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THIOPHENESULFONAMIDES AS ENDOTHELIN RECEPTOR ANTAGONISTS[1]

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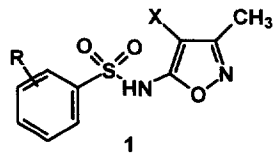
Abstract: The synthesis and in vitro binding affinities of a series of thiophenesulfonamides as ET_A selective endothelin receptor antagonists is described. The most potent inhibitor displayed an IC_{50} of 43 nM and 3 μM to ET_A and ET_B receptors, respectively.

In 1988 Yanagisawa and coworkers isolated endothelin-1 and subsequently two more isopeptides, endothelin-2 and endothelin-3, were identified.[2] These unique bicyclic peptides containing two disulfide bridges are the most potent endogenous vasoactive peptides known. Another group of polypeptides, the sarafotoxins,[3] have a high degree of primary sequence homology with the endothelins and show similar biological function. Both endothelins and sarafotoxins are known to mediate their biological functions through cell surface receptors, namely ET_A and ET_B, although other receptor subtypes have been identified.[4] Various pharmacological actions have been attributed to the endothelins.[5] The recent disclosure of a number of small molecule antagonists[6] provides useful pharmacological tools to study the biological roles of endothelins and may have clinical potential in the therapeutic intervention of endothelin mediated disorders. As part of our ongoing program,[7] we have designed thiophenesulfonamides as endothelin receptor antagonists based on molecular modeling and isosteric replacement of the phenyl ring in benzenesulfonamide[7] endothelin receptor antagonists (Figure 1) with a thiophene ring.



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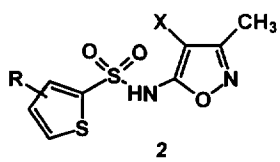
X = Br, Cl, CH_3



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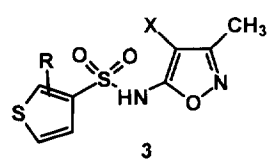

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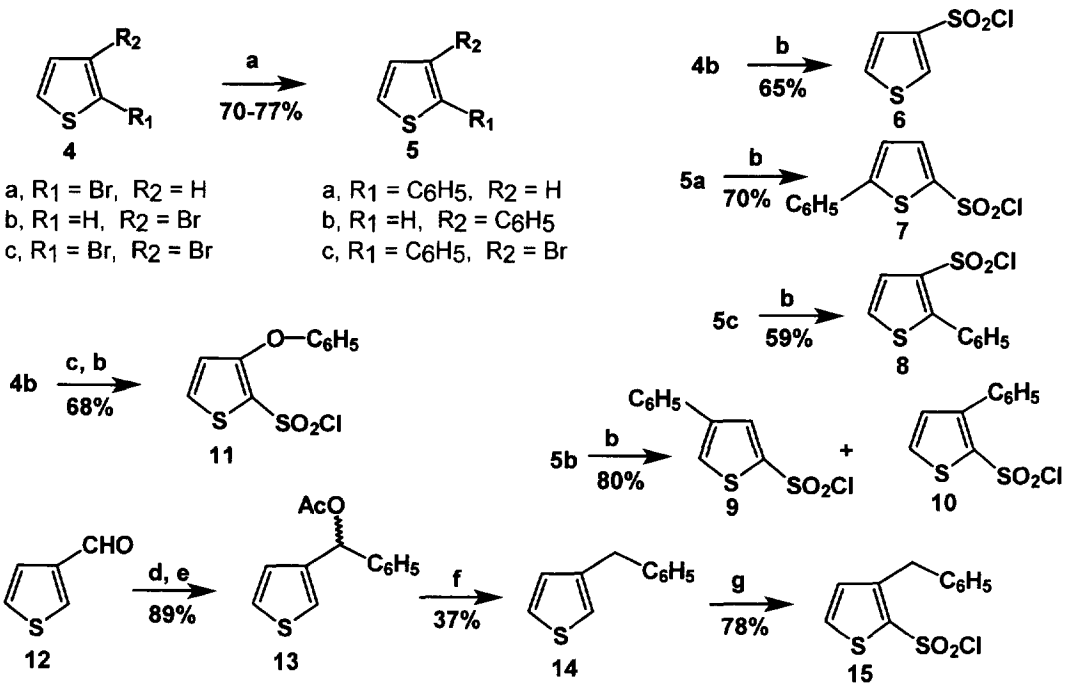
Figure 1

