Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 16, pp. 2093-2098, 1997
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0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00367-3

2-ARYLOXYCARBONYLTHIOPHENE-3-SULFONAMIDES: HIGHLY POTENT AND ET_A SELECTIVE ENDOTHELIN RECEPTOR ANTAGONISTS[1]

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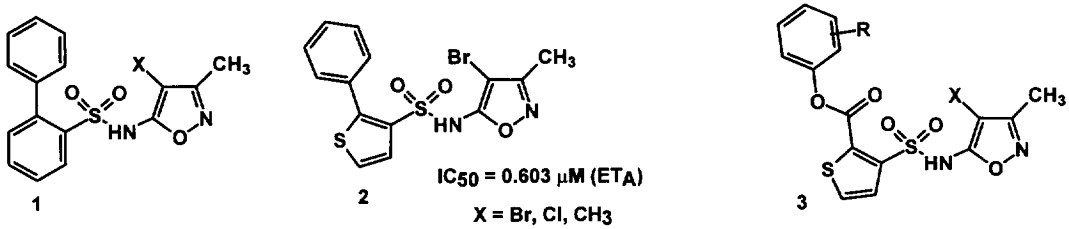
Abstract: A series of 2-aryloxycarbonylthiophene-3-sulfonamides were synthesized and evaluated to determine their antagonistic activity at the endothelin receptors. N-(4-chloro-3-methyl-5-isoxazolyl)-2-[(3,4-methylenedioxy)phenoxycarbonyl]thiophene-3-sulfonamide was identified as a highly selective, potent (IC_{50} = 8.3 nM) and low molecular weight nonpeptide antagonist. © 1997 Elsevier Science Ltd.

The isolation and characterization of the endothelins[2] (ET-1, ET-2, and ET-3) has triggered intensive research in the area due to their unique vasoactive properties. The snake venom peptides, sarafotoxins,[3] are highly homologues to endothelins and like the endothelins are known to exert their biological functions through well characterized specific cell surface receptors,[4] namely ET_A and ET_B. There are indications of the presence of other receptor subtypes in certain tissues. Elevated levels of the endothelins are found in various diseases[5] such as hypertension, angina, congestive heart failure, acute renal failure, myocardial ischaemia, cyclosporin induced renal toxicity, and pulmonary diseases. Thus, small molecule endothelin receptor antagonists are valuable pharmacological tools to study the biological roles of endothelins, and may have clinical potential in the treatment of endothelin mediated disorders.



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IC_{50} = 0.603 μM (ET_A)

X = Br, Cl, CH_3

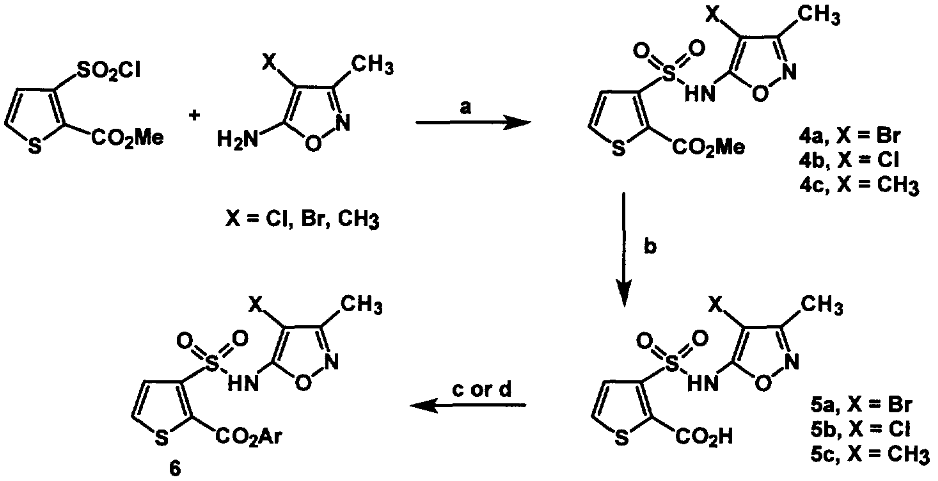
3

Figure 1

Several reports of small molecule antagonists[6] have appeared in literature over the past few years. Among them, sulfonamides[6a-i] are a class of endothelin antagonists that were reported from this and other research laboratories. Earlier studies from this laboratory have described that the replacement of the benzene ring, in a series of benzenesulfonamide endothelin receptor antagonists, with a thiophene ring (Figure 1) have resulted in either ET_A or ET_B selective endothelin receptor antagonists,[6b,e] depending on the substitution pattern. In continuation of the structure-activity relationship (SAR) studies on thiophenesulfonamides, we report here that the insertion of a carboxyl functional group between the phenyl and thiophene rings (Figure 1) resulted in dramatic improvement in ET_A potency.

