Discovery and Structure–Activity Relationships of Sulfonamide ET_A-Selective Antagonists

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Random screening of compounds in an ET_A receptor binding assay led to the discovery of a class of benzenesulfonamide ligands. Optimization led to the development of 5-amino-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulfonamides which were functional antagonists. Structural features which were important to activity included a 1,5-substitution pattern on the naphthalene ring; a sulfonamide NH with a pK value < 7; an amine, preferably with alkyl substituents, at the 5-position; and methyl groups on both the 3- and 4-positions of the isoxazole.

Introduction

The endothelins comprise a family of potent vasoconstrictor peptides which also include the highly homologous snake venom peptides, the sarafotoxins.[1-3] Although originally isolated from endothelial cells, endothelin is produced by a number of other cell types, including kidney cells,[4] macrophages,[5] and human cancer cell lines.[6] The biological effects of ET are mediated through interaction with specific G protein coupled receptors. The ET-1 selective ET_A subtype is the predominant vascular smooth muscle receptor.[7] The isopeptide nonselective ET_B receptor[8] was originally believed to exclusively be a vasodilator receptor. However, it is now apparent that, depending upon the tissue type, the ET_B receptor can mediate either vasodilation or vasoconstriction.[9,10] A putative ET_C receptor subtype was recently cloned from dermal melanophores of *Xenopus laevis*;[11] however, the mammalian equivalent of this frog receptor has not yet been identified. In addition to its contractile and vasodilatory effects, endothelin functions as a growth factor,[6] causes the release of substances such as ANP from myocytes[12] and aldosterone from adrenal cells,[13] and inhibits the release of substances such as renin.[14] Endothelin has been implicated in the pathophysiology of a large number of diseases, including, among others, hypertension, pulmonary hypertension, myocardial ischemia, subarachnoid hemorrhage, and renal failure.[15] While initial evidence for the involvement of endothelin in these diseases was indirect, the development of small molecule endothelin antagonists has more directly and convincingly begun to demonstrate that endothelin can be a causative agent of some diseases.

A large number of structurally diverse endothelin antagonists of differing subtype selectivity have been discovered. Among the ET_A selective agents are the cyclic pentapeptides[16-18] such as BQ-123, related acyltripeptides such as FR139317,[19] myriceron caffeoyl ester,[20,21] a family of anthraquinone derivatives,[22,23] *N*-isoxazolyl-1-naphthalenesulfonamides,[24] and a recently disclosed class of butenolides.[25] Asterric acid[26] is a weak ET_A ligand whose ET_B affinity was not reported. Ligands which are relatively nonselective in their respective ET_A/ET_B affinity include a series of hexapeptide analogues derived from the C-terminal tail of endothelin,[27,28] a group of cyclic depsipeptides,[29] *N*-pyrimidinylbenzenesulfonamides,[30] indancarboxylic acids,[31] and, most recently, phenoxyphenylacetic acids.[32]

Prior to the discovery of orally active endothelin antagonists, infusion of BQ-123 was used to demonstrate the role of endothelin in various rat hypertension models and intraperitoneal injection of FR139317 was used in a rat model of renal disease progression.[33,34] With reports of the utility of the orally active antagonists in various models, it is clear that compounds are at hand with the characteristics to clearly define the pathophysiological role of endothelin and to eventually become therapeutic agents. For example, Ro 46-2005 has been shown to be effective in animal models of subarachnoid hemorrhage, renal vasoconstriction following renal artery clamping, and hypertension,[30] BMS-182874 has been shown to be effective in an animal model of hypertension,[24] and Ro 47-0203 has been shown to be without effect in an animal model of ischemia and reperfusion.[35]

We have previously described the discovery of benzenesulfonamide ET_A receptor antagonists and the development of the orally active, highly selective ET_A receptor antagonist BMS-182874.[24] In this report, we describe in greater detail the structure–activity relationships of this class of ET_A antagonists.

Chemistry

Nearly all of the sulfonamides were prepared by the condensation of sulfonyl chlorides with isoxazolamines in pyridine, at temperatures ranging from room temperature to 80 °C. Selected compounds (e.g., **33**, **73**, **74**, **75**) were prepared by the reaction of commercially

