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SEARCH FOR SURROGATES: A STUDY OF ENDOTHELIN RECEPTOR ANTAGONIST STRUCTURE ACTIVITY RELATIONSHIPS[1]

B. Raju,*[#] Ilya Okun, Fiona Stavros,[#] and Ming Fai Chan
ImmunoPharmaceutics Inc. (A Wholly-owned Subsidiary of Texas Biotechnology Corp.), 11011 Via Frontera, San Diego, CA 92127, USA

Abstract: The aryloxymethylene group was used as a replacement for an ester or amide bond present in a series of ET_A selective endothelin antagonists. The effect of such replacement on the binding affinity is described. © 1997 Elsevier Science Ltd.

The endothelins[2] (ETs) and sarafotoxins,[3] a family of bicyclic polypeptides with 21 amino acids, are the most potent vasoconstrictors known. The knowledge that these are widely distributed in various tissues and that elevated levels of endothelins have been associated with a variety of diseases suggests that regulation of these polypeptides or their receptor binding are attractive therapeutic targets.[4] ETs are known to mediate their biologic functions through specific cell surface receptors, namely ET_A and ET_B.[5] The recent discovery of selective as well as nonselective nonpeptide endothelin receptor antagonists[6,7] may help in the identification of specific roles for individual endothelins and receptors in endothelin mediated disorders and further lead to clinically valuable endothelin receptor antagonists.

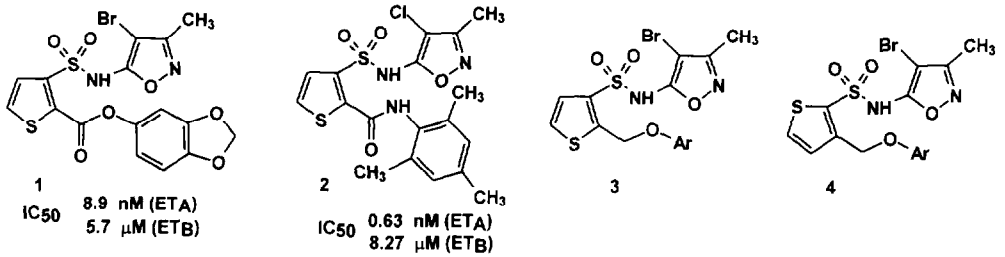


Figure 1

Recent publications from this laboratory have described a series of 2-aryloxycarbonylthiophene-3-sulfonamides[7c] **1** and 2-arylaminocarbonylthiophene-3-sulfonamides[7d] **2** (Figure 1) as potent and ET_A selective endothelin receptor antagonists. Although the sulfonamides **1** and **2** are high affinity ligands to the ET_A receptor, based on in vitro binding assays, their in vivo efficacy was negligible. One of the reasons may be the proteolytic liability of the ester and amide functional groups present in these classes of compounds, which prompted us to investigate replacement with stable surrogates. In this communication, we report the effect of replacement of -COOAr group with $-CH_2$-O-Ar, an ether linkage,[8] on binding affinity. The ether linkage

provides information on the effect of the carbonyl group in 2-aryloxycarbonylthiophene-3-sulfonamides on binding affinity.

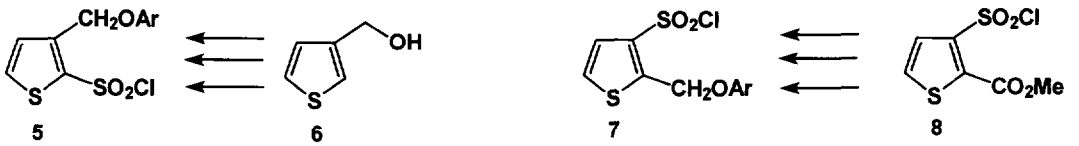
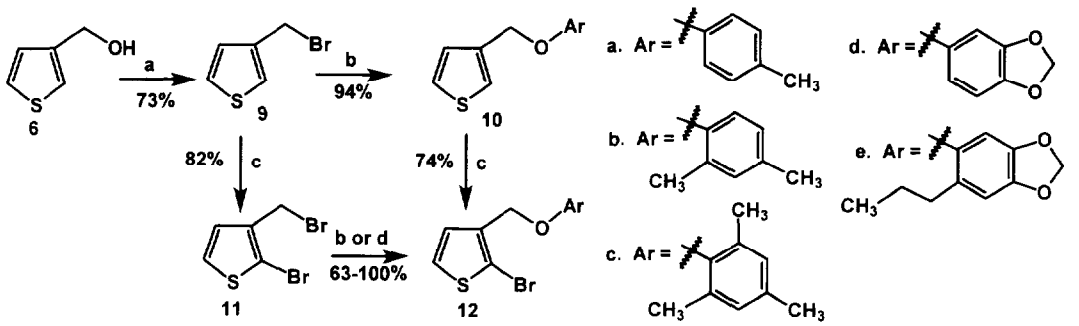


