15 mL). 4-Bromo-*N*-(3-methyl-5-isoxazolyl)benzenesulfonamide (obtained above, 951 mg, 3.0 mmol), 4-methylbenzeneboronic acid (560 mg, 4.0 mmol) and tetrakis-(triphenylphosphine)palladium(0) (300 mg, 0.26 mmol) were added. The reaction mixture was stirred at 80 °C under a nitrogen atmosphere for 24 h, then cooled, diluted with water (50 mL) and extracted with ether (50 mL) to remove neutral impurities and excess 4-methylbenzeneboronic acid. The aqueous phase was acidified to pH 2 with concentrated HCl to form a solid precipitate which was filtered, dried under vacuum and recrystallized from hexane/EtOAc to give 4-(4-tolyl)-*N*-(3-methyl-5-isoxazolyl)benzenesulfonamide (**17**, Ar = $CH_3C_6H_4$; 1.0 g, 100% yield), mp 194–198 °C. IR (KBr) 3033, 2831, 2814, 2692, 1613, 1497, 1350, 1169, 1152, 1094, 883, 808, 785 cm^{-1}; ^1H NMR (DMSO-d_6): 7.90 (s, 2H), 7.64 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 5.77 (s, 1H), 3.37 (br s, 1H), 2.35 (s, 3H), 2.10 (s, 3H).

(b) 4-(4-Tolyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)benzenesulfonamide (4h). *N*-Bromosuccinimide (NBS) (178 mg, 1.0 mmol) was added to a stirred suspension of 4-(4-tolyl)-*N*-(3-methyl-5-isoxazolyl)benzenesulfonamide (327 mg, 1.0 mmol) in $CHCl_3$ (12 mL). The reaction mixture was stirred for 10 min and diluted with CH_2Cl_2 (50 mL). The resulting solution was washed with water (2×50 mL), dried over $MgSO_4$ and concentrated. The crude product was recrystallized from hexane/EtOAc to give **4h** (350 mg, 86% yield), mp 153–156 °C. IR (KBr) 3216, 1621, 1578, 1345, 1172, 1080, 810, 750 cm^{-1}; ^1H NMR (DMSO-d_6): 7.88–7.90 (m, 4H), 7.66 (d, J = 8.1 Hz, 2H), 7.33 (d, J = 8.1 Hz, 2H), 2.37 (s, 3H), 2.15 (s, 3H). HRMS(EI) calcd for $C_{17}H_{15}BrN_2O_3S$: 407.9966, found: 407.9968. Anal. calcd for $C_{17}H_{15}BrN_2O_3S$: C, 50.13; H, 3.71; N, 6.87; S, 7.87. Found: C, 50.10; H, 3.84; N, 6.73; S, 7.96.

The following compounds were prepared using Method E:

4-[(4-Trifluoromethyl)phenyl]-*N*-(4-bromo-3-methyl-5-isoxazolyl)benzenesulfonamide (4i). Mp 113–117 °C (EtOAc/hexanes). IR (KBr) 3450, 1715, 1612, 1593, 1497, 1416, 1327, 1250, 1169, 1132, 1109, 1070, 821 cm^{-1}; ^1H NMR (DMSO-d_6): 7.78–7.94 (m, 8H), 1.95 (s, 3H). Anal. calcd for $C_{17}H_{12}BrF_3N_2O_3S \cdot 0.5TFA$: C, 41.72; H, 2.43; N, 5.40; S, 6.19. Found: C, 41.62; H, 2.70; N, 5.73; S, 6.54.

4-(2-Methoxyphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-benzenesulfonamide (4j). Mp 208 °C (dec) (EtOAc/hexanes). IR (KBr) 2900–3500, 1589, 1262, 1235, 1134, 1107 cm^{-1}; ^1H NMR (DMSO-d_6): 7.76 (d, J = 8.1 Hz, 2H), 7.49 (d, J = 8.1 Hz, 2H), 7.36 (br t, J = ca. 8 Hz, 1H), 7.30 (dt, J = 1.0, 7.4 Hz, 1H), 7.11 (d, J = 8.2 Hz, 1H), 7.06 (t, J = 7.4 Hz, 1H), 3.34 (s, 3H), 1.94 (s, 3H). Anal. calcd for $C_{17}H_{15}BrN_2O_4S \cdot 1.5H_2O$: C, 45.34; H, 4.03. Found: C, 45.52; H, 3.90.