Retrosynthetic analysis of thiophenesulfonamides **2** and **3** (Figure 1) leads to substituted sulfonyl chloride and isoxazoleamine moieties. Substituted thiophenesulfonyl chlorides were prepared as described in Schemes I and II.[8,9] Phenylthiophenes **5a** and **5b** (Scheme I) were prepared from 2-bromothiophene and 3-bromothiophene, respectively, under Suzuki coupling conditions[10] using phenylboronic acid. Under similar reaction conditions, 2,3-dibromothiophene was coupled with phenylboronic acid to get 3-bromo-2-phenylthiophene **5c** as a single product. Thiophenes **4b**, **5a-c** were treated with n-butyllithium, the anions quenched with sulfur dioxide and further oxidation of the resultant sulphinates with N-chlorosuccinimide gave

the sulfonyl chlorides,[9a] either as a single isomer (**6**, **7**, and **8**) or as a mixture of regioisomers (**9** and **10**). 3-Phenoxythiophene was prepared from 3-bromothiophene and phenol using cuprous chloride in pyridine as the solvent. Regioselective deprotonation with n-butyllithium, followed by trapping of the anion with sulfur dioxide and further oxidation of the sulphinate gave the sulfonyl chloride[9a] **11**. The acetate **13**, which was obtained from thiophene-3-carboxaldehyde by treatment with phenylmagnesium bromide and subsequent acetylation of the alcohol with acetic anhydride in pyridine, was reductively cleaved using lithium in liquid ammonia to get 3-benzylthiophene **14**. This was subjected to regioselective chlorosulphonation using chlorosulphonic acid to obtain the sulfonyl chloride[9b] **15**.

Scheme I

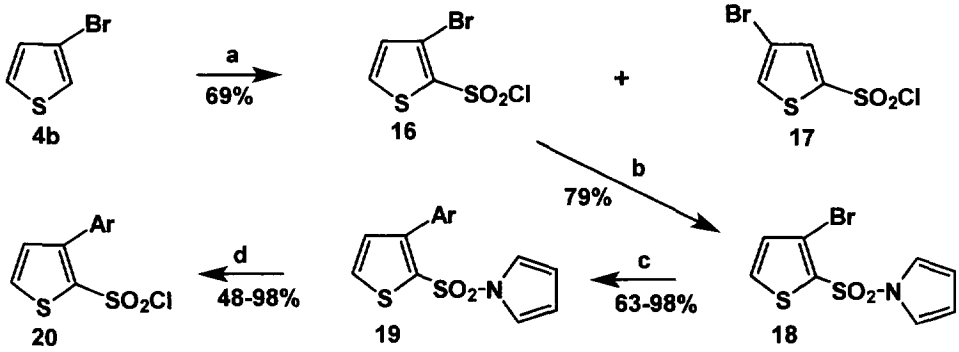


Reagents: (a) $C_6H_5B(OH)_2$, $Pd(PPh_3)_4$, toluene, EtOH, 2 M Na_2CO_3, 90 °C; (b) n-BuLi, THF, -78 °C; SO_2 gas; N-chlorosuccinimide; (c) phenol, CuCl, pyridine, 100 °C; (d) C_6H_5MgBr, ether, rt; (e) Ac_2O, pyridine 100 °C; (f) Li, liquid NH_3; (g) 1.1 equiv $ClSO_3H$, CH_2Cl_2, -15 °C to rt; $POCl_3$, PCl_5, rt.

3-Arylthiophene-2-sulfonyl chlorides were prepared as outlined in Scheme II. Regioselective chlorosulfonation of 3-bromothiophene with chlorosulfonic acid gave a 10:1 mixture of sulfonyl chlorides **16** and **17**, respectively, which were separated by flash column chromatography.[9c] Protection of the sulfonyl group with pyrrole[11] gave **18**, which was subjected to Suzuki coupling using substituted phenylboronic acids[12] to get **19**. Removal of the pyrrole protecting group in **19** by basic hydrolysis,[11] followed by conversion of the resultant sulfonic acid salt by treatment with phosphorus oxychloride and phosphorus pentachloride gave the

sulfonyl chlorides **20**. Sulfonyl chlorides **21a-v**, **23a**, and **23b** were coupled with 5-amino-4-bromo-3-methylisoxazole using sodium hydride as base[7] to afford sulfonamides[13] **22a-v**, **24a**, and **24b** (Scheme III).