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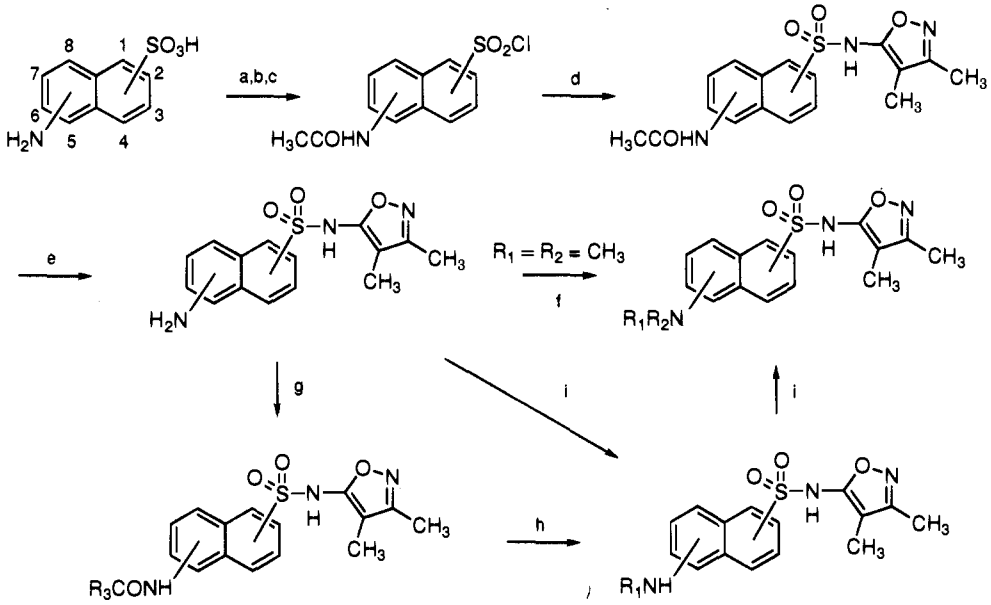
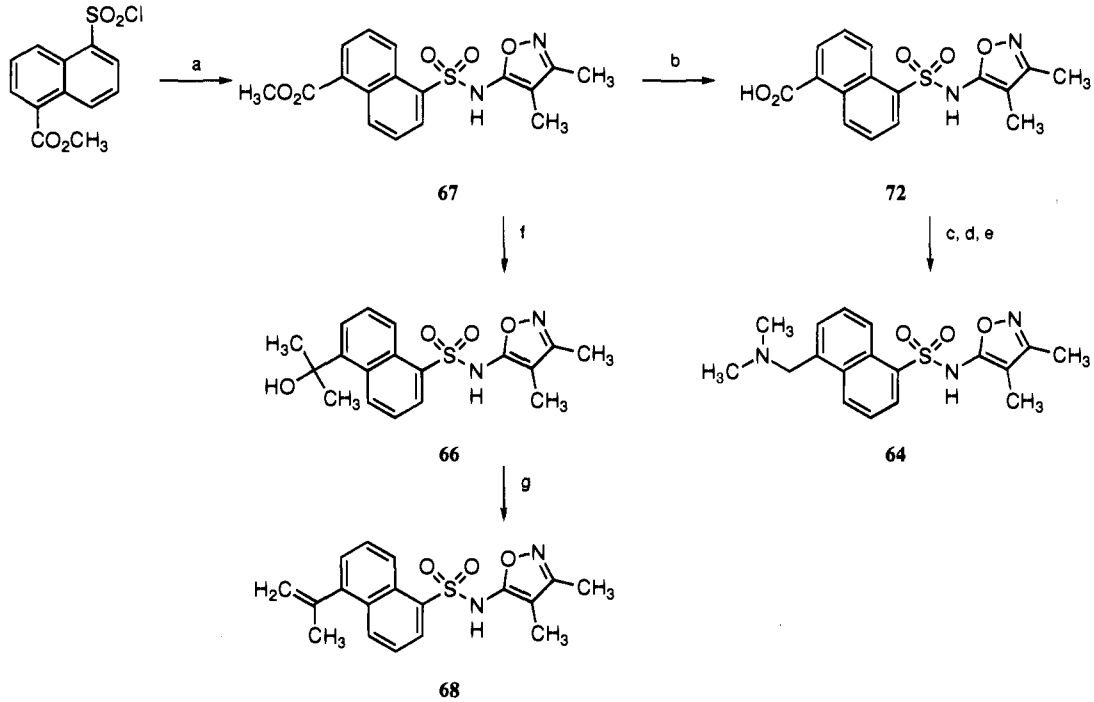
Scheme 1[a]



[a] (a) NaOH; (b) Ac_2O, 100 °C; (c) PCl_5 or $ClSO_3H$; (d) 3,4-dimethyl-5-isoxazolamine, DMAP, pyridine, room temperature to 70 °C; (e) NaOH; (f) 37% H_2CO, $NaCNBH_3$, H_2SO_4, aqueous THF; (g) R_3COCl, pyridine; (h) borane, THF; (i) ketone or aldehyde, $NaCNBH_3$, H^+.

Scheme 2[a]



[a] (a) 3,4-dimethyl-5-isoxazolamine, DMAP, pyridine, 70 °C; (b) NaOH, aqueous MeOH; (c) BH_3·THF; (d) PCC; (e) Me_2NH, $NaBH(OAc)_3$, THF; (f) CH_3MgBr, THF; (g) CF_3CO_2H, CH_2Cl_2, reflux.

available arenesulfonyl chlorides with commercially available isoxazolamines. Known sulfonyl chlorides were prepared by literature methods (**67**,[36] **69**[37]). In general, the corresponding aminonaphthalenesulfonic acids were used to prepare the aminonaphthalene derivatives. As shown in Scheme 1, following formation of the sodium salt and acetylation of the amine, the sulfonyl chlorides were prepared using either phosphorus pentachloride or chlorosulfonic acid. Following condensation with the isoxazolamine, the primary amine analogues were prepared by hydrolysis of the acetamide with aqueous sodium hydroxide and the dimethylamine analogues were prepared by reductive amination using sodium cyanoborohydride and aqueous formaldehyde. Secondary amines were prepared either by reductive amination using the appropriate ketone (e.g., **50**) or aldehyde (e.g., **60**) or by borane reduction of the appropriate amides, which were prepared from the appropriate acid chloride. As shown in Scheme 2, several other targets were prepared beginning with 5-(chlorosulfonyl)-1-naphthalenecarboxylic acid, methyl ester using standard synthetic procedures.

Dansylisoxazoles were generally prepared by the reaction of dansyl chloride with the corresponding isoxazolamine in pyridine. Isoxazolamines which were not commercially available were prepared by the condensation of hydroxylamine hydrochloride with the corresponding keto nitrile.[38-40] The keto nitriles were

prepared by the acylation of the corresponding nitrile with the requisite ester.[41] The isoxazolecarboxylic acid, ethyl ester **79** was prepared by the reaction of dansyl amide with 5-bromo-3-methyl-4-isoxazolecarboxylic acid, ethyl ester in DMF in the presence of cesium carbonate. The requisite bromoisoxazole was prepared as a mixture of regioisomers by the cycloaddition of ethyl 3-bromopropiolate[42] and acetaldoxime, using NaOCl to generate the nitrile oxide.[43]

Biological Testing

Compounds from the Bristol-Myers Squibb collection, selected without regard to chemical structure, were tested for their ability to inhibit [^{125}I]ET-1 binding to vascular smooth muscle VSM-A10 cells. From the large group of heterologous samples screened, one, sulfathiazole (**7**) (Table 2), showed reproducible binding activity. This compound was a relatively weak ET_A binding inhibitor, with an IC_{50} value of 69 μM. Additional screening of related compounds from the BMS collection was performed, and IC_{50} values were determined for compounds which displayed >30% inhibition of ET-1 binding at a concentration of 32 μM. Functional antagonist testing was performed by obtaining ET-1 concentration–response curves in rabbit carotid artery rings in the presence or absence of antagonist and calculating K_B values.

