Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 20, pp. 2393-2398, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00441-6

HALOGEN SUBSTITUTION AT THE ISOXAZOLE RING ENHANCES THE ACTIVITY OF N-(ISOXAZOLYL)SULFONAMIDE ENDOTHELIN ANTAGONISTS[1]

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Abstract: Replacement of the 4-methyl group in a series of N-(3,4-dimethylisoxazolyl)benzenesulfonamide endothelin antagonists with a bromine or chlorine atom resulted in a three- to ten-fold increase in the binding affinity for both the ET_A and ET_B receptors. This potentiation in activities was also observed for naphthalene and biphenylsulfonamide endothelin antagonists. Copyright © 1996 Elsevier Science Ltd

The endothelin family of peptides (ET-1, ET-2, ET-3, and the sarafotoxins) has been identified as the most potent endogenous vasoconstrictor.[2,3] They are widely distributed in various tissues,[4] and exert their biological actions through distinct cell surface receptors. Two receptor subtypes, ET_A and ET_B, have been identified and there is evidence to support the presence of a third receptor subtype in certain tissues.[5] Endothelin receptor antagonists may have clinical potential in endothelin mediated disorders and may be useful pharmacological tools to study the biological roles of the endothelins.

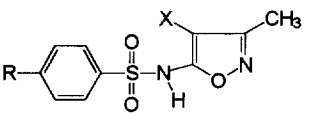
Several different classes of endothelin antagonists have been identified.[4,6] We reported the discovery of sulfisoxazole (**1**), a sulfanilamide antibacterial agent, as a subtype selective ET antagonist through a pharmacophore directed screening approach.[7] Optimization of **1** led to the discovery of the N-(3,4-dimethyl-5-isoxazolyl)-2,5-disubstituted benzenesulfonamides that are potent and highly selective antagonists for the ET_A receptor (**25a**, **26a**, and **27a**).[8] In this Letter, we would like to report that replacement of the 4-methyl group on the isoxazole ring by a bromine atom results in a three- to ten-fold increase in the binding affinity (Table 1).

Chemistry: The sulfonamides were synthesized by reacting the corresponding aminoisoxazole and sulfonyl chloride either in pyridine (with or without 4-dimethylaminopyridine as a catalyst) or in dry THF using sodium hydride as a base as previously described.[8,9] 5-Amino-4-bromo-3-methylisoxazole and 5-amino-4-chloro-3-methylisoxazole were prepared by treating 5-amino-3-methylisoxazole with the corresponding N-halosuccinimide in dichloromethane at 0 °C.[10] The sulfonyl chlorides were prepared according to reported procedures.[8]

Pharmacology: Radioreceptor binding studies were carried out in triplicate at 4 °C with either TE 671 (ATCC # HTB 139) cell membrane preparation containing human ET_A receptors or transfected COS 7 cell membrane preparation containing human ET_B receptors. Smooth muscle contraction experiments were carried out at 37 °C

using rat thoracic aortic rings. The pA_2 values were calculated from the average of at least three experiments. Details of these experiments have been described in previous publications.[8,11]

Table 1. Binding Affinity of Representative Benzene Sulfonamides

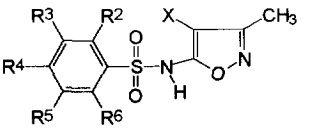


No.	R	X	IC_{50} (μM)	
			ET_A	ET_B
1	NH_2	Me	0.85 ± 0.25 (4)	24 ± 9 (5)
2a	H	Me	0.50 ± 0.05 (2)	88 ± 5 (3)
2b	H	Br	0.079 ± 0.04 (7)	20 ± 4 (8)
2c	H	Cl	0.14 ± 0.02 (2)	27 ± 1.5 (3)

