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Communications to the Editor

The Discovery of Sulfonamide Endothelin Antagonists and the Development of the Orally Active ET_A Antagonist 5-(Dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulfonamide

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The endothelins (ETs) are a family of potent vasoconstrictor peptides originally isolated from endothelial cells.[1] These peptides are now known to be produced by a number of other cell types and to elicit activities in addition to vasoconstriction (reviewed in ref 2). ET exerts its biological effects through interaction with specific receptors, of which three subtypes have been cloned and expressed.[3-5] The ET_A subtype, which is selective for ET-1 over ET-3, appears to be the predominant vascular smooth muscle receptor while the isopeptide nonselective ET_B receptor appears to mediate either vasodilation or vasoconstriction, depending upon the tissue type.[6,7] The ET_C receptor subtype, which specifically binds ET-3, was recently cloned from dermal melanophores of *Xenopus laevis*.

ET has been suggested to play a role in the pathophysiology of a large number of diseases (see citations in ref 2). Much of this work involved monitoring the effects of administered ET in animal models of disease or the presence of elevated serum levels of ET in patients with these diseases. Proof that ET is a causative agent has remained elusive, but the recent discovery of ET receptor antagonists will surely remedy this situation.

Structurally diverse ET antagonists of differing subtype selectivity have been discovered, including cyclic pentapeptides,[8-10] related acyl tripeptides,[11] hexapeptide analogues,[12] a family of anthraquinone derivatives,[13,14] myriceron caffeoyl ester,[15] asterric acid,[16] a group of cyclic depsipeptides,[17] and most recently *N*-pyrimidinylbenzenesulfonamides[18] and indanecarboxylic acids.[19] While the cyclic pentapeptide and acyl tripeptide antagonists have proven useful in testing the role of ET in some disease models,[20,21] orally active antagonists with long half-lives such as the recently reported ET_A/ET_B nonselective benzenesulfonamide antagonists[18] would be optimal tools which have the potential to become therapeutic agents.

In this report, we describe the discovery of benzenesulfonamide ET_A receptor antagonists and structure–activity studies which have led to the identification of the naphthalenesulfonamide **11**, a potent, orally active, highly selective ET_A receptor antagonist.

Chemistry. Nearly all of the syntheses involved the condensation of sulfonyl chlorides with isoxazolamines in pyridine, at temperatures ranging from room temperature to 80 °C. In some cases (e.g., **11**, **19**), compounds were prepared by the reaction of commercially available arenesulfonyl chlorides with commercially available isoxazolamines. Known sulfonyl chlorides were prepared by literature methods (**16**[22]). The 5-amino-1-naphthalenesulfonamide derivatives were prepared from 5-amino-1-naphthalenesulfonic acid. Following formation of the sodium salt and acetylation of the amine, the sulfonyl chloride was prepared using PCl_5. Condensation with the isoxazolamine afforded the amide **12**, and hydrolysis yielded the primary amine **14**. Several other targets (**13**, **15**, and **18**) were prepared from 5-(chlorosulfonyl)-1-naphthalenecarboxylic acid, methyl ester using standard methods.

Results and Discussion. Samples from the Bristol-Myers Squibb compound collection were screened for their ability to inhibit [^{125}I]ET-1 binding to vascular smooth

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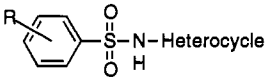
Table 1. *N*-Heterocyclic-benzenesulfonamide Analogs



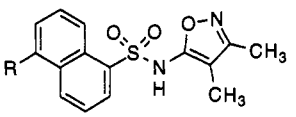
compd	heterocycle	R	binding IC_{50} (μM) (n)	K_{Bapp} (μM)
1	2-thiazolyl	4-NH_2	69 ± 6 (3)	>100
2	3,4-dimethyl-5-isoxazolyl	4-NH_2	0.78 ± 0.06 (3)	>100
3	3-methyl-5-isoxazolyl	4-NH_2	28 (1)	
4	3-pentyl-5-isoxazolyl	4-NH_2	>32 (1)	
5	3-phenyl-5-isoxazolyl	4-NH_2	>32 (1)	
6	3,4-dimethyl-5-isoxazolyl	4-$NH(CH_2)_2CH_3$	27 ± 5.6 (2)	40 ± 10
7	3,4-dimethyl-5-isoxazolyl	4-OH	9.2 ± 1.9 (3)	>100
8	3,4-dimethyl-5-isoxazolyl	4-$NHCH_2C_6H_5$	9.8 ± 5.3 (3)	100 ± 50
9	3,4-dimethyl-5-isoxazolyl	4-$N(CH_3)_2$	84 (1)	
10	3,4-dimethyl-5-isoxazolyl	4-CN	>32 (1)	