Figure 2. Retrosynthetic Analysis

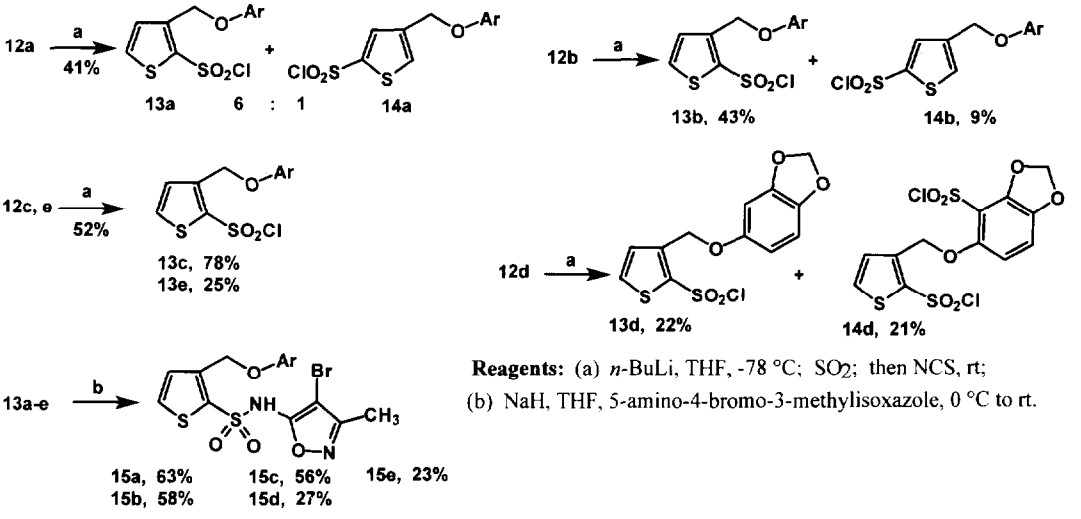
The regioisomeric thiophenesulfonamides **3** and **4** (Figure 1) were synthesized from the sulfonyl chloride and isoxazoleamine moieties. Thus, the required sulfonyl chlorides were derived from commercially available thiophene derivatives **6** and **8** as summarized in Figure 2.

Scheme I



Reagents: (a) Br_2, PPh_3, CH_2Cl_2, pyridine, 0 °C; (b) ArOH, NaH, THF or DMF, 0°C to rt; (c) NBS, AcOH : $CHCl_3$ (1:1), rt; (d) ArOH, K_2CO_3, acetone, reflux.

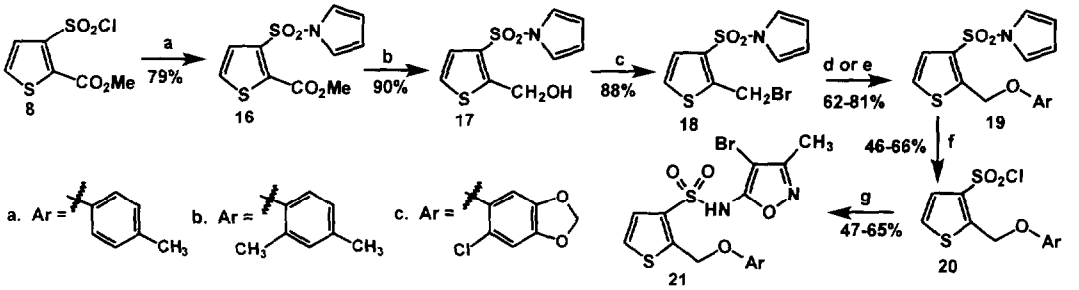
Scheme II



Reagents: (a) *n*-BuLi, THF, -78 °C; SO_2; then NCS, rt;
(b) NaH, THF, 5-amino-4-bromo-3-methylisoxazole, 0 °C to rt.

The synthesis of 3-aryloxymethylthiophene-2-sulfonamides is summarized in the Schemes I and II. 3-Bromomethylthiophene **9**, obtained from 3-hydroxymethylthiophene **6** by treatment with bromine-triphenylphosphine adduct, was used to alkylate *p*-cresol to obtain 3-[(4-methylphenoxy)methyl]thiophene **10a**. Bromination of the ether **10a** under acidic conditions using NBS gave 2-bromo-3-[(4-methylphenoxy)methyl]thiophene **12a** as the major product.[9] Alternatively, 2-bromo-3-aryloxymethylthiophenes **12b-e** were synthesized by alkylation of substituted phenols with 2-bromo-3-bromomethylthiophene **11**, which was prepared from 3-bromomethylthiophene **9** by reacting with NBS under acidic conditions.[9] The bromine substituent on the thiophenes **12a-e** was utilized to introduce the sulfonyl chloride functionality in the 2-position.[7b] This was achieved by treatment with *n*-butylithium to effect lithium-halogen exchange, followed by quenching of the anion with sulfur dioxide and oxidation of the resultant sulphinates to sulfonyl chlorides **13a-e** using NCS in a one-pot reaction. In some cases, other regioisomeric sulfonyl chlorides were also isolated along with the desired sulfonyl chlorides. The regioisomeric sulfonyl chlorides were separated by flash column chromatography, except **13a** and **14a** which were used as a mixture in the next step. The sulfonyl chlorides **13a-e** were reacted with 5-amino-4-bromo-3-methylisoxazole[7k] using sodium hydride as a base to afford sulfonamides[10] **15a-e**.