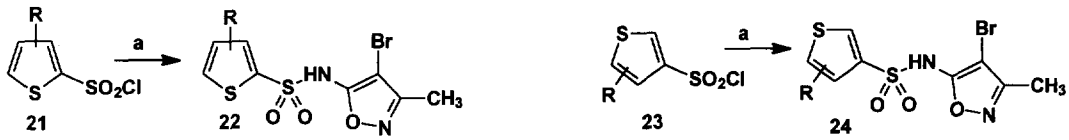
Scheme II



Reagents: (a) $ClSO_3H$, CH_2Cl_2, -78 °C to rt; (b) NaH, THF, pyrrole, 0 °C to rt; (c) arylboronic acids[12] $Pd(PPh_3)_4$, toluene, EtOH, 2M Na_2CO_3, 90 °C; (d) NaOH, EtOH, reflux; $POCl_3$, PCl_5, rt.

The binding affinity of the sulfonamides were evaluated in vitro using a competitive inhibition radio receptor assay for both human ET_A and ET_B receptors.[14] The IC_{50} values are tabulated in Table 1. The binding affinities of thiophenesulfonamides **22a** and **24a** to the ET_A receptor are very similar to that of benzenesulfonamide **25a**. This observation is parallel with the isosteric nature of benzene with thiophene. The early success in identifying thiophenesulfonamides **22a** and **24a** as promising leads prompted us to develop structure-activity relationship (SAR) studies of the thiophenesulfonamides. Our earlier studies[15] on benzenesulfonamides showed that 2-biphenylsulfonamide **25b** is a potent ET_A selective ligand whereas 4-biphenylsulfonamide is an ET_B selective ligand. Accordingly, four of the several possible regioisomers of phenylthiophenesulfonamides were synthesized (compounds **22b-d** and **24b**). As anticipated, compounds **22b** and **24b** are ET_A selective and **22d** is ET_B selective with modest potency. Surprisingly, the thiophenesulfonamides **22b** and **24b** are about 40- to 50-fold less active than the corresponding 2-biphenylsulfonamide **25b**. Increasing the distance between the phenyl and thiophene rings by the insertion of an oxygen atom, analogue **22u**, or a methylene group, analogue **22v**, did not significantly change the binding affinity compared to the sulfonamide **22b**. A series of analogues of 3-phenylthiophenesulfonamide **22b** were synthesized for further SAR development. Studies on the 3-(2-tolyl)- (**22e**), 3-(3-tolyl)- (**22f**) and 3-(4-tolyl)thiophene-2-sulfonamide (**22g**) have shown that the *ortho* and *para* substituted analogues **22e** and **22g** display higher affinity to the ET_A receptor than the *meta* substituted analogue **22f**. Similar studies using 3-(methoxyphenyl)thiophene-2-sulfonamides **22h-j** have indicated that the *para* substituted derivative **22j** has a higher affinity to the ET_A receptor than both *ortho* and *meta*-substituted sulfonamides **22h** and **22i**.

Scheme III



a. R = H, 35%
b. R = 3-C6H5, 35%
c. R = 4-C6H5, 15%
d. R = 5-C6H5, 59%
e. R = 3-C6H4(2-CH3), 70%
f. R = 3-C6H4(3-CH3), 65%
g. R = 3-C6H4(4-CH3), 67%
h. R = 3-C6H4(2-OCH3), 72%
i. R = 3-C6H4(3-OCH3), 80%
j. R = 3-C6H4(4-OCH3), 79%
k. R = 3-C6H4(4-Et), 58%
l. R = 3-C6H4(4-*n*Pr), 71%
m. R = 3-C6H4(4-*i*Pr), 75%
n. R = 3-C6H4(4-*n*Bu), 67%
o. R = 3-C6H4(4-*i*Bu), 36%
p. R = 3-C6H4(4-*i*Pen), 39%
q. R = 3-C6H3(2,4-di-CH3), 67%
r. R = 3-C6H3(2-CH3, 4-*n*Pr), 53%
s. R = 3-C6H3(2-CH3, 4-*i*Bu), 50%
t. R = 3-C6H3(2-CH3, 4-*i*Pen), 43%
u. R = 3-OC6H5, 58%
v. R = 3-CH2C6H5, 24%

a. R = H, 62%
b. R = 2-C6H5, 55%

a. R = H, 71%
b. R = C6H5, 30%

Reagents: a. NaH, THF, 5-amino-4-bromo-3-methylisoxazole,[7] 0 °C to rt.