4-(3-Methoxyphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-benzenesulfonamide (4k). Mp 140–144 °C (EtOAc/hexanes). IR (KBr) 3209, 1622, 1397, 1211, 1167, 1082 cm^{-1}; ^1H NMR ($CDCl_3$): 8.03 (d, J = 8.5 Hz, 2H), 7.74 (d, J = 8.5 Hz, 2H), 7.41 (t, J = 7.9 Hz, 1H), 7.19 (dd, J = 0.75, 7.6 Hz, 1H), 7.12 (br s, 1H), 6.98 (dd, J = 2.3, 8.2 Hz, 1H), 6.92 (br s, 1H), 3.88 (s, 3H), 2.24 (s, 3H). Anal. calcd for $C_{17}H_{15}BrN_2O_4S \cdot 0.5TFA$: C, 45.01; H, 3.25; N, 5.83; S, 6.68. Found: C, 44.65; H, 3.58; N, 5.69; S, 6.62.

4-(4-Methoxyphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-benzenesulfonamide (4l). Mp 205–209 °C (dec) (EtOAc/hexanes). IR (KBr) 3200–2600, 1593, 1487, 1454, 1416,

1292, 1252, 1136, 1107 cm^{-1}; ^1H NMR (DMSO-d_6): 7.78 (d, J=8.3 Hz, 2H), 7.64 (d, J=8.3 Hz, 2H), 7.64 (d, J=8.7 Hz, 2H), 7.03 (d, J=8.7 Hz, 2H), 3.80 (s, 3H), 1.93 (s, 3H). Anal. calcd for $C_{17}H_{15}BrN_2O_4S \cdot 0.5TFA$: C, 45.01; H, 3.25; N, 5.83; S, 6.68. Found: C, 44.66; H, 3.44; N, 5.70; S, 6.41.

4-(3,4-Methylenedioxyphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)benzenesulfonamide (4m). Mp 172–174 °C (HPLC). IR (KBr) 3210, 1620, 1593, 1480, 1406, 1356, 1231, 1169, 1082, 1038 cm^{-1}; ^1H NMR (DMSO-d_6): 7.80–7.90 (m, 4H), 7.37 (d, J=1.4 Hz, 1H), 7.27 (dd, J=1.4, 8.1 Hz, 1H), 7.05 (d, J=8.1 Hz, 1H), 6.10 (s, 2H), 2.15 (s, 3H). Anal. calcd for $C_{17}H_{13}BrN_2O_5S$: C, 46.69; H, 2.99; N, 6.40; S, 7.33. Found: C, 46.56; H, 3.08; N, 6.22; S, 7.16.

The following compounds were prepared using Method E but substituting 5-bromo-2-thiophenesulfonyl chloride for 4-bromobenzenesulfonyl chloride.

5-(3-Aminophenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5n). Low melting colorless solid (HPLC). IR (KBr) 2500–3600, 1680, 1624, 1535, 1522, 1408, 1352, 1082, 688 cm^{-1}; ^1H NMR (DMSO-d_6): 8.46 (d, J=1.8 Hz, 1H), 8.22 (dd, J=8.1, 1.7 Hz, 1H), 8.16 (d, J=8.1 Hz, 1H), 7.69–7.78 (m, 2H), 7.49–7.58 (m, 1H), 2.09 (s, 3H). Anal. calcd for $C_{14}H_{12}BrN_3O_3S_2 \cdot 1.0TFA$: C, 36.37; H, 2.48; N, 7.95. Found: C, 36.67; H, 2.55; N, 7.76.

5-(2-Formylphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5o). Pale-brown oil (HPLC). IR (neat) 2700–3200, 1697, 1684, 1167, 1082 cm^{-1}; ^1H NMR (DMSO-d_6): 10.08 (s, 1H), 7.94 (d, J=8 Hz, 1H), 7.78 (t, J=8 Hz, 1H), 7.60–7.70 (overlapping d and t, J=ca. 8 Hz, 2H), 7.56 (d, J=3.9 Hz, 1H), 7.27 (t, J=3.9 Hz, 1H), 2.09 (s, 3H).

5-(2-Pyridyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5v). Mp 186–188 °C (EtOAc). IR (KBr) 3314, 3239, 1331, 1152, 783 cm^{-1}; ^1H NMR (DMSO-d_6): 7.87 (AB q, J=8.5 Hz, 2H), 7.56 (s, 1H), 7.52 (d, J=8.0 Hz, 1H), 7.38–7.42 (m, 2H), 7.25 (d, J=7.5 Hz, 1H), 2.39 (s, 3H). HRMS(EI) calcd for $C_{13}H_{10}BrN_3O_3S_2$: 398.9347, found: 398.9354. Anal. calcd for $C_{13}H_{10}BrN_3O_3S_2 \cdot 0.25$ EtOAc: C, 40.20; H, 2.89; N, 10.05. Found: C, 40.09; H, 2.75; N, 10.13.