Structure–Activity Relationships

Benzenesulfonamides. Following the identification of the screening hit **7** as a weak ET_A receptor ligand, an initial group of diverse archival compounds was screened. These compounds were selected to probe the importance of the aminophenyl, phenylsulfonyl, sulfonamide, and isoxazole portions of **7** to receptor binding. The only compounds which showed reproducible binding at a concentration of 10 μM were 4-hydroxy-*N*-(2-thiazolyl)benzenesulfonamide (**5**) and sulfamethoxazole (**2**) (Table 2). These findings suggested that the core structure responsible for ET_A binding consisted of a 5-membered aminoheterocycle with an *N*-phenylsulfonyl group. An additional set of archival compounds was then tested to confirm and extend these conclusions. This screen uncovered a number of active analogues of varied potency including the thiadiazole **6**, a third class of *N*-heterocyclic benzenesulfonamide ET_A ligands. However, N-isoxazolylbenzenesulfonamides remained the most potent class, with sulfisoxazole (**1**; IC_{50} = 780 nM) displaying the highest affinity. In VSM-A10 cells, **1** inhibited the ET-1-induced increase in intracellular Ca^{2+} with an IC_{50} value of 40 ± 3 μM, indicating that this compound was a functional antagonist in a cell-based assay. Nevertheless, at a concentration of 100 μM it did not produce a rightward shift of the ET-1 concentration–response curve in rabbit carotid artery rings, indicating that it was not a functional antagonist under the conditions of this tissue-based experiment. In spite of this, efforts were undertaken to produce potent endothelin antagonists using the benzenesulfonamide **1** as a starting point.

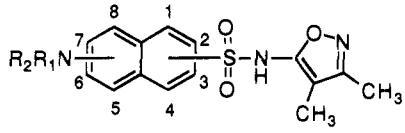
The requirements of the central sulfonamide portion of **1** were investigated by the synthesis of selected analogues (Table 3). The poor affinity of the *N*-methyl analogue **8** highlighted the importance of the sulfonamide hydrogen. The extremely poor affinity of the carboxamide analogue **10** suggested that the sulfonamide serves as more than a simple hydrogen bond donor. The pK_a values of a variety of N-substituted benzenesulfonamides have been determined, and *N*-isoxazolyl analogues are among the most acidic.[44] For example, the pK_a of 4-amino-*N*-(3-methyl-5-isoxazolyl)-benzenesulfonamide (**11**, Table 4) was reported to be 4.2, quite similar to the pK_a of a carboxylic acid. This information suggested that the sulfonamidate was the receptor-bound form of these endothelin receptor ligands. This hypothesis was especially attractive because both endothelin and most of the known endothelin receptor antagonists possess a carboxylic acid or an appropriate surrogate.[18,27,30,31,45–47] Nevertheless, the carboxyl-substituted sulfonamide **9** showed only weak receptor affinity, perhaps because of incorrect spacing of the anion in the (carboxymethyl)sulfonamide compared to the sulfonamidate.

A large number of benzenesulfonamides which contained phenyl groups and six-membered heterocycles attached to the sulfonamide nitrogen were screened. Some of these analogues contained groups which acidified the sulfonamide to the point where a significant proportion of sulfonamidate would exist at pH 7. Nevertheless, only marginal binding affinity was observed with any of these compounds. Therefore, our hypothesis at this time was that acidification of the sulfonamide by the nitrogen substituent was a necessary but not a sufficient characteristic for ET_A binding affinity. To further support this contention, several *N*-phenyl-4-aminobenzenesulfonamide analogues were prepared with strongly electron-withdrawing substituents on the *N*-phenyl ring attached to the sulfonamide nitrogen. Neither the 3,5-dinitrophenyl analogue (pK = 6.2[48]) nor the 3,5-bis(trifluoromethyl)phenyl analogue showed any binding at a concentration of 32 μM (data not shown).

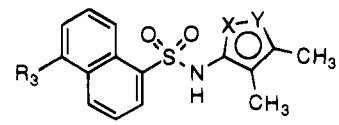
In order to identify the additional characteristics of the isoxazole ring which were important to binding, analogues were prepared in both the 3- and 5-isoxazolamine series (Table 4). Comparison of **11** with **1** and comparison of **2** with **12** indicated that the "inner" methyl group (the 4-methyl group in either series) is required for potent binding. Replacement of the "outer" methyl group (the 3-methyl group in the 5-isoxazolamine series or the 5-methyl group in the 3-isoxazolamine series) with larger substituents such as *tert*-butyl, *n*-pentyl,[24] or phenyl led to large losses in affinity. This suggested that these substituents may have exceeded the size requirements of a binding pocket on the receptor. The benzisoxazole **15** showed weak binding affinity.