Table1. IC_{50} Values for thiophenesulfonamides

No.	IC_{50} (μM)[a] ET_A	ET_B	% Purity[c]	R_t[d]	No.	IC_{50} (μM)[a] ET_A	ET_B	% Purity[c]	R_t[d]
22a	1.91	>100[b]	98	15.06	22n	0.1596	6.1067	100	26.35
22b	0.819	64.45	98	20.04	22o	0.0823	2.895	100	25.80
22c	3.39	13.50	98	21.35	22p	0.182	3.275	100	26.55
22d	36.60	2.40	100	21.59	22q	0.1002	60.3	99	22.98
22e	0.306	>100[b]	98	21.68	22r	0.04285	2.91	97	25.50
22f	0.832	21.20	100	22.12	22s	0.0479	1.505	97	27.17
22g	0.3155	95.55	100	21.47	22t	0.115	2.45	97	28.38
22h	1.71	59.1	100	21.63	22u	1.45	35.5	100	19.84
22i	1.32	56.25	96	21.62	22v	2.603	39.77	100	21.46
22j	0.334	62.05	100	20.48	24a	2.75	>100	99	16.22
22k	0.184	43.85	100	23.98	24b	0.603	74.10	98	21.04
22l	0.0873	8.16	100	25.32	25a	2.51	>100[b]	100	20.77
22m	0.2175	28.25	100	25.15	25b	0.0162	>100[b]	96	16.63

[a] an average of two runs; [b] highest concentration of sulfonamide present in assay mixture; [c] Analytical HPLC[13]; [d] Retention time in minutes.

In continuation of SAR studies to further improve the binding affinity, a series of *para* substituted phenylthiophenesulfonamides **22k-p** were synthesized. There was a modest increase in the ET_A binding affinity as the length of the *para*-alkyl chain increased. The ET_A affinity was optimum with 4-n-propyl and 4-isobutyl substitutents as seen in sulfonamides **22l** and **22o**, respectively. The branched chain analogues **22m** and **22p** showed no improvement in binding affinity. The modest increase in the ET_A binding affinity of 3-(2-tolyl)thiophene-2-sulfonamide **22e** and 3-(4-tolyl)thiophene-2-sulfonamide **22g** over 3-phenylthiophene-2-

sulfonamide **22b** prompted us to study the cumulative effect of the dimethyl derivative **22q** on the binding affinity. Thus the analogue **22q** was about 3-fold better than the monosubstituted derivatives **22e** and **22g**. The optimized alkyl groups at the 4-position and methyl group at the 2-position on the phenyl ring were incorporated in analogues **22r-t**. Both the sulfonamides **22r** and **22s** showed slightly better potency than **22l** and **22o**, respectively. The ET_B receptor binding affinity of phenylthiophenesulfonamides (Table 1) varied from 1.5 μM to being undetectable at 100 μM.

In conclusion, the phenylthiophenesulfonamides **22b** and **24b** are less potent than the corresponding 2-biphenylsulfonamide **25b**. However, through systematic studies, the binding affinity of the thiophenesulfonamide **22a** was improved from micromolar to low nanomolar affinity to the ET_A receptor (**22r-s**). The phenylthiophenesulfonamides can be either ET_A or ET_B selective endothelin receptor antagonists which is determined by the substitution pattern between the sulfonylisoxazole and aryl moieties on the thiophene ring.