Scheme III

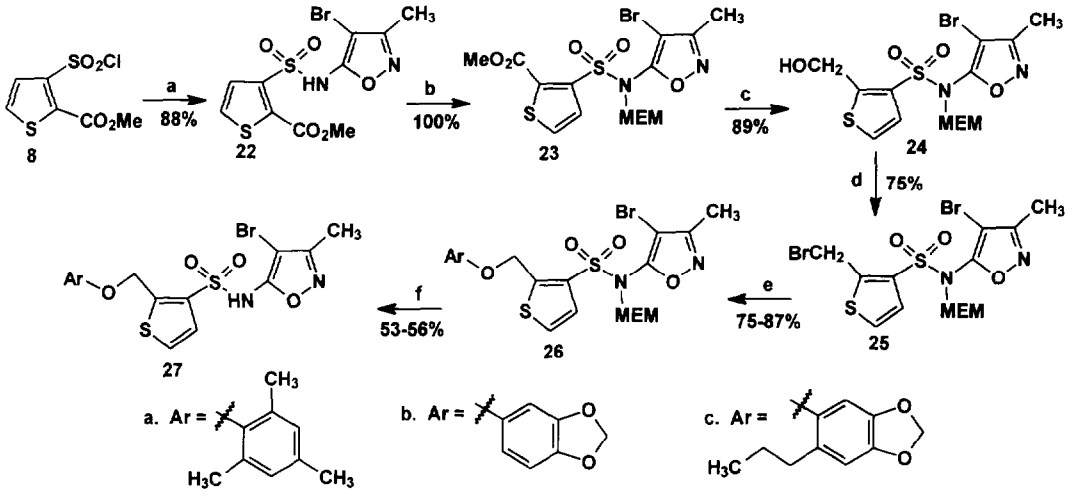


Reagents: (a) NaH, pyrrole, THF, 0 °C to rt; (b) $NaBH_4$, MeOH, THF, rt; (c) PPh_3, Br_2, CH_2Cl_2, pyridine, 0 °C; (d) ArOH, K_2CO_3, acetone, reflux; (e) ArOH, NaH, THF or DMF, 0°C to rt; (f) KOH, MeOH, H_2O, reflux; $POCl_3$, PCl_5, rt; (g) NaH, THF, 5-amino-4-bromo-3-methylisoxazole, 0 °C to rt.

2-Methoxycarbonylthiophene-3-sulfonyl chloride **8** was used as a starting material in the synthesis of 2-aryloxymethylthiophene-3-sulfonamides **21a-c** and **27a-c** as outlined in e Schemes III and IV, respectively. Protection of the sulfonyl group in 2-methoxycarbonylthiophene-3-sulfonyl chloride **8** with pyrrole[11] gave N-(2-methoxycarbonylthiophene-3-sulfonyl)pyrrole **16** in good yield. Reduction of the ester group in **16**, using sodium borohydride in methanol and THF mixture, followed by conversion of hydroxymethyl to bromomethyl gave the synthon **18** in excellent yield. Etherification of substituted phenols using bromomethylthiophene sulfonamide **18** gave the ethers **19a-c**. The subsequent transformation of these derivatives **19a-c** to the required sulfonyl chlorides **20a-c** was effected by the removal of pyrrole protecting group,[11] by basic

hydrolysis, followed by conversion of the resultant sulfonates to sulfonyl chlorides using $POCl_3$ and PCl_5. Under these conditions, chlorination of the electron rich aromatic ring system **20c** was observed. The sulfonyl chlorides **20a-c** were coupled with 5-amino-4-bromo-3-methylisoxazole under basic conditions to afford the sulfonamides **21a-c** (Scheme III).

Scheme IV



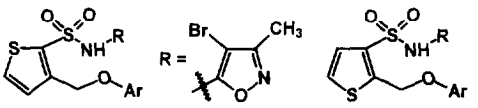
Reagents: (a) NaH, THF, 5-amino-4-bromo-3-methylisoxazole, 0 °C to rt; (b) Methoxyethylmethyl chloride, DIEA, EtOAc, rt; (c) $LiBH_4$, THF, rt; (d) PPh_3, Br_2, CH_2Cl_2, pyridine, 0 °C; (e) K_2CO_3, acetone, reflux; (f) 4 N HCl : MeOH (1:3), reflux.