The synthesis of the thiophenesulfonamides are illustrated[7] in Scheme I. 2-Carbomethoxythiophene-3-sulfonyl chloride was reacted with either 5-amino-4-halo-3-methylisoxzole[8] or 5-amino-3,4-dimethylisoxzole using sodium hydride in THF to afford 2-carbomethoxythiophene-3-sulfonamides **4a-c**. The acids **5a-c**, obtained by basic hydrolysis of the ester derivatives[7] **4a-c**, were activated either using carbonyldiimidazole or phosphonitrilic chloride trimer[9] and treated with the appropriate alcohol or phenol to get the corresponding esters **6**.

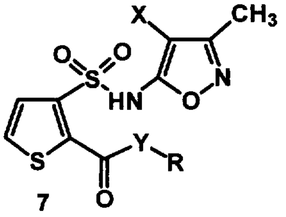
Scheme I



Reagents: (a) NaH, THF, 5-amino-4-halo-3-methylisoxazole or 5-amino-3,4-dimethylisoxazole, 0 °C to rt; (b) NaOH, water, rt; (c) carbonyldiimidazole, THF, ArOH, Et$_3$N, reflux; (d) phosphonitrilic chloride trimer, THF, Et$_3$N, ArOH, rt.

Binding affinities of thiophene-3-sulfonamides[10] (Table 1) were determined in in vitro radioligand binding experiments by competition with ^{125}I-labeled endothelin-1 for both ET$_A$ and ET$_B$ receptors.[6a,11] The results are summarized in Table 1. The methyl esters **7a-c** are weak ET$_A$ selective antagonists. The acid **7d** is about 10-fold less active at the ET$_A$ receptor than the related ester **7a**. On the other hand, the acids **7e** and **7f** have no significant difference in binding affinity compared to **7b** and **7c**, respectively. Also, the binding affinity of methyl ester **7a** is very similar to that of phenylthiophenesulfonamide **2** (Figure 1). These observations prompted us to study the effect of an increase in bulkiness of the substituent on the carboxylic group. The phenolic esters **7g-i** are slightly less active or equipotent to the methyl esters **7a-c**. On the other hand, the amides **7j-l** derived from aniline were at least 6 and 10 fold better than their respective methyl esters and phenolic esters, respectively, in their ET$_A$ inhibitory activity. Further substitution on the benzene ring of the phenolic esters with a methyl group gave **7m-q**. The *p*-tolyl derivative **7m** is at least 200-fold better in ET$_A$ potency than the corresponding phenolic ester **7g**. Also, the *p*-tolyl ester **7m** displayed about 70- to 100-fold better ET$_A$ binding affinity than the *o*-tolyl or *m*-tolyl esters **7o** and **7n**, respectively. Among the *p*-tolyl ester derivatives, both the chloro and dimethyl isoxazole derivatives **7p** and **7q** are about 200-fold less active than the bromo derivative **7m**. The methylenedioxyphenoxy esters **7r** and **7s** have similar binding affinity to the ET_A receptor and these derivatives are about 1000-fold better than **7t**, the diemthylisoxazole analog. The dimethyl phenoxy esters **7u-w** were almost equipotent in their ET_A receptor binding affinity. The thiophenesulfonamides described in this series have ET_B receptor binding affinity from the low micromolar to being undetectable at 100 µM. The best inhibitor **7s** has an IC_{50} of 8 µM to ET_B receptor and this compound is very highly selective for the ET_A receptor (IC_{50} = 8.3 nM).