Using *N*-(5-methyl-3-isoxazolyl)benzenesulfonamide and *N*-(3,4-dimethyl-5-isoxazolyl)benzenesulfonamide, the effects of phenyl substituents were studied (Table 5). In the former series, the 4-hydroxy analogue **16** showed modest affinity, while 4-substitution with acetamido, carboxy, or methoxy afforded inactive analogues (data not shown, IC_{50} > 10 μM). While both the amino and hydroxy groups are electron-donating and able to act as hydrogen bond donors, the poor activity of the methoxy analogue suggests that it is not electron-donating ability which is contributing to affinity. The 3-nitro-4-hydroxy substitution pattern, which further acidifies the 4-hydroxyl, also afforded an inactive analogue (IC_{50} > 32 μM, data not shown). Using *N*-(3,4-dimethyl-5-isoxazolyl)benzenesulfonamide as the frame-

Table 1. Physical Properties of *N*-Isoxazolylnaphthalenesulfonamide Analogues



A



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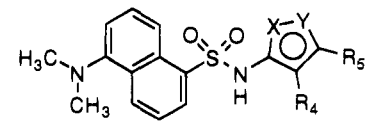


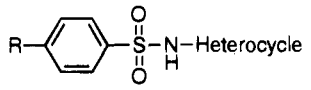
C

compd	structural type	substn pattern	R groups	X	Y	mp, °C	formula	anal.	recrystn solvent
29	A	1,4	$R_1 = R_2 = H$	O	N	152–154	$C_{15}H_{15}N_3O_3S \cdot 0.2H_2O$	C,H,N	
30	A	1,4	$R_1 = H$, $R_2 = Ac$	O	N	199–205	$C_{17}H_{17}N_3O_4S \cdot 0.2H_2O$	C,H,N,S	EtOH/H_2O
31	A	1,5	$R_1 = R_2 = H$	O	N	121–127	$C_{15}H_{15}N_3O_3S \cdot 1.0H_2O$	C,H,N,S	EtOH/H_2O
32	A	1,5	$R_1 = H$, $R_2 = Ac$	O	N	216–222	$C_{17}H_{17}N_3O_4S \cdot 0.4H_2O$	C,H,N,S	
33	A	1,5	$R_1 = R_2 = CH_3$	O	N	126–129	$C_{17}H_{19}N_3O_3S$	C,H,N,S	
34	A	1,6	$R_1 = R_2 = H$	O	N	179–180	$C_{15}H_{15}N_3O_3S \cdot 0.1H_2O$	C,H,N,S	MeOH/H_2O
35	A	1,6	$R_1 = H$, $R_2 = Ac$	O	N	232–236	$C_{17}H_{17}N_3O_4S$	C,H,N,S	MeOH/H_2O
36	A	1,6	$R_1 = R_2 = CH_3$	O	N	182–183	$C_{17}H_{19}N_3O_3S$	C,H,N,S	EtOH/H_2O
37	A	1,7	$R_1 = R_2 = H$	O	N	182–183	$C_{15}H_{15}N_3O_3S \cdot 0.5H_2O$	C,H,N,S	EtOH/H_2O
38	A	1,7	$R_1 = H$, $R_2 = Ac$	O	N	140–143	$C_{17}H_{17}N_3O_4S \cdot 1.1H_2O$	C,H,N,S	EtOH/H_2O
39	A	1,7	$R_1 = R_2 = CH_3$	O	N	222–223	$C_{17}H_{19}N_3O_3S \cdot 0.1H_2O$	C,H,N,S	EtOH/H_2O
40	A	2,5	$R_1 = R_2 = H$	O	N	152–153	$C_{15}H_{15}N_3O_3S$	C,H,N,S	toluene
41	A	2,5	$R_1 = H$, $R_2 = Ac$	O	N	210–212	$C_{17}H_{17}N_3O_4S \cdot 0.3H_2O$	C,H,N,S	EtOH/$CHCl_3$
42	A	2,6	$R_1 = R_2 = H$	O	N	174–176	$C_{15}H_{15}N_3O_3S \cdot 0.2H_2O$	C,H,N,S	EtOH/H_2O
43	A	2,6	$R_1 = H$, $R_2 = Ac$	O	N	206–207	$C_{17}H_{17}N_3O_4S \cdot 0.2H_2O$	C,H,N,S	MeOH/H_2O
44	A	2,7	$R_1 = R_2 = H$	O	N	193–194	$C_{15}H_{15}N_3O_3S \cdot 0.1H_2O$	C,H,N,S	EtOH/H_2O
45	A	2,7	$R_1 = H$, $R_2 = Ac$	O	N	192–194	$C_{17}H_{17}N_3O_4S$	C,H,N,S[a]	EtOH/H_2O
46	A	2,7	$R_1 = R_2 = CH_3$	O	N	131–132	$C_{17}H_{19}N_3O_3S \cdot 0.4H_2O$	C,H,N,S[b]	bnz/hex
47	A	2,8	$R_1 = R_2 = H$	O	N	198–202	$C_{15}H_{15}N_3O_3S$	C,H,N,S	EtOH/H_2O
48	A	2,8	$R_1 = H$, $R_2 = Ac$	O	N	133–134	$C_{17}H_{17}N_3O_4S \cdot 1.1H_2O$	C,H,N,S	EtOH/H_2O
49	A	2,8	$R_1 = R_2 = CH_3$	O	N	155–156	$C_{17}H_{19}N_3O_3S \cdot 0.1H_2O$	C,H,N,S	EtOH/H_2O
50	B	1,5	$R_3 = NHCH(CH_3)_2$	O	N	156–159	$C_{18}H_{21}N_3O_3S$	C,H,N,S	