RESULTS AND DISCUSSION

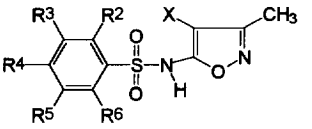
In our initial SAR study of the benzene sulfonamide ET antagonists, substituent effects on the isoxazole ring were systematically studied. The binding affinity was greatly influenced by the size of the substituents. The electronic nature of the substituent has only a minor effect. Thus, while both methyl and trifluoromethyl groups are well tolerated, groups larger than an ethyl group cause a rapid decline in the binding affinity. As a result, the 3,4-dimethylisoxazolyl group was used throughout our previous study due to its ready availability and the high activity of the resulting sulfonamides.[8] Further studies on the substituent effect, however, revealed that replacement of the 4-methyl group on the isoxazole ring with a chlorine or bromine atom further enhanced the binding of these ET antagonists (compounds **2a**, **2b**, and **2c**). A bromine atom is generally more effective than chlorine, resulting in a three- to ten-fold enhancement over the methyl analog. The enhancement in binding is observed for both the ET_A and ET_B receptor (Tables 2 and 3). The rank order of activity and selectivity of the compounds are not usually affected by the bromine replacement except in very active compounds (**25b-27b**) where the rank order is changed. This effect seems to be universal and applies equally well to the naphthalene and biphenyl sulfonamides (Table 4). Replacement of the 4-methyl group in the potent, orally-active ET_A selective antagonist BMS-182874 (**33a**)[9] resulted in a five-fold increase in the binding affinity without appreciably affecting the ET_A selectivity. The antagonistic activity of the corresponding bromo analog TBC-10616 (**33b**), as measured by the inhibition of contraction of rat aortic smooth muscle, was also increased (pA_2 7.5 vs. 7.3 for BMS-182874).

Table 2. Effect of Isoxazole Substituents on the ET_A Binding Affinity of Benzenesulfonamide Antagonists



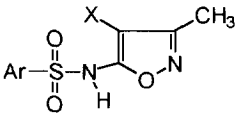
R^2	R^3	R^4	R^5	R^6	X = CH_3		X = Br		Ratio
					Cpd	IC_{50} (μM)[8]	Cpd	IC_{50} (μM)	CH_3/Br
H	H	H	H	H	**2a**	0.50 ± 0.05	**2b**	0.079 ± 0.04	6.3
CH_3	H	H	H	H	**3a**	1.0 ± 0.1	**3b**	0.14 ± 0.03	7.1
F	H	H	H	H	**4a**	0.77 ± 0.20	**4b**	0.16 ± 0.04	4.8
Cl	H	H	H	H	**5a**	0.98 ± 0.25	**5b**	0.071 ± 0.006	14
Br	H	H	H	H	**6a**	0.62 ± 0.09	**6b**	0.040 ± 0.02	16
H	CH_3	H	H	H	**7a**	0.72 ± 0.23	**7b**	0.12 ± 0.01	6.0
H	Cl	H	H	H	**8a**	1.5 ± 0.3	**8b**	0.23 ± 0.06	6.5
H	Br	H	H	H	**9a**	1.8 ± 0.2	**9b**	0.23 ± 0.06	7.7
H	NO_2	H	H	H	**10a**	14 ± 10	**10b**	2.7 ± 0.1	5.2
H	H	CH_3	H	H	**11a**	4.2 ± 1.5	**11b**	1.4 ± 1	3.0
H	H	F	H	H	**12a**	6.7 ± 3	**12b**	13 ± 0.1	5.1
H	H	Cl	H	H	**13a**	10 ± 2	**13b**	2.0 ± 1	5.0
H	H	Br	H	H	**14a**	8.9	**14b**	3.0 ± 0.04	3.0
H	H	NO_2	H	H	**15a**	90 ± 30	**15b**	19 ± 5	4.7
CH_3	Cl	H	H	H	**16a**	0.99 ± 0.11	**16b**	0.17 ± 0.13	5.8
Cl	H	Cl	H	H	**17a**	14 ± 7	**17b**	2.5 ± 0.5	5.6
H	Cl	Cl	H	H	**18a**	3.8 ± 1.5	**18b**	0.55 ± 0.12	6.9
H	Cl	H	Cl	H	**19a**	0.43 ± 0.07	**19b**	0.062 ± 0.02	6.9
H	CH_3O	CH_3O	H	H	**20a**	1.9	**20b**	0.49 ± 0.18	3.9
CH_3	H	H	CH_3	H	**21a**	0.27 ± 0.03	**21b**	0.044 ± 0.03	6.1
CH_3	H	H	Br	H	**22a**	0.84 ± 0.11	**22b**	0.24	3.5
CH_3NH	H	H	CH_3	H	—	—	**23b**	0.0081 ±	—
CH_3O	H	H	Br	H	**24a**	0.47 ± 0.24	**24b**	0.072 ± 0.03	6.5
C_2H_5	H	H	C_2H_5	H	**25a**	0.027 ± 0.01	**25b**	0.0062 ± 0.003	4.4
C_2H_5	H	H	Br	H	**26a**	0.053 ± 0.015	**26b**	0.0028 ±	19
n-C_3H_7	H	H	Br	H	**27a**	0.015 ± 0.002	**27b**	0.011 ± 0.007	1.4
Cl	H	H	Cl	H	**28a**	0.28 ± 0.15	**28b**	0.11 ± 0.02	2.5
Br	H	H	Br	H	**29a**	0.49 ± 0.05	**29b**	0.14 ± 0.01	3.5
Br	H	H	C_2H_5	H	**30a**	0.27 ± 0.06	**30b**	0.029 ± 0.01	9.3
CH_3	H	CH_3	H	CH_3	**31a**	53 ± 6	**31b**	5.9 ± 0.9	9.0