Method F

(a) 5-(2-Tolyl)-2-thiophenesulfonyl chloride. An aqueous solution of sodium carbonate (2 M, 15 mL, 30 mmol) and 2-methylbenzeneboronic acid (1.63 g, 12 mmol) were added to a solution of 2-bromothiophene (1.63 g, 10 mmol) and tetrakis(triphenylphosphine)palladium(0) (300 mg, 0.26 mmol) in toluene (15 mL) and ethanol (15 mL) under nitrogen. The mixture was heated under reflux for 2 h, cooled to room temperature and extracted with EtOAc (2×50 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel using hexane as eluent to afford 1.2 g (69% yield) of 2-(2-tolyl)thiophene as a colorless gum.

To a cold (−5 to 0 °C) solution of 2-(2-tolyl)thiophene (0.87 g, 5.0 mmol) in CH_2Cl_2 (1 mL) was added chlorosulfonic acid (0.33 mL, 5.0 mmol) over 15 min with constant stirring. After 10 min, phosphorous oxychloride (2.0 mL, 21.5 mmol) and phosphorous pentachloride (2.08 g, 10.0 mmol) were added. The reaction mixture was allowed to attain ambient temperature and stirred for 3 h. The mixture was poured onto crushed ice (50 g) and extracted with EtOAc (2×50 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (2% EtOAc in hexanes) to give 5-(2-tolyl)-2-thiophenesulfonyl chloride (1.0 g, 73% yield).

(b) 5-(2-Tolyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5b). Using the procedures described in Method C, reaction of 5-(2-tolyl)-2-thiophenesulfonyl chloride (114 mg, 0.42 mmol) with 5-amino-4-bromo-3-methylisoxazole (89 mg, 0.50 mmol) gave a crude product which was purified by column chromatography (10% MeOH/$CHCl_3$) to give a gum (120 mg). This gum was dissolved in 0.28 mL of 1.0 M NaOH and the solution was concentrated in vacuo. The sodium salt of **5b** was obtained as a light brown solid (125 mg, 67% yield), mp 184–188 °C. IR (KBr) 2700–3600, 1588, 1443, 1433, 1425, 1414, 1252, 1128, 1094 cm^{-1}; ^1H NMR (DMSO-d_6): 7.20–7.40 (m, 5H), 7.06 (d, J=3.7 Hz, 1H), 2.34 (s, 3H), 1.98 (s, 3H). Anal. calcd for $C_{15}H_{12}BrNaN_2O_3S_2 \cdot 2.5H_2O$: C, 37.51; H, 3.57. Found: C, 37.22; H, 3.49.

The following compounds were prepared according to Method F:

5-(3-Tolyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5c). (Na^+ salt): hygroscopic gum (HPLC). IR (KBr) 2600–3600, 1589, 1493, 1449, 1416, 1271, 1132, 1098, 1067, 916 cm^{-1}; ^1H NMR (DMSO-d_6): 7.46 (s, 1H), 7.43 (d, J=8.1 Hz, 1H), 7.26–7.35 (m, 2H), 7.15 (d, J=7.5 Hz, 1H), 7.10 (br s, 1H), 2.34 (s, 3H), 1.97 (s, 3H). Anal. calcd for $C_{15}H_{12}BrNaN_2O_3S_2 \cdot 2.5H_2O$: C, 37.51; H, 3.57. Found: C, 37.67; H, 3.40.

5-(4-Tolyl)-*N*-(4-bromo-5-methyl-3-isoxazolyl)-2-thiophenesulfonamide (5d). Mp 175 °C (dec) (free acid from column chromatography using MeOH/$CHCl_3$ as eluent). IR (KBr) 3580, 1597, 1437, 1416, 1248, 1134, 1101,

902, 746, 733, 719, 646 cm^{-1}; ^1H NMR (DMSO-d_6): 7.53 (d, J=8 Hz, 2H), 7.31 (s, 2H), 7.23 (d, J=8 Hz, 2H), 2.32 (s, 3H), 1.98 (s, 3H). Anal. calcd for $C_{15}H_{12}BrNaN_2O_3S_2$: C, 41.39; H, 2.77; N, 6.49; S, 14.86. Found: C, 41.46; H, 2.92; N, 6.43; S, 14.73.