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References and Notes

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9. (a) To a stirred solution of 3-bromothiophene (1.5 g, 9.2 mmol) in dry ether (15 mL) at -78 °C was added *t*-BuLi (5.95 mL of 1.7 M in hexane, 10 mmol) dropwise. The reaction mixture was stirred at -78 °C for 30 min. Sulfur dioxide gas was bubbled in slowly for 10 min and the reaction mixture was allowed to attain room temperature slowly. N-Chlorosuccinimide (1.51 g, 11.04 mmol) was added and after 1 h the reaction mixture was diluted with water and extracted with methylene chloride (2 x 40 mL). The combined organic

layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product was purified by flash column chromatography using 2% ethyl acetate in hexane as eluent to obtain thiophene-3-sulfonyl chloride (1.24 g, 74%).

(b) To a stirred solution of 3-benzylthiophene (0.875 g, 5 mmol) in methylene chloride (2 mL) at -5 °C was added chlorosulfonic acid (0.33 mL, 5 mmol). The resultant reaction mixture was stirred at -5 °C for 30 min. To this was added phosphorusoxy chloride (2 mL) and phosphorus pentachloride (1.5 g) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured onto ice and stirred for 1h and was extracted with methylene chloride (2 x 50 mL). The combined organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The residue was purified by flash column chromatography using 2% ethyl acetate in hexane as eluent to afford 3-benzylhiophene-2-sulfonyl chloride (1.2 g, 78%).

(c) To a stirred solution of 3-bromothiophene (20 g, 123 mmol) in methylene chloride (50 mL) at -78 °C was added chlorosulfonic acid (50 mL, 756 mmol) dropwise over 1 h. This mixture was slowly allowed to attain ambient temperature where stirring continued for 3 h. This was then carefully poured onto ice (1500 g) and extracted with methylene chloride (4 x 100 mL). The combined organic layer was dried over anhydrous magnesium sulfate, filtered and the solvent removed under reduced pressure. The residue was purified by flash column chromatography using 3% ethyl acetate in hexane as eluent to obtain 4-bromothiophene-2-sulfonyl chloride (1.6 g, 5%) and 3-bromothiophene-2-sulfonyl chloride (20.5 g, 64%)

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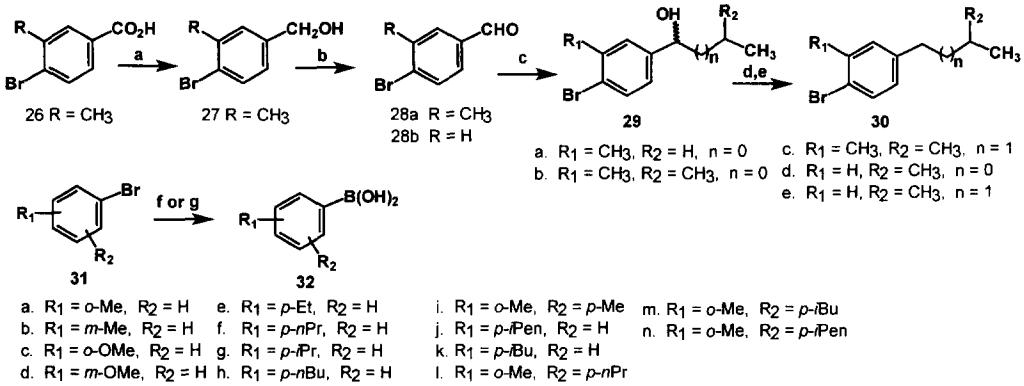
12. Benzeneboronic acid, 4-methyl- and 4-methoxyphenylboronic acids were purchased from a commercial source (Lancaster). Substituted phenylboronic acids were prepared as described below.



Reagents: (a) LAH, ether, rt; (b) MnO_2, CH_2Cl_2, rt; (c) ethylmagnesium bromide or isopropylmagnesium chloride or isobutylmagnesium chloride, ether, rt; (d) Ac_2O, pyridine, 80 °C; (e) Et_3SiH, $BF_3.OEt_2$, rt; (f) Mg, THF or ether; triisopropyl borate, -78 °C to rt; H^+; (g) n-BuLi, THF, -78 °C ; triisopropyl borate, -78 °C to rt; H^+.

13. All sulfonamides were characterized by high resolution FAB MS analysis, high field PMR, IR. The purities of sulfonamides were determined by analytical HPLC on C18 reverse phase column (250 mm X 4.6 mm) eluting with water and acetonitrile mixture containing 0.1% TFA from 5% acetonitrile to 95% acetonitrile (Linear gradient) over 30 min period.
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