muscle (vsm)-A10 cells using modifications of a previously described procedure.[23,24] Sulfathiazole (1; Table 1) was discovered to be a weak inhibitor of binding to ET_A receptors (IC_{50} = 69 μM). Additional screening of related compounds led to the identification of sulfisoxazole (2; IC_{50} = 780 nM) as a relatively potent ET_A ligand. While 2 inhibited the increase in intracellular Ca^{2+} in vsm-A10 cells elicited by 3 nM ET-1 (IC_{50} = 40 ± 3 μM),[25] 2 at a concentration of 100 μM did not produce a rightward shift of the ET-1 concentration–response curve in rabbit carotid artery rings,[26] indicating that it was not a functional antagonist under the conditions of this experiment. Nevertheless, 2 was used as a starting point for efforts aimed at optimizing the sulfonamide ET_A ligands.

The poor affinity of 4-amino-*N*-(3,4-dimethyl-5-isoxazolyl)-*N*-methylbenzenesulfonamide (data not shown; no inhibition at 32 μM) indicated that an unsubstituted sulfonamide nitrogen was critical to the receptor affinity of this class of ET antagonists. Studies of the isoxazole substituents using 4-amino-*N*-(5-isoxazolyl)benzenesulfonamide indicated that the 4-methyl group is required for potent binding (3) while replacement of the 3-methyl group with larger substituents led to large losses in affinity (4, 5). Using *N*-(3,4-dimethyl-5-isoxazolyl)benzenesulfonamide, the effects of phenyl substituents were studied. While a number of substituents afforded analogues with IC_{50} values in the low micromolar range, only the analogues which contained alkylamino (6) and aralkylamino groups (8) were functional antagonists with K_B or apparent K_B values ≤100 μM (K_{Bapp} values were obtained from experiments using only 1 concentration of antagonist).[7]

Prompted by the functional antagonist activity of aminobenzene sulfonamides of increased lipophilicity, *N*-(3,4-dimethyl-5-isoxazolyl)naphthalenesulfonamides containing aromatic nitrogen substituents were prepared for nearly all of the possible substitution patterns (data not shown). The 1,5-substitution pattern provided the most potent analgoues (Table 2). The dimethylamino analogue 11 (BMS-182874) displayed an IC_{50} value of 150 nM (K_i value = 55 nM) and was a relatively potent functional antagonist, with an IC_{50} value of 570 ± 70 nM as an antagonist of the ET-1-induced increase in intracellular Ca^{2+} in vsm-A10 cells and a K_B value of 520 nM in rabbit carotid artery rings (for comparison, respective values for BQ-123: K_i = 18 ± 4.2 nM; IC_{50} value for intracellular Ca^{2+} increase = 26 ± 7 nM; K_B = 35 ± 14 nM). The reasons for the substantial difference between the K_i value and the K_B value is not understood, but this phenomenon has been observed with other endothelin antagonists (e.g., FR 139317, IC_{50} = 0.53 nM, pA_2 = 7.2).[27] In the 1,5-substitution pattern, compounds with a variety of other

Table 2. 5-Substituted *N*-(3,4-Dimethyl-5-isoxazolyl)-1-naphthalenesulfonamides



compd	R	binding IC_{50} (μM) (n = 2)	K_B* or K_{Bapp} (μM)
11	$N(CH_3)_2$	0.15 ± 0.01	0.52 ± 0.10*
12	$NHCOCH_3$	0.88 ± 0.12	11 ± 0.27*
13	$CH_2N(CH_3)_2$	2.8 ± 0.7	20 ± 6
14	NH_2	4.0 ± 0.8	>10
15	$C(=CH_2)(CH_3)$	5.7 ± 2.4	
16	OCH_3	6.5 ± 1.4	
17	OH	7.8 ± 0.1	100 ± 40
18	CO_2H	13.0 ± 1.0	
19	H	20.0 ± 1.0	

5-substituents displayed much lower affinity and much less efficacy as functional antagonists. The receptor subtype specificity of 11 was evaluated by determining its binding affinity in rat cerebellar membranes, an ET_B-containing tissue. With a K_i value of >200 μM, 11 showed greater than 3600-fold selectivity for the ET_A receptor.