51	B	1,5	$R_3 = c\text{-}(N(CH_2)_4)$	O	N	170–173	$C_{19}H_{21}N_3O_3S \cdot 0.5H_2O$	C,H,N,S	
52	B	1,5	$R_3 = NHCH_2CH_3$	O	N	75–85	$C_{17}H_{19}N_3O_3S \cdot 0.2EtOAc$	C,H,N,S	
53	B	1,5	$R_3 = NH(CH_2)_4CH_3$	O	N	142–145	$C_{20}H_{25}N_3O_3S$	C,H,N,S	
54	B	1,5	$R_3 = NHCH_3$	O	N	92–105	$C_{16}H_{17}N_3O_3S \cdot 0.6H_2O$	C,H,N,S	
55	B	1,5	$R_3 = N(CH_3)CH(CH_3)_2$	O	N	132–133	$C_{19}H_{23}N_3O_3S \cdot 1.2H_2O$	C,H,N,S	Hex/EtOAc
56	B	1,5	$R_3 = c\text{-}(NCO(CH_2)_3)$	O	N	183–187	$C_{19}H_{19}N_3O_4S \cdot 0.8H_2O$	C,H,N,S	Hex/EtOAc
57	B	1,5	$R_3 = NHCSNHPh$	O	N	137–138	$C_{22}H_{20}N_4O_3S_2 \cdot 1.9H_2O$	C,H,N,S[c]	
58	B	1,5	$R_3 = c\text{-}(NCO(CH_2)_4)$	O	N	203–208	$C_{20}H_{21}N_3O_4S \cdot 0.1H_2O$	C,H,N,S	
59	B	1,5	$R_3 = NHCH(CH_3)CO_2Et$	O	N	62–65	$C_{20}H_{23}N_3O_5S \cdot 0.1H_2O$	C,H,N,S[d]	
60	B	1,5	$R_3 = NHCH_2Ph$	O	N	140–142	$C_{22}H_{21}N_3O_3S$	C,H,N,S	
61	B	1,5	$R_3 = NHCOCH(CH_3)_2$	O	N	177–180	$C_{19}H_{21}N_3O_4S$	C,H,N,S	MeOH/H_2O
62	B	1,5	$R_3 = c\text{-}(N(CH_2)_5)$	O	N	89–93	$C_{20}H_{23}N_3O_3S \cdot 1.3H_2O$	C,H,N,S[e]	
63	B	1,5	$R_3 = NH(CH_2)_3CO_2H$	O	N	128–132	$C_{19}H_{21}N_3O_5S \cdot 1.2H_2O$	C,H,N,S[f]	
64	B	1,5	$R_3 = CH_2N(CH_3)_2$	O	N	143–146	$C_{18}H_{21}N_3O_3S \cdot 1.7H_2O$	C,H,N,S[g]	
65	B	1,5	$R_3 = NH(CH_2)_5Ph$	O	N	120–123	$C_{26}H_{28}N_3O_3SNa \cdot 0.7H_2O$	C,H,N,S	
66	B	1,5	$R_3 = C(OH)(CH_3)_2$	O	N	97–101	$C_{18}H_{20}N_2O_4S$	C,H,N,S	
67	B	1,5	$R_3 = CO_2CH_3$	O	N	173–178	$C_{17}H_{16}N_2O_5S \cdot 0.2H_2O$	C,H,N,S	
68	B	1,5	$R_3 = C(=CH_2)(CH_3)$	O	N	65–70	$C_{18}H_{18}N_2O_3S$	C,H,N,S	
69	B	1,5	$R_3 = OCH_3$	O	N	72–75	$C_{16}H_{16}N_2O_4S \cdot 0.25H_2O$	C,H,N,S	
70	B	1,5	$R_3 = NH(CH_2)_2CHPh_2$	O	N	171–177	$C_{30}H_{29}N_3O_3S \cdot 0.8H_2O$	C,H,N,S	ether
71	B	1,5	$R_3 = OH$	O	N	201–203	$C_{15}H_{14}N_2O_4S$	C,H,N,S	EtOH/H_2O
72	B	1,5	$R_3 = CO_2H$	O	N	202–204	$C_{16}H_{14}N_2O_5S \cdot 0.3H_2O$	C,H,N,S	
73	B	1,5	$R_3 = N((CH_2)_3CH_3)_2$	O	N	130–135	$C_{23}H_{30}N_3O_3SNa \cdot 0.6H_2O$	C,H,N,S	
74	B	1,5	$R_3 = H$	O	N	54–58	$C_{15}H_{14}N_2O_3S$	C,H,N,S	
75	B	1,5	$R_3 = Cl$	O	N	155–158	$C_{15}H_{13}ClN_2O_3S$	C,H,N,S,Cl	
76	B	1,5	$R_3 = N{=}CPh_2$	O	N	220–225	$C_{28}H_{23}N_3O_3S$	C,H,N,S	MeOH
77	B	1,5	$R_3 = NHCOCH_2CHPh_2$	O	N	200–204	$C_{30}H_{27}N_3O_4S \cdot 0.2H_2O$	C,H,N,S	MeOH/H_2O
78	A	1,5	$R_1 = R_2 = CH_3$	N	O	190–192	$C_{17}H_{19}N_3O_3S$	C,H,N,S	EtOH/H_2O
79	C	1,5	$R_4 = Et$, $R_5 = CH_3$	O	N	55–85	$C_{18}H_{21}N_3O_3S$	C,H,N,S	
80	C	1,5	$R_4 = CH_3$, $R_5 = Et$	O	N	51–68	$C_{18}H_{21}N_3O_3S \cdot 0.5H_2O$	C,H,N,S	
81	C	1,5	$R_4 = CH_3$, $R_5 = H$	O	N	57–67	$C_{16}H_{17}N_3O_3S \cdot 0.4H_2O$	C,H,N,S	
82	C	1,5	$R_4 = H$, $R_5 = CH_3$	O	N	183–187	$C_{16}H_{17}N_3O_3S \cdot 0.5H_2O$	C,H,N,S	EtOAc/Hex
83	C	1,5	$R_4 = Ph$, $R_5 = CH_3$	O	N	78–88	$C_{22}H_{21}N_3O_3S \cdot 0.4H_2O$	C,H,N,S	
84	C	1,5	$R_4 = NO_2$, $R_5 = CH_3$	O	N	222–228	$C_{16}H_{16}N_4O_5S$	C,H,N,S	
85	C	1,5	$R_4 = CO_2Et$, $R_5 = CH_3$	O	N	146–148	$C_{19}H_{21}N_3O_5S$	C,H,N,S	ether
86	C	1,5	$R_4 = CH_2Ph$, $R_5 = CH_3$	O	N	59–65	$C_{23}H_{23}N_3O_3S \cdot 0.1H_2O$	C,H,N,S	
87	C	1,5	$R_4, R_5 = -(CH_2)_4-$	O	N	69–80	$C_{19}H_{21}N_3O_3S \cdot 0.8H_2O$	C,H,N,S	