Method G

(a) 5-(4-Ethylphenyl)-2-thiophenesulfonyl chloride. A solution of pyrrole (385 mg, 5.8 mmol) in dry THF (2 mL) was added dropwise over 10 min to a suspension of sodium hydride (60% oil dispersion, 191 mg, 4.8 mmol) in dry THF (2 mL) at 0 °C (ice bath). After the addition was complete, the ice bath was removed and the solution was stirred at room temperature until gas evolution ceased (15 min), whereupon a solution of 5-bromo-2-thiophenesulfonyl chloride (1.0 g, 3.8 mmol) in THF (4 mL) was added dropwise through a steel cannula. After stirring for 1 h at room temperature, the mixture was filtered through Celite. The filter pad was rinsed with THF, and the filtrate was evaporated. The light brown solid that remained was recrystallized from MeOH to produce *N*-(5-bromo-2-thiophenesulfonyl)-pyrrole as a white powder (821 mg, 74% yield) which was coupled with 4-ethylbenzeneboronic acid under Suzuki conditions as described in Method E, Part (a) to give *N*-[5-(4-ethylphenyl)-2-thiophenesulfonyl]pyrrole as a tan solid in 81% yield after purification by column chromatography using 10% EtOAc/hexanes.

A solution of *N*-[5-(4-ethylphenyl)-2-thiophenesulfonyl]pyrrole (100 mg, 0.32 mmol) and 6 N sodium hydroxide (1 mL) in MeOH (1.5 mL) was heated under reflux for 6 h. Evaporation of solvents in vacuo gave an oil which was treated with phosphorus oxychloride (258 mL, 2.5 mmol) and phosphorus pentachloride (131 mg, 0.63 mmol). The resulting brown suspension was heated at 50 °C for 3 h to give a clear brown solution which was carefully added to 20 mL of crushed ice and then extracted with EtOAc (3×25 mL). The combined organic phase was washed with brine (2×5 mL), dried ($MgSO_4$) and evaporated to leave an oily residue. Flash chromatography over silica gel (2% EtOAc/hexanes) yielded 53 mg of 5-(4-ethylphenyl)-2-thiophenesulfonyl chloride (59%) of a pale-yellow oil.

(b) 5-(4-Ethylphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5i). Reaction of 5-(4-ethylphenyl)-2-thiophenesulfonyl chloride (47 mg, 0.11 mmol) with 5-amino-4-bromo-3-methylisoxazole (29 mg, 0.16 mmol) as described in Method C yielded **5i** as a pale brown solid (46 mg, 66% yield) after flash chromatography (10% MeOH/$CHCl_3$), mp 172–175 °C. IR (KBr) 2800–3600, 1589, 1416, 1248, 1132, 1099, 623 cm^{-1}; ^1H NMR (DMSO-d_6): 7.55 (d, J=8.0 Hz, 2H), 7.32 (br s, 2H), 7.25 (d, J=8.0 Hz, 2H), 2.61 (q, J=7.5 Hz, 2H), 1.98 (s, 3H), 1.19 (t, J=7.5 Hz, 3H). HRMS(EI) calcd for $C_{16}H_{15}BrN_2O_3S_2$: 427.9687, found: 427.9678.

The following compounds were prepared according to Method G:

5-Phenyl-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5a). Mp 132 °C (dec) (purified by column chromatography with 10% MeOH/$CHCl_3$). IR (KBr) 3412, 1591, 1495, 1451, 1414, 1281, 1246, 2236, 1099, 756 cm^{-1}; ^1H NMR (DMSO-d_6): 7.67 (d, J=8.0 Hz, 1H), 7.42 (d, J=8.0 Hz, 1H), 7.32–7.46 (m, 5H), 1.99 (s, 3H). Anal. calcd for $C_{14}H_{10}BrNaN_2O_3S_2$: C, 39.92; H, 2.39; N, 6.65; S, 15.22. Found: C, 40.36; H, 2.35; N, 6.61; S, 15.26.

4-[(4-Trifluoromethyl)phenyl]-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5e). Mp 156 °C (purified by column chromatography with 10% MeOH/$CHCl_3$). IR (KBr) 2700–3600, 1599, 1499, 1416, 1332, 1015, 918, 808, 745, 667 cm^{-1}; ^1H NMR (DMSO-d_6): 7.90 (d, J=8 Hz, 2H), 7.76 (d, J=8 Hz, 2H), 7.54 (d, J=3 Hz, 1H), 7.37 (d, J=3 Hz, 1H), 1.98 (s, 3H). Anal. calcd for $C_{15}H_9BrF_3NaN_2O_3S_2$: C, 36.82; H, 1.85; N, 5.72; S, 13.11. Found: C, 36.53; H, 2.11; N, 5.53; S, 13.40.

5-(2-Methoxyphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5f). Mp 172–174 °C (HPLC). IR (KBr) 3198, 1632, 1418, 1343, 1258, 1161, 1082, 1020 cm^{-1}; ^1H NMR ($CDCl_3$): 7.71 (s, 1H), 7.69 (d, J=3.1 Hz, 1H), 7.46 (d, J=3.9 Hz, 1H), 7.37 (t, J=8 Hz, 1H), 7.13 (br s, 1H), 7.00–7.08 (m, 2H), 3.98 (s, 3H), 2.24 (s, 3H). HRMS(EI) calcd for $C_{15}H_{13}BrN_2O_4S_2$: 429.9480, found: 429.9502. Anal. calcd for $C_{15}H_{13}BrN_2O_4S_2$: C, 41.96; H, 3.05; N, 6.52; S, 14.93. Found: C, 42.09; H, 3.15; N, 6.48; S, 15.18.