Table 1. IC_{50} values of thiophene-3-sulfonamides



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				IC_{50} (µM)	
Entry	Y	X	R	ET_A	ET_B
7a	-O-	-Br	$-CH_3$	0.74	$>100^a$
7b	-O-	-Cl	$-CH_3$	1.09	$>100^a$
7c	-O-	$-CH_3$	$-CH_3$	3.47	$>100^a$
7d	-O-	-Br	-H	10.21	$>100^a$
7e	-O-	-Cl	-H	1.06	$>100^a$
7f	-O-	$-CH_3$	-H	6.06	$>100^a$
7g	-O-	-Br	$-C_6H_5$	2.71	$>100^a$
7h	-O-	-Cl	$-C_6H_5$	4.40	$>100^a$
7i	-O-	$-CH_3$	$-C_6H_5$	4.12	$>100^a$
7j	-NH-	-Br	$-C_6H_5$	0.166	77.60^b
7k	-NH-	-Cl	$-C_6H_5$	0.148	$>100^b$
7l	-NH-	$-CH_3$	$-C_6H_5$	0.55	$>100^b$
7m	-O-	-Br	$-C_6H_4(p\text{-}CH_3)$	0.0093	9.21
7n	-O-	-Br	$-C_6H_4(m\text{-}CH_3)$	1.2	$>100^a$
7o	-O-	-Br	$-C_6H_4(o\text{-}CH_3)$	0.68	$>100^a$
7p	-O-	-Cl	$-C_6H_4(p\text{-}CH_3)$	3.22	$>100^a$
7q	-O-	$-CH_3$	$-C_6H_4(p\text{-}CH_3)$	2.52	$>100^a$
7r	-O-	-Br	$-C_6H_3(3,4\text{-}OCH_2O\text{-})$	0.0089	5.74
7s	-O-	-Cl	$-C_6H_3(3,4\text{-}OCH_2O\text{-})$	0.0083	7.94
7t	-O-	$-CH_3$	$-C_6H_3(3,4\text{-}OCH_2O\text{-})$	6.14	48.68
7u	-O-	-Br	$-C_6H_3(2,4\text{-di-}CH_3)$	0.29	43.2
7v	-O-	-Cl	$-C_6H_3(2,4\text{-di-}CH_3)$	0.65	68.4
7w	-O-	$-CH_3$	$-C_6H_3(2,4\text{-di-}CH_3)$	0.27	$>100^a$

[a] Compound concentration of 100 µM fails to inhibit receptor binding more than 50%.

[b] Detailed SAR was presented in reference 12c.

Previously we have shown that a 4-halosubstitutent on the isoxazole moiety enhances the potency compared to a 4-methyl substituent,[6d] and that among the halogens a bromine atom at the 4-position is slightly preferred over a chlorine based on the in vitro binding data. In the present series of thiophenesulfonamides however, these preferences are not always maintained as illustrated by: (1) the dramatic difference in binding affinity between a bromo and a chloro derivative (**7m** vs. **7p**), (2) very similar binding affinities among the halo derivatives (**7r** and **7s**), and (3) equipotency between the 4-halo derivatives and the 4-methyl derivatives (**7u**, **7v**, and **7w**). The above observations coupled with about 10-fold difference in binding affinity of the related esters and amides (**7g-i** vs. **7j-l**) lead us to test the stability of the esters under the radioligand assay conditions. All the phenolic ester derivatives are partially hydrolyzed under the assay conditions (buffer pH 7.5) in the presence of membrane preparation.[13] The widespread SAR and dramatic difference in the binding affinity of related analogues (**7m** vs. **7p**) may be due to the instability of the compounds under the assay conditions. However, compounds that showed significantly greater potency than **7a-c** could not be false positive due to hydrolysis.

In summary, the compounds described in this communication represents a new class of sulfonamide endothelin antagonists[14] with nanomolar binding affinity and high selectivity (**7r** or **7s**, 1000-fold) for the ET_A receptor. These leads helped to design metabolically more stable analogues through systematic studies in this laboratory.[12]

Acknowledgments: The authors wish to thank Dr. Timothy P. Kogan and Dr. Richard A. F. Dixon for helpful discussions.