[a] Anal. fits for $0.03H_2O$, 0.04 EtOAc. [b] Anal. fits for 0.4(benzene). [c] Anal. fits for $0.75NH_3$. [d] Anal. fits for 0.15(hexanes), $0.12H_2O$. [e] Anal. fits for $0.5CF_3CO_2H$. [f] Anal. fits for $1.0CH_2Cl_2$. [g] Anal. fits for $1.2CF_3CO_2H$.

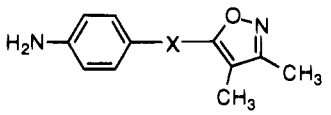
work, the moderate affinity of a 4-hydroxy analogue (**25**) was confirmed, and it was discovered that a 4-fluoro substituent also provided a relatively potent analogue (**17**). Several 3-substituted analogues displayed moderate affinity. Because the 4-amino compound remained the analogue with the highest affinity, a number of analogues were prepared which contained substituted amino groups. While none of these compounds displayed affinity as high as **1**, several alkylamino (**19**, **22**) and aralkylamino analogues (**27**), as well as the 4-fluoro

Table 2. Biological Activity of Screening Hits



compd	R	heterocycle	IC_{50}, μM (n)
1	NH_2	3,4-dimethyl-5-isoxazolyl	0.78 ± 0.06 (3)
2	NH_2	5-methyl-3-isoxazolyl	4.0 (1)
3	$NHCOCH_2NH_2$	3,4-dimethyl-5-isoxazolyl	6.5 (1)
4	$NHCOCH_3$	3,4-dimethyl-5-isoxazolyl	13 (1)
5	OH	2-thiazolyl	30 (1)
6	NH_2	1,3,4-thiadiazol-2-yl	57 (1)
7	NH_2	2-thiazolyl	69 ± 6 (3)

Table 3. Biological and Physical Data for Sulfonamide Modified Analogues

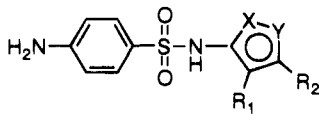


compd	X	IC_{50}, μM (n)	mp, °C	recrystn solvent
1	SO_2NH	0.78 ± 0.06 (3)		
8	SO_2NCH_3	>32 (1)	175–176	EtOH
9	$SO_2NCH_2CO_2H$	>32 (1)	202–205	EtOH
10	CONH	>32 (1)	180–181	EtOH/H_2O

analogue, were functional antagonists with apparent K_B values in the double digit micromolar range.

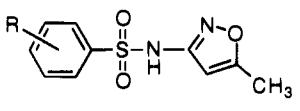
Naphthalenesulfonamides. Prompted by the functional antagonist activity of aminobenzenesulfonamides of increased lipophilicity, we investigated the activity of naphthalenesulfonamides (Table 6). In addition to their increased lipophilicity, the different substitution patterns of aminonaphthalenesulfonamides allow a systematic analysis of the optimal spacing of the critical amine and sulfonamide pharmacophores. Using the *N*-(3,4-dimethyl-5-isoxazolyl)naphthalenesulfonamide framework, amino-, acetylamino-, and dimethylamino-substituted analogues were prepared for nearly all of the possible substitution patterns. Across this series of compounds, there did not appear to be a consistent rank order of binding potency for the three derivatives prepared. For example, the dimethylamino derivative **33** was the most potent 1,5 analogue, the acetylamino derivative **38** was the most potent 1,7 analogue, while the amino derivative **47** was the most potent 2,8 analogue. The 1,5-substitution pattern provided the highest affinity analogues. The dimethylamino derivative **33** (BMS-182874) displayed an IC_{50} value of 15 ± 1 nM (K_i = 55 ± 3.7 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 ± 100 nM in rabbit carotid artery rings (for comparison, respective values for BQ-123: K_i = 18 ±

Table 4. *N*-Isoxazolylbenzenesulfonamide Analogues

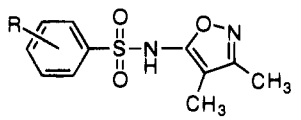


compd	X	Y	R_1	R_2	binding IC_{50}, μM (n)	$[Ca^{2+}]_i$ IC_{50}, μM	mp, °C	recrystn solvent
1	O	N	CH_3	CH_3	0.78 ± 0.06 (3)	40 ± 3		
11	O	N	H	CH_3	28 (1)		164–165	H_2O
12	N	O	CH_3	CH_3	0.26 (1)	40 ± 5	192–193	EtOH
2	N	O	H	CH_3	4 (1)			
13	N	O	H	$C(CH_3)_3$	>32 (1)		172–174	benzene
14	N	O	H	C_6H_5	>32 (1)		150–152	MeOH
15	N	O	$-(CH)_4-$		32 (1)		151–152	EtOH/H_2O