5-(3-Methoxyphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5g). Mp 112–114 °C (EtOAc/hexanes). IR (KBr) 3300–3600, 3066, 2870, 2822, 1626, 1473, 1397, 1167 cm^{-1}; ^1H NMR (DMSO-d_6): 7.56 (s, 2H), 7.38 (t, J=8.0 Hz, 1H), 7.24–7.28 (m, 2H), 6.95 (dd, J=2.0, 8.0 Hz, 1H), 3.82 (s, 3H), 2.12 (s, 3H). Anal. calcd for $C_{15}H_{13}BrN_2O_4S_2$: C, 41.96; H, 3.05; N, 6.52; S, 14.93. Found: C, 42.20; H, 3.06; N, 6.33; S, 14.78.

5-(4-Methoxyphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophensulfonamide (5h). Mp 128–130 °C (EtOAc/hexanes). IR (KBr) 3300–3600, 2930, 1624, 1505, 1429, 1416, 1352, 1256, 1161, 1082, 1031 cm^{-1}; ^1H NMR (DMSO-d_6): 7.66 (d, J=8.7 Hz, 2H), 7.55 (d, J=4 Hz, 1H), 7.44 (d, J=4 Hz, 1H), 7.02 (d, J=8.7 Hz, 2H), 3.81 (s, 3H), 2.14 (s, 3H). Anal. calcd for $C_{15}H_{13}BrN_2O_4S_2$: C, 41.96; H, 3.05; N, 6.52; S, 14.93. Found: C, 42.00; H, 3.16; N, 6.61; S, 14.80.

5-(4-Propylphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5j). Light-tan oil (HPLC). IR (KBr) 3463, 1588, 1428, 1291, 988 cm^{-1}; ^1H NMR (DMSO-d_6): 7.62 (d, J=8.5 Hz, 2H), 7.52 (d, J=3 Hz, 1H), 7.47 (d, J=3 Hz, 1H), 7.26 (d, J=8.5 Hz, 2H), 2.58 (t, J=6 Hz, 2H), 2.12 (s, 3H), 1.58 (q, J=6 Hz, 2H), 0.90 (t, J=6 Hz, 3H). HRMS(EI) calcd for $C_{17}H_{17}BrN_2O_3S_2$: 441.9844, found: 441.9837.

5-(4-Isopropylphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5k). Mp 114–116 °C (HPLC). IR (KBr) 3459, 1591, 1497, 1435, 1416, 1283, 1248, 1132, 1067 cm^{-1}; ^1H NMR (DMSO-d_6): 7.62 (d, J=8.5 Hz, 2H), 7.54 (d, J=3 Hz, 1H), 7.48 (d, J=3 Hz, 1H), 7.25 (d, J=8.5 Hz, 2H), 2.90 (*heptet*, J=6 Hz, 1H), 2.13 (s, 3H), 1.20 (d, J=6 Hz, 6H). HRMS(EI) calcd for $C_{17}H_{17}BrN_2O_3S_2$: 441.9844, found: 441.9829.

5-(4-Methoxycarbonylphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5l). Mp 158–160 °C (CH_3CN/H_2O/MeOH). IR (KBr) 2500–3200, 1719, 1624, 1431, 1422, 1279, 1163, 1090, 1024, 855, 768 cm^{-1}; ^1H NMR (DMSO-d_6): 8.02 (d, J=8.4 Hz, 2H), 7.88 (d, J=8.4 Hz, 2H), 7.70 (d, J=4.0 Hz, 1H), 7.60 (d, J=4.0 Hz, 1H), 3.88 (s, 3H), 2.12 (s, 3H). Anal. calcd for $C_{16}H_{13}BrN_2O_5S_2$: C, 42.02; H, 2.86; N, 6.12; S, 14.02. Found: C, 41.78; H, 3.07; N, 5.80; S, 14.04.

5-[3,5-Bis(trifluoromethyl)phenyl]-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5p). Mp 140 °C (dec) (HPLC). IR (KBr) 2500–3200, 1634, 1624, 1507, 1375, 1364, 1290, 1181, 1173, 1142, 1088, 1032 cm^{-1}; ^1H NMR (DMSO-d_6): 8.35 (s, 2H), 8.11 (s, 1H), 7.89 (d, J=3.9 Hz, 1H), 7.59 (d, J=3.9 Hz, 1H), 2.09 (s, 3H). HRMS(EI) calcd for $C_{16}H_9BrF_6N_2O_3S_2$: 535.9122, found: 535.9128. Anal. calcd for $C_{16}H_9BrF_6N_2O_3S_2$: C, 35.90; H, 1.69. Found: C, 35.84; H, 1.66.