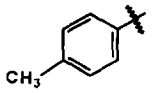
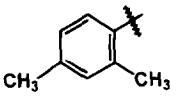
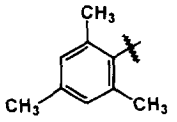
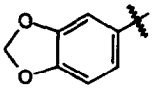
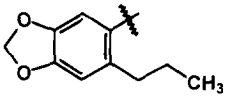
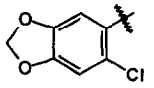
In an alternative approach to prepare 2-aryloxymethylthiophene-3-sulfonamide, 2-methoxycarbonylthiophene-3-sulfonyl chloride **8** was coupled with 5-amino-4-bromo-3-methylisoxazole to afford the sulfonamide **22** (Scheme VI). Protection of the sulfonamide with a methoxyethylmethyl (MEM) group and reduction of the ester group using lithium borohydride gave the alcohol **24**. The bromide **25** was prepared by reacting **24** with bromine-triphenylphosphine adduct. Alkylation of substituted phenols using bromide **25** followed by removal of the MEM group gave the sulfonamides **27a-c**.

Table 1 lists IC_{50} values obtained for aryloxymethylthiophenesulfonamides using ^{125}I-ET-1 in a competitive radioligand assay for both the cloned human ET_A and ET_B receptors.[7a,12] Substitution of the phenyl ring of **15a** at the 2-position with a methyl group gave **15b** which was 4-fold better in its ET_A and ET_B potency. Similarly, sulfonamide **21b** is 4-fold more potent than **21a** on both the receptors. The regioisomeric trimethyl derivatives **15c** and **27a** are about 20-fold better in their ET_A affinity than the monomethyl derivatives **15a** and **21a**, respectively. Similarly, the *ortho* substitution of the phenyl ring in methylenedioxy derivative **15d** gave **15e** which resulted in reasonable improvement in both the ET_A and ET_B receptor affinity. Similar improvement is displayed by the regioisomeric thiophenesulfonamides **27b** vs. **27c** and **27b** vs. **21c**. The

increase in binding affinity by the substitution of the phenyl ring at appropriate positions with a methyl group is parallel with as seen in amide series.[7d] More surprisingly, there is a dramatic improvement in the ET_B receptor binding affinity in analog **27c** compared to **27b** and other analogs in this series. In general, the binding affinities of regioisomeric aryloxymethylthiophenesulfonamides are very similar.

Table 1. IC_{50} Values for the Aryloxymethylthiophenesulfonamides.



		IC_{50} (μM)			IC_{50} (μM)	
No.	Ar	ET_A	ET_B	No.	ET_A	ET_B
15a		1.64 ± 0.085	23.00 ± 2.97	21a	1.2 ± 0.042	15.60 ± 0.989
15b		0.346 ± 0.062	7.275 ± 0.941	21b	0.308 ± 0.056	4.48 ± 0.438
15c		0.054 ± 0.012	4.28 ± 0.559	27a	0.062 ± 0.009	3.07 ± 0.453
15d		0.513 ± 0.00	9.55 ± 0.311	27b	0.299 ± 0.005	5.93 ± 0.962
15e		0.134 ± 0.019	1.305 ± 0.021	27c	0.104 ± 0.005	0.335 ± 0.102
			a	21c	0.129 ± 0.053	0.765 ± 0.209

[a] This regioisomer was not prepared

In summary, the regioisomeric aryloxymethylthiophenesulfonamides **15c** and **27a** displayed the best ET_A potency (IC_{50} = 54 nM and 62 nM, respectively), while the analog **27c** is the most ET_B (IC_{50} = 0.34 μM) active ligand. There is about a 100-fold loss in ET_A binding by substituting the carbonyl group, present in 2-aryloxycarbonylthiophene-3-sulfonamides or 2-arylaminocarbonylthiophene-3-sulfonamides, by a methylene group, as in the present series of compounds. On the other hand, there is a substantial improvement in the ET_B binding affinity as in analog **27c** compared to the corresponding ester derivative. Further studies are necessary to exploit this observation to arrive at nonselective or ET_B selective endothelin antagonists. The substantial loss in ET_A binding affinity may indicate that the carbonyl group in **1** and **2** (Figure 1) may impose some

conformational feature in the spatial display of the phenyl ring and/or may have favorable interactions with receptor elements.

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References and Notes

* Address for correspondence. Phone: (713)796-8822; Fax: (713)796-8232; e-mail: braju@tbc.com
# Present address: Texas Biotechnology Corporation, 7000 Fannin, Suite 1920, Houston, TX 77030

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