Table 3. Effect of Isoxazole Substituents on the ET_B Binding Affinity of Benzenesulfonamide Antagonists



R^2	R^3	R^4	R^5	R^6	X = CH_3		X = Br		Ratio
					Cpd	IC_{50} (μM)[8]	Cpd	IC_{50} (μM)	CH_3/Br
H	H	H	H	H	**2a**	88 ± 15	**2b**	20 ± 4 (8)	4.4
CH_3	H	H	H	H	**3a**	39 ± 3%[a]	**3b**	45 ± 20	—
F	H	H	H	H	**4a**	44 ± 4%[a]	**4b**	35 ± 6	—
Cl	H	H	H	H	**5a**	27 ± 2%[a]	**5b**	37 ± 2	—
Br	H	H	H	H	**6a**	34 ± 2%[a]	**6b**	39 ± 4	—
H	CH_3	H	H	H	**7a**	60 ± 10%[a]	**7b**	13 ± 1	—
H	Cl	H	H	H	**8a**	90 ± 5	**8b**	13 ± 0.2	6.9
H	Br	H	H	H	**9a**	49 ± 3	**9b**	9.4 ± 1.4	5.2
H	NO_2	H	H	H	**10a**	37 ± 3%[a]	**10b**	38 ± 2	—
H	H	CH_3	H	H	**11a**	15 ± 4	**11b**	4.0 ± 1	3.8
H	H	F	H	H	**12a**	57 ± 12	**12b**	14 ± 1.5	4.1
H	H	Cl	H	H	**13a**	29 ± 0.8	**13b**	7.0 ± 2	4.1
H	H	Br	H	H	**14a**	14 ± 5	**14b**	3.4 ± 0.4	4.1
H	H	NO_2	H	H	**15a**	30 ± 9	**15b**	6.8 ± 3	4.4
CH_3	Cl	H	H	H	**16a**	74 ± 7	**16b**	22 ± 15	3.4
Cl	H	Cl	H	H	**17a**	105 ± 12	**17b**	24 ± 7	4.4
H	Cl	Cl	H	H	**18a**	25 ± 9	**18b**	6.7 ± 1.8	3.7
H	Cl	H	Cl	H	**19a**	47 ± 6	**19b**	14 ±1	3.4
H	CH_3O	CH_3O	H	H	**20a**	44 ± 4%[a]	**20b**	24 ± 5	—
CH_3	H	H	CH_3	H	**21a**	58 ± 10	**21b**	15 ± 3	3.9
CH_3	H	H	Br	H	**22a**	59 ± 19	**22b**	13 ± 2	4.5
CH_3NH	H	H	CH_3	H	—	—	**23b**	0.93 ± 0.1	—
CH_3O	H	H	Br	H	**24a**	20 ± 4	**24b**	5.3 ± 0.4	3.8
C_2H_5	H	H	C_2H_5	H	**25a**	17 ± 7	**25b**	5.2 ± 0.8	3.3
C_2H_5	H	H	Br	H	**26a**	23 ± 9	**26b**	5.2 ± 1.1	4.4
n-C_3H_7	H	H	Br	H	**27a**	26 ± 4	**27b**	6.6 ± 0.2	3.9
Cl	H	H	Cl	H	**28a**	80 ± 1	**28b**	25 ± 3	3.2
Br	H	H	Br	H	**29a**	33 ± 4	**29b**	11 ± 2	3.0
Br	H	H	C_2H_5	H	**30a**	27 ± 11	**30b**	4.7 ± 1.1	5.7
CH_3	H	CH_3	H	CH_3	**31a**	27 ± 1%[a]	**31b**	46 ± 4	—