5-(5-Methyl-2-thienyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5s). Mp 161–162 °C (dec) (HPLC). IR (KBr) 2400–3100, 2920, 2818, 2700, 1624, 1456, 1419, 1362, 1161, 1088, 1013, 853, 787 cm^{-1}; ^1H NMR (DMSO-d_6): 7.50 (d, J=3.8 Hz, 1H), 7.29 (d, J=3.6 Hz, 1H), 7.23 (d, J=3.8 Hz, 1H), 6.84 (d, J=3.6 Hz, 1H), 2.47 (s, 3H), 2.08 (s, 3H). HRMS(EI) calcd for $C_{13}H_{11}BrN_2O_3S_3$: 419.9095, found: 419.9099. Anal. calcd for $C_{13}H_{11}BrN_2O_3S_3$: C, 37.23; H, 2.64; N, 6.68. Found: C, 37.26; H, 2.52; N, 6.37.

5-(5-Ethyl-2-thienyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5t). Mp 126 °C (dec) (HPLC). IR (KBr) 2800–3700, 2920, 1591, 1497, 1452, 1416, 1285, 1258, 1134, 1096, 1065, 1011, 920, 619 cm^{-1}; ^1H NMR (DMSO-d_6): 7.25 (d, J=3.5 Hz, 1H), 7.15 (d, J=3.5 Hz, 1H), 7.06 (d, J=3.5 Hz, 1H), 6.83 (d, J=3.5 Hz, 1H), 2.81 (q, J=7.5 Hz, 2H), 1.99 (s, 3H), 1.24 (t, J=7.5 Hz, 3H). HRMS(EI) calcd for $C_{14}H_{13}BrN_2O_3S_3$: 433.9251, found: 433.9236.

5-(3-Thienyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5u). Mp 129–131 °C (HPLC). IR (KBr) 3300–3500, 1624, 1418, 1362, 1161, 1088 cm^{-1}; ^1H NMR (DMSO-d_6): 7.96 (br s, 2H), 7.69 (dd, J=3, 5 Hz, 1H), 7.48–7.52 (m, 2H), 7.41 (d, J=3 Hz, 1H), 2.12 (s, 3H). Anal. calcd for $C_{12}H_9BrN_2O_3S_3$: C, 35.56; H, 2.24; N, 6.91; S, 23.74. Found: C, 35.42; H, 2.23; N, 6.68; S, 24.03.

5-(1-Napthyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5x). mp 110 °C (dec) (HPLC). IR (KBr) 2600–3600, 1589, 1414, 1263, 1130, 1094, 1011, 920, 774, 650, 608 cm^{-1}; ^1H NMR (DMSO-d_6): 8.15 (dd, J=3.2, 6.2 Hz, 1H), 7.99–8.05 (m, 2H), 7.58 (AB q, J=2 Hz, 4H), 7.47 (d, J=3.7 Hz, 1H), 7.22 (d, J=3.7 Hz, 1H), 2.01 (s, 3H). Anal. calcd for $C_{18}H_{13}BrN_2O_3S_2 \cdot 1.0TFA$: C, 42.64; H, 2.50. Found: C, 42.28; H, 2.86.

5-(Benzo[b]thien-2-yl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5y). mp 164 °C (HPLC). IR (KBr) 2900–3600, 1628, 1424, 1414, 1358, 1165, 1186, 1017, 855, 606 cm^{-1}; ^1H NMR (DMSO-d_6): 7.99 (d, J=7 Hz, 1H), 7.87 (d, J=7 Hz, 1H), 7.83 (s, 1H), 7.53 (br s, 1H), 7.40–7.53 (m, 3H), 2.11 (s, 3H). HRMS(EI): calcd for $C_{16}H_{11}BrN_2O_3S_3$: 455.9095, found: 455.9107. Anal. calcd for $C_{16}H_{11}BrN_2O_3S_3$: C, 42.20; H, 2.43; N, 6.15; S, 21.12. Found: C, 42.20; H, 2.42; N, 5.81; S, 21.07.