References and Notes

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7. (a) To a stirred suspension of sodium hydride (8.0 g, 330 mmol, prewashed with hexane) in dry THF (400 mL) at 0 °C was added 5-amino-4-chloro-3-methylisoxazole (20 g, 150 mmol) in small portions. After complete addition the reaction mixture was stirred at room temperature for 30 min. (in the case of dimethyl isoxazole the reaction mixture was stirred at ambient temperature for 10 h). This slurry was cannulated slowly into a solution of 2-methoxycarbonylthiophene-3-sulfonyl chloride (36.2 g, 150 mmol) in dry THF at 0 °C. The resultant reaction mixture was stirred at ambient temperature for 1 h. The excess sodium hydride was decomposed by addition of methanol and then added to water (1000 mL). This mixture was extracted with ether (2 x 200 mL) to remove the neutral impurities. The aqueous layer was adjusted to pH 2-3 using concentrated hydrochloric acid and cooled to 10 °C with stirring. The resultant solid was filtered and dried under high vacuum and used in the next step without any further purification. For analytical purpose a small quantity of sample was purified by column chromatography; (b) The above esters were dissolved in sodium hydroxide (5%, 300 mL) and stirred at room temperature for 8 h. The reaction was monitored by TLC; (c) To a stirred solution of N-(4-chloro-3-methyl-5-isoxazolyl)-2-carboxylthiophene-3-sulfonamide (1 g, 3.2 mmol) in dry THF (5 mL) was added carbonyl diimidazole (0.533 g, 3.28 mmol), imidazole (225 mg, 3.2 mmol), and sesamol (884 mg, 6.4 mmol). The reaction mixture was heated under reflux for 4 h. THF was removed under reduced pressure and the residue was partitioned between 1 N HCl and ethyl acetate. The organic layer was dried over magnesium sulfate and the solvent removed under reduced pressure. Purification by HPLC using acetonitrile and water containing 0.1 % TFA (0 to 100% over 20 min, linear gradient) on a reverse phase C18 column gave the product; (d) To a stirred mixture of N-(4-chloro-3-methyl-5-isoxazolyl)-2-carboxylthiophene-3-sulfonamide (0.323 g, 1 mmol), phenol (188 g, 2 mmol) and triethylamine (0.834 ml, 6 mmol) in dry THF (3 mL) was added phosphonitrilic chloride trimer (0.381 g, 1.1 mmol) in one lot which resulted in a slightly exothermic reaction. The resultant reaction mixture stirred at ambient temperature for 30 min. This was diluted with water, the pH adjusted to 2-3 using 6 N HCl, extracted with ethyl acetate, and the combined organic layer was dried over magnesium

sulfate. Removal of the solvent under reduced pressure and purification by flash chromatography gave N-(4-bromo-3-methyl-5-isoxazolyl)-2-phenoxycarbonylthiophene-3-sulfonamide (256 mg, 65%).

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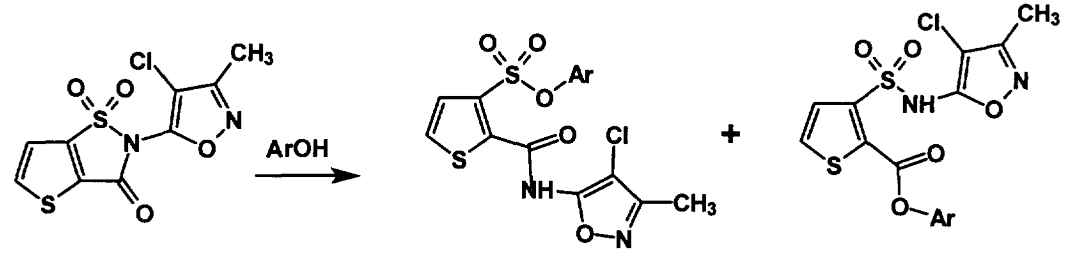
9. Using carbonyldiimdazole as a coupling reagent under refluxing conditions in the presence of a phenol resulted in the formation of two products (**9** and **10**, as shown below) as determined by NMR of the mixture. This was explained by the formation of lactam **8** which was isolated and characterized. Therefore, phosphonitrilic chloroide trimer was identified as a coupling reagent for the formation of esters (**6**, Scheme I) which gave only the required ester **10**.



8 **9** **10**

10. All sulfonamides are characterized by high resolution FAB MS analysis, C, H, N analysis, PMR, IR and purities were further judged by analytical HPLC on a C18 reverse-phase chromatography.
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13. Incubation experiments: Compounds were dissolved in DMSO (0.04 M), diluted 100-fold with buffer [150 mM NaCl, 5 mM $MgCl_2$, 30 mM of HEPES buffer (pH 7.4) and 0.5 mg/mL bacitracin] to get 400 μM concentration. 50 μL of membrane (2 μg of protein) and 50 μL (20 nmol) of the stock solution of the compound were diluted to 0.2 mL with the buffer. After incubating for 24 h, an aliquot of this solution was analysed by analytical HPLC to get two peaks corresponding to the initial ester (**6**, Scheme I) and the respective acid (**5**, Scheme I), one of the cleaved product.
14. ET-1 causes a dose dependent accumulation of phosphoinositide in TE 671 cells in the presence of lithium.[11] Addition of **7r** resulted in a parallel shift to the right of the ET-1 dose responce curve in the TE 671 cells indicating that **7r** acts as functional antagonist of ET-1 in this system.

(*Received in USA* 30 *April* 1997; *accepted* 8 *July* 1997)