Table 5. *N*-Isoxazolylbenzenesulfonamide Analogues



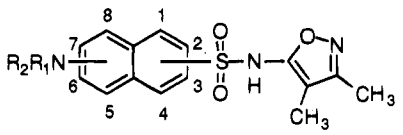
A



B

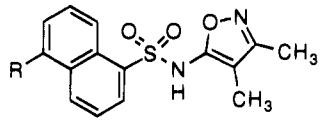
compd	isoxazole	R	IC_{50}, μM (n)	K_B* or $K_{B_{app}}$, μM	mp, °C	recrystn solvent
2	A	4-NH_2	4.0 (1)	>100		
16	A	4-OH	53 (1)	>100	133–135	benzene
1	B	4-NH_2	0.78 ± 0.06 (3)	>100		
17	B	4-F	1 (1)	40 ± 20	151–154	
18	B	3-NH_2	1.3 (1)		147–150	
19	B	3-$NH(CH_2)_2CH_3$	2.1 (1)	70 ± 41*	171–173	
20	B	4-$NHCSNHC_6H_5$	4.1 (1)		178–179	
21	B	4-$NHCH_2CH_3$	5 (1)		138–139	CH_2Cl_2
22	B	4-$NH(CH_2)_2CH_3$	27 ± 5.6 (2)	40 ± 10	87–88	
23	B	3-C_6H_5	5.4 (1)		gum	
24	B	3-NO_2	7.8 (1)		176–178	EtOH
25	B	4-OH	9.2 ± 1.9 (3)	>100	185–195	EtOH/H_2O
26	B	4-$NHCH_2CO_2H$	12 (1)		159–163	
27	B	4-$NHCH_2C_6H_5$	9.8 ± 5.3 (3)	100 ± 50	58–62	
28	B	4-$NHCONHC_6H_5$	38 (1)		132–138	EtOAc/hexanes

Table 6. *N*-Isoxazolylnaphthalenesulfonamide Analogues



compd	substitution pattern	R_1	R_2	IC_{50}, μM (n)	K_B* or $K_{B_{app}}$, μM
29	1,4	H	H	3.5 (1)	
30	1,4	H	$COCH_3$	3.4 (1)	
31	1,5	H	H	4.0 ± 0.8 (2)	>10
32	1,5	H	$COCH_3$	0.88 ± 0.12 (2)	11 ± 0.27*
33	1,5	CH_3	CH_3	0.015 ± 0.01 (2)	0.52 ± 10*
34	1,6	H	H	2.4 (1)	
35	1,6	H	$COCH_3$	1.3 (1)	
36	1,6	CH_3	CH_3	0.84 (1)	
37	1,7	H	H	1.4 (1)	
38	1,7	H	$COCH_3$	0.87 (1)	
39	1,7	CH_3	CH_3	5.6 (1)	
40	2,5	H	H	24 (1)	
41	2,5	H	$COCH_3$	2.7 ±	40 ± 20
42	2,6	H	H	0.66 ±	43 ± 10*
43	2,6	H	$COCH_3$	3.8 (1)	
44	2,7	H	H	10 (1)	
45	2,7	H	$COCH_3$	42 (1)	
46	2,7	CH_3	CH_3	1.4 (1)	
47	2,8	H	H	9.8 (1)	
48	2,8	H	$COCH_3$	27.5 (1)	
49	2,8	CH_3	CH_3	31.5 (1)	

Table 7. 5-Substituted *N*-Isoxazolylnaphthalenesulfonamide Analogues



compd	R	IC_{50}, μM	K_B* or K_{Bapp}, μM
50	$NHCH(CH_3)_2$	0.02 ± 0.001	0.33 ± 0.09*
51	c-($N(CH_2)_4$)	0.03 ± 0.01	0.28 ± 0.04*
52	$NHCH_2CH_3$	0.06 ± 0.005	0.50 ± 0.07*
53	$NH(CH_2)_4CH_3$	0.08 ± 0.005	0.27 ± 0.04*
54	$NHCH_3$	0.13 ± 0.04	1.60 ± 0.18*
55	$N(CH_3)CH(CH_3)_2$	0.13 ± 0.01	0.70 ± 0.16
56	c-($NCO(CH_2)_3$)	0.13 ± 0.007	9.6 ± 1.7*
33	$N(CH_3)_2$	0.15 ± 0.01	0.52 ± 0.10*
57	$NHCSNHC_6H_5$	0.15 ± 0.04	13 ± 4.0
58	c-($NCO(CH_2)_4$)	0.27 ± 0.06	7.0 ± 1.0
59	$NHCH(CH_3)CO_2CH_2CH_3$	0.40 ± 0.54	3.1 ± 1.0*
60	$NHCH_2C_6H_5$	0.59 ± 0.15	0.90 ± 0.25
61	$NHCOCH(CH_3)_2$	0.88 ± 0.05	
62	c-($N(CH_2)_5$)	1.76 ± 0.09	4.0 ± 0.8
63	$NH(CH_2)_3CO_2H$	1.96 ± 0.48	30 ± 7
64	$CH_2N(CH_3)_2$	2.8 ± 0.7	20 ± 6
65	$NH(CH_2)_5C_6H_5$	3.05 ± 0.76	
66	$C(OH)(CH_3)_2$	2.89 ± 0.05	
67	CO_2CH_3	3.38 ± 0.59	
68	$C(=CH_2)(CH_3)$	5.7 ± 2.4	
69	OCH_3	6.5 ± 1.40	
70	$NH(CH_2)_2CH(C_6H_5)_2$	7.8 ± 0.1	
71	OH	7.8 ± 0.1	100 ± 40
72	CO_2H	13 ± 1.0	
73	$N((CH_2)_3CH_3)_2$	15.9 ± 0.3	
74	H	20. ± 1.0	
75	Cl	24 ± 3.2	
76	$N=C(C_6H_5)_2$	39 ± 1.9	
77	$NHCOCH_2CH(C_6H_5)_2$	46 ± 7.3	

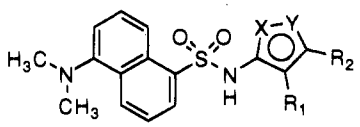
4.2 nM; IC_{50} value for intracellular Ca^{2+} increase = 26 ± 7 nM; K_B = 35 ± 14 nM).