Method H

5-(4-Carboxyphenyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5m). A solution of lithium hydroxide (13 mg, 0.32 mmol) in MeOH (2 mL) was added to a solution of **5l** (121 mg, 0.27 mmol) in MeOH (5 mL). The solution was stirred at room temperature for 18 h and the MeOH removed in vacuo. The residue was dissolved in water, acidified to pH 2 with 4 N HCl and extracted with EtOAc (3×25 mL). The combined organic phase was washed with water (10 mL), brine (10 mL) and dried over $MgSO_4$. Evaporation of the solvent gave 50 mg (43% yield) of a pale-yellow solid, which was further purified by preparative HPLC to give a white solid, mp 219–220 °C. IR (KBr) 2400–3500, 3217, 1680, 1620, 1607, 1425, 1408, 1397, 1348, 1321, 1302, 1163, 1078, 1024, 694, 617 cm^{-1}; ^1H NMR (DMSO-d_6): 7.99 (d, J=8.3 Hz, 2H), 7.85 (d, J=8.3 Hz, 2H), 7.67 (d, J=3.6 Hz, 1H), 7.58 (d, J=3.6 Hz, 1H), 2.13 (s, 3H). Anal. calcd for $C_{15}H_{11}BrN_2O_5S_2$: C, 40.64; H, 2.50; N, 6.32; S, 14.47. Found: C, 40.65; H, 2.31; N, 5.91; S, 14.43.

Method I

(a) 5-(5-Methyl-2-furyl)-2-thiophenesulfonyl chloride. *tert*-Butyllithium (1.7 M solution in pentane, 7.9 mL, 14.6 mmol) was added dropwise under a nitrogen atmosphere to a solution of 2-methylfuran (1.0 g, 12 mmol) in THF (20 mL) at −78 °C. The solution was warmed to −10 °C and stirring continued for 45 min. The solution was added to an anhydrous solution of zinc chloride in THF (0.5 M, 27 mL, 13.5 mmol) at −30 °C and slowly warmed to room temperature. Stirring was continued for 1 h, resulting in a pale yellow clear solution. The solution was then transferred via a steel cannula under nitrogen to a solution of *N*-(5-bromo-2-thiophenesulfonyl)pyrrole (3.5 g, 12.0 mmol) and tetrakis(triphenylphosphine)palladium(0) (693 mg, 0.6 mmol) in THF (15 mL) at −78 °C. The solution was warmed to room temperature and stirred for 2 h. Purification by column chromatography using 2% EtOAc/hexanes gave 680 mg (19% yield) of *N*-[5-(5-methyl-2-furyl)-2-thiophenesulfonyl]pyrrole as a pale-yellow powder.

Using to the procedures of Method G, Part (a) *N*-[5-(5-methyl-2-furyl)-2-thiophenesulfonyl]pyrrole (300 mg, 1.02 mmol) was converted to 5-(5-methyl-2-furyl)-2-thiophenesulfonyl chloride as a pale yellow solid in 53% yield after column chromatography using 2% EtOAc/hexanes.

(b) 5-(5-Methyl-2-furyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5q). Reaction of 5-(5-methyl-2-furyl)-2-thiophenesulfonyl chloride (55 mg, 0.21 mmol) with 5-amino-4-bromo-3-methylisoxazole (41 mg, 0.21 mmol) as described in Method C gave, after purification by column chromatography (10% MeOH/$CHCl_3$), 45 mg (54% yield) of **5q** as a brown solid, mp 123–124 °C. IR (KBr) 3000–3600, 1592, 1427, 1416, 1260, 1130, 1099, 1007, 606 cm^{-1}; ^1H NMR (DMSO-d_6): 7.27 (d, J = 3.8 Hz, 1H), 7.12 (d, J = 3.8 Hz, 1H), 6.71 (d, J = 3.0 Hz, 1H), 6.20 (d, J = 3,0 Hz, 1H), 2.32 (s, 3H), 1.98 (s, 3H). HRMS(FAB) calcd for $C_{13}H_{12}BrN_2O_4S_2$ ($[M+1]^+$): 402.9422, found: 402.9440. Anal. calcd for $C_{13}H_{11}BrN_2O_4S_2 \cdot 1.0TFA$: C, 34.83; H, 2.34. Found: C, 34.95; H, 2.74.

Method J

(a) (2,2′-Bithiophene)-5-sulfonyl chloride. *tert*-Butyllithium (1.7 M solution in pentane, 4.3 mL, 7.3 mmol) was added over 20 min to a cold (−78 °C), stirred solution of 5-bromo-2,2′-bithiophene[30] (1.5 g, 6.1 mmol) in anhydrous Et_2O (10 mL) under an argon atmosphere. Stirring was continued at this temperature for an additional 20 min. Sulfur dioxide gas was then bubbled in at −78 °C until a yellow precipitate was formed. Bubbling of sulfur dioxide gas was continued for an additional 3 min and a solution of *N*-chlorosuccinimide (NCS, 902 mg, 6.8 mmol) in THF (5 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirring was continued for an additional 1.5 h. The mixture was then concentrated and the residue dissolved in ether. The organic layer was washed with water, brine and dried over $MgSO_4$. Evaporation of solvent left a pale yellow solid, which was recrystallized from hexane to give 700 mg (44%) of a yellow solid, mp 63–64 °C.