11 was tested for oral activity in DOCA-salt rats. When one kidney rats are implanted with a deoxycorticosterone acetate (DOCA) pellet and given saline to drink, they respond by developing hypertension. The thoracic aorta and mesenteric vascular bed of DOCA-salt hypertensive rats contain significantly more ET-1 than do the uninephrectomized control rats,[28] although there is no difference in the circulating levels of ET-1 in the two models.[29] The high levels of tissue ET-1 in DOCA-salt rats suggested that ET-1 might play a role in this model of hypertension. 11 was tested at a single oral dose of 100 μmol/kg in 5% $NaHCO_3$. In the first hour after dosing, mean arterial pressure slowly fell by 25% from a control level of 183 ± 4 mmHg (Figure 1). Between 12 and 24 h after dosing, mean arterial pressure was still 12% below the control level. Thus, in this *in vivo* model, 11 is an orally active antihypertensive agent with a long duration of effect. Previously, BQ-123 was shown to produce a small but statistically significant hypotensive effect in a similar model.[30] The poorer hypotensive effect of BQ-123 compared to 11 is likely due to the extremely short half-life of BQ-123 *in vivo* (Dr. Richard Morrison, unpublished results).

In summary, optimization of benzenesulfonamide ligands discovered through random screening in an ET_A binding assay led to the development of *N*-isoxazolyl-1-naphthalenesulfonamide ligands. The 5-dimethylamino analogue 11 is an orally active, non-peptide, highly ET_A selective

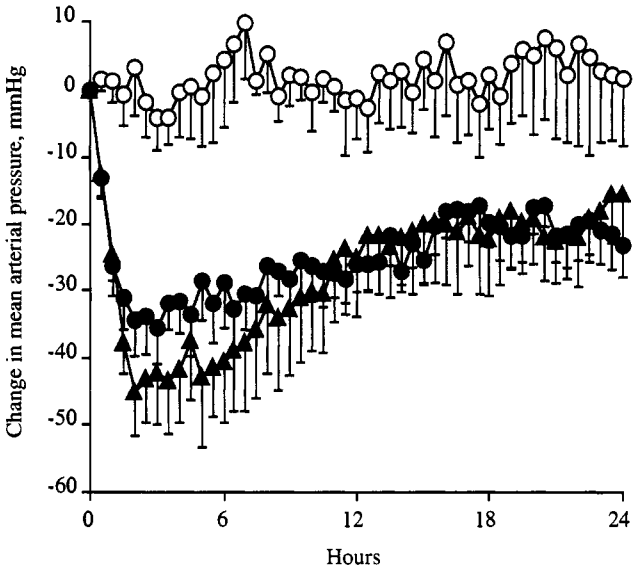


Figure 1. Antihypertensive effect of vehicle (open circles), a single dose of 11 iv (100 μmol/kg in 5% $NaHCO_3$; filled circles), and a single dose of 11 po (100 μmol/kg in 5% $NaHCO_3$; filled triangles) administered at time 0 in one kidney DOCA-salt hypertensive rats. Data are plotted as mean ± SEM; $n = 6$ animals.

receptor antagonist which is proving useful in elucidating the role of endothelin in animal models of human disease. These results will be reported elsewhere.

Supplementary Material Available: Experimental procedures for the preparation of 11, 13, 15, 17, and 18 as well as spectral data (5 pages). Ordering information is given on any current masthead page.

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(24) Receptor binding assay modifications used for screening in intact cells: Confluent vsm-A10 cells were harvested by scraping, collected by centrifugation, resuspended in phosphate buffered saline (PBS) supplemented with 0.1% glucose and 0.1% BSA, and incubated (3 h at 4 °C) with 0.4 nM [^{125}I]ET-1 (2,200 Ci/mmol, NEN) in the presence and absence of 1 μM ET-1 or synthetic compound. Bound and free [^{125}I]ET-1 were separated by filtration on a Tomtec cell harvester and bound radioactivity was quantified by scintillation counting.

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