[a] % inhibition at 100 μM



32 Ar = 1-naphthyl

33 Ar = 

34 Ar = 2-biphenyl
35 Ar = 3-biphenyl
36 Ar = 4-biphenyl

Incorporation of this observation into our earlier work on the benzenesulfonamide ET antagonists led to the identification of potent N-(4-bromo-3-methyl-5-isoxazolyl)-2,5-disubstituted benzenesulfonamides such as TBC-10688 (**23b**, IC_{50}=8.1 nM, pA_2=7.5), TBC-10654 (**25b**, IC_{50}=6.2 nM, pA_2=6.5), and TBC-10662 (**26b**, IC_{50}=2.8 nM, pA_2=6.4) that are also highly selective (ET_A/ET_B > 1000) ET_A receptor antagonists.

CONCLUSIONS

Replacement of the 4-methyl group in N-(3,4-dimethyl-5-isoxazolyl)sulfonamide endothelin antagonists with a bromine or chlorine atom results in a three- to ten-fold increase in the binding affinity for both the ET_A and ET_B receptors. This effect seems to be universal and applies to other types of sulfonamide endothelin antagonists. This increase in binding affinity is accompanied by a potentiation of the antagonistic effect as measured by the attenuation of the ET-1 induced rat aortic smooth muscle contraction. Several potent, ET_A selective, low molecular weight, nonpeptide endothelin antagonists were identified by incorporation of this observation with our earlier SAR studies in the benzenesulfonamide antagonists.

Table 4. Effect of Isoxazole Substituent on the Binding Affinity of Naphthyl and Biphenyl Antagonists

Compound	X	IC_{50} (μM)	
		ET_A	ET_B
32a	CH_3	0.44 ± 0.05	49 ± 5
32b	Br	0.11 ± 0.04	16 ± 4
33a	CH_3	0.0058 ± 0.0008	14 ± 2
33b	Br	0.0011 ± 0.0002	3.3 ± 0.8
34a	CH_3	0.0083 ± 0.0014	55
34b	Br	0.0014 ± 0.0005	7.9 ± 2
34c	Cl	0.0038 ± 0.0016	13 ± 3
35a	CH_3	0.58 ± 0.05	4.9 ± 1.5
35b	Br	0.120 ± 0.002	1.1 ± 0.2
35c	Cl	0.16 ± 0.03	1.2 ± 0.3
36a	CH_3	9.9 ± 2.1	0.61 ± 0.15
36b	Br	2.9 ± 0.83	0.14 ± 0.04

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10. A typical experimental procedure is as follows: N-Bromosuccinimide (4.45 g, 25.0 mmol) was added to a solution of 5-amino-3-methylisoxazole (2.45 g, 25.0 mmol) in $CHCl_3$ (200 mL) at 0 °C. After 1 h, the reaction was judged complete by TLC and the solvent was removed under reduced pressure. The residue was diluted with ether (200 mL) and washed with water (3 × 100 mL). The aqueous phase was back-extracted once with ether (100 mL) and discarded. The combined organic phase was dried over $MgSO_4$, filtered, and concentrated. The resulting solid was recrystallized from $CHCl_3$/hexanes at -20 °C to give 3.11 g (70%) of 5-amino-4-bromo-3-methylisoxazole as light yellow needles, mp 67-68 °C. [Lit.[12] mp 67-69 °C]
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(*Received in USA* 26 *June* 1996; *accepted* 12 *September* 1996)