(b) 5-(2-Thienyl)-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5r). (The title compound was prepared in the same manner as described in Method C from (2,2′-bithiophene)-5-sulfonyl chloride (300 mg, 1.14 mmol) and 5-amino-4-bromo-3-methylisoxazole (183 mg, 1.03 mmol). After purification by column chromatography (10% MeOH/$CHCl_3$), 430 mg (94%) of a pale brown solid was obtained which was further purified by recrystallization form EtOAc/hexanes, mp 210 °C (dec). IR (KBr) 3000–3500, 3102, 3059, 1701, 1593, 1495, 1447, 1416, 1292, 1226, 1099, 1076, 1064, 1013 cm^{-1}; ^1H NMR (DMSO-d_6): 7.54 (d, J = 6 Hz, 1H), 7.35 (d, J = 5 Hz, 1H), 7.26 (d, J = 5 Hz, 1H), 7.13 (d, J = 5 Hz, 1H), 7.09 (dd, J = 5, 6 Hz, 1H), 5.80 (br s, 1H), 1.98 (s, 3H). HRMS(FAB) calcd for $C_{12}H_{10}BrN_2O_3S_3$ ($[M+1]^+$): 404.9037, found: 404.9052. Anal. calcd for $C_{12}H_9BrN_2O_3S_3 \cdot 1.0H_2O$: C, 34.04; H, 2.62; N, 6.62; S, 20.72. Found: C, 34.11; H, 2.49; N, 6.41; S, 20.55.

Method K

(a) 5-Benzyl-2-thiophenesulfonyl chloride. To a solution of 2-benzylthiophene (0.875 g, 5.0 mmol) in $CHCl_3$ (2 mL) at 0 °C was added chlorosulfonic acid dropwise and the reaction stirred at 0 °C for 30 min. The reaction mixture was poured onto crushed ice (20 g) and after the ice had melted, was extracted with EtOAc, dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure to give 5-benzyl-2-thiophenesulfonic acid.

Phosphorous pentachloride (2.08 g, 40 mmol) was added to a solution of 5-benzyl-2-thiophenesulfonic acid in phosphorous oxychloride (6.0 g, 40 mmol) at 0 °C. The reaction mixture was kept at 50 °C for 1 h, cooled to room temperature, poured onto crushed ice (50 g) and extracted with EtOAc (2×30 mL). Removal of the solvent under reduced pressure gave a crude product, which was purified by column chromatography (3% EtOAc in hexane) to give 5-benzyl-2-thiophenesulfonyl chloride (0.60 g, 39% yield).

(b) 5-Benzyl-*N*-(4-bromo-3-methyl-5-isoxazolyl)-2-thiophenesulfonamide (5w). The title compound was prepared in the same manner as described in Method C from

5-amino-4-bromo-3-methylisoxazole and 5-benzyl-2-thiophenesulfonyl chloride in 22% yield. The product was purified by HPLC to give a solid, mp 49–51 °C. IR (KBr): 3300–3600, 3098, 3025, 1630, 1416, 1360, 1173, 1086, 1015 cm^{-1}; ^1H NMR (DMSO-d_6): 7.44 (d, J = 3.7 Hz, 1H), 7.20–7.38 (m, 5H), 6.97 (d, J = 3.7 Hz, 1H), 4.22 (s, 2H), 2.14 (s, 3H). Anal. calcd for $C_{16}H_{16}BrN_2O_3S_2$: C, 44.86; H, 3.76; N, 6.54; S, 14.97. Found: C, 44.76; H, 3.96; N, 6.29; S, 15.23.

Membrane preparation

A membrane preparation containing human ET_B receptor was prepared from COS 7 cells which were transfected with DNA encoding the human ET_B receptor as described previously.[33] 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 being resuspended in 30 mM HEPES buffer, pH 7.4, containing aprotinin (100 KIU/mL) to give a final membrane concentration of 2 mg/mL. Aliquots of membrane were stored at −70 °C until use. Protein content was determined using the Pierce BCA assay kit with bovine serum albumin (BSA) as a standard.

A membrane preparation containing human ET_A receptors was prepared as described above from TE 671 cells (ATCC # HTB 139) which naturally express ET_A receptors.[33]

Receptor 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 4 °C, followed by addition of 1 mL PBS and centrifugation at 2000 g for 25 min at 0 °C. The supernatant was decanted and the membrane bound radioactivity counted on a Genesys gamma counter.

Phosphoinositide hydrolysis in cells

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 as described before.[16] The total cell protein in each well was measured using the Pierce BCA assay after solubilizing the cells in 0.1 M NaOH.

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