In the optimal 1,5-substitution pattern, a variety of analogues were prepared in order to probe the structure–activity requirements of the 5-position (Table 7). A number of amine substituents, including secondary amines, acyclic and cyclic tertiary amines, thioureas, amides, and lactams provided analogues which displayed high affinity. Amines, amides, or imines which contained very large substituents were of considerably lower affinity, suggesting a steric limit to the size of the 5-substituent. There are a number of physical properties of aromatic amines which might be important to receptor binding. The pK_a values of 1-aminonaphthalene, 1-(methylamino)naphthalene, and 1-(dimethylamino)naphthalene were reported to be 3.92, 3.70, and 4.88, respectively.[49] At the pH of the receptor binding assay, therefore, all of the aminonaphthalenesulfonamides should exist as the sulfonamidate with a neutral 5-amino group. Indeed, the poor activity of **71**, the analogue with a 5-hydroxy substituent which is capable of acting as a hydrogen bond donor, supports the hypothesis that a 5-ammonium ion does not play a role in receptor binding. An aromatic amine is also electron-donating and capable of acting as a hydrogen bond acceptor. The poor activity of the 5-methoxy analogue **69** suggests that electron donation to the naphthalene ring is not an important feature of the 5-amine substituent. Small lipophilic substituents on the 5-amino group led to increases in both binding affinity and functional potency. The poor affinity of the 5-isopropenyl analogue **68** indicates that a 5-lipophilic substituent alone is insufficient to provide a potent ligand.

Despite the fact that a large variety of compounds with 5-nitrogen substituents provided ligands with IC_{50} values below 1 μM, only analogues containing secondary and tertiary amines at the 5-position were potent functional antagonists. The most potent analogues, which displayed K_B values between 250 and 500 nM, contained isopropylamine (**50**), pyrrolidine (**51**), ethylamine (**52**), butylamine (**53**), and dimethylamine (**33**) at the 5-position. The reasons for the large differences between the K_i values and the K_B values is not understood, but this phenomenon has been observed with other endothelin antagonists (e.g., FR 139317, K_i = 0.53 nM, K_B = 63 nM).[50] Wu-Wong et al. have shown that the irreversibility of ET binding to ET_B receptors compared to the binding of the anagonists Ro46-2005 or PD142893 results in an increase of binding IC_{50} values with increasing incubation time.[51] They speculate that this irreversibility of ET binding may affect the functional potency of ET antagonists. We have reported that, for BMS-182874, the K_i value for ET_A receptor binding is similar both to the apparent K_B value for inhibition of ET-1 stimulated phosphoinositide turnover (75 nM) as well as to the K_B value for ET-1 induced increases in intracellular Ca^{2+} (140 nM) in VSM-A10 cells.[52] The similarities between binding affinity and the K_B values in these selected cell-based functional assays lead us to speculate that the differences in binding affinity and functional potency reflect differences in cellular versus tissue preparations.

With the identification of the 1,5-naphthalene substitution pattern containing a 5-alkylamine as optimal, the structure–activity requirements of the isoxazole were reinvestigated. Using dansyl as the naphthalenesulfonamide, the dimethyl analogues containing either 5-isoxazolamine (**33**) or 3-isoxazolamine (**78**) were equipotent (Table 8). Further studies were conducted using the 5-isoxazolamine pattern. Analogues **84** and **85** containing strongly electron-withdrawing substituents at the 4-position were poorly active, suggesting that

Table 8. *N*-Isoxazolylnaphthalenesulfonamide Analogues



compd	R_1	R_2	X	Y	IC_{50}, μM	K_B* or $K_{B_{app}}$, μM
78	CH_3	CH_3	N	O	0.06 ± 0.01	0.98 ± 0.28
33	CH_3	CH_3	O	N	0.15 ± 0.01	0.52 ± 0.10
79	Et	CH_3	O	N	0.29 ± 0.06	4 ± 1
80	CH_3	Et	O	N	1.17 ± 0.5	3.0 ± 0.8
81	CH_3	H	O	N	4.9 ± 0.86	
82	H	CH_3	O	N	8.9 ± 1.1	
83	Ph	CH_3	O	N	15.9 ± 0.6	
84	NO_2,	CH_3	O	N	17.3 ± 0.8	>100
85	CO_2Et	CH_3	O	N	31 ± 1.4	
86	CH_2Ph	CH_3	O	N	50 ± 7	
87	$R_1, R_2 = -(CH_2)_4-$		O	N	153 ± 11	

these acidifying groups lowered the pK of the sulfonamide below an optimal range. At both the 3- and 4-position, replacement of the methyl group with hydrogen caused large reductions in potency (**81**, **82**), suggesting that the methyl groups provide hydrophobic binding interactions. Alternate explanations are that the methyl groups increase the electron density of the isoxazole ring or that at least the "inner" methyl group is important for conformationally biasing the molecule. Attempts to improve affinity by inserting larger hydrophobic groups indicated that the steric constraints at these positions are quite stringent. Both the 3-methyl-4-ethyl analogue **79** and the 3-ethyl-4-methyl analogue **80** were somewhat less potent than the dimethyl analogues. Larger groups at the 4-position led to large reductions in potency and constraining the 3- and 4-substituents into a 6-membered ring (**87**) also produced an analogue with low affinity.

Additional Characterization of Compound 33. In order to gain insight into the conformational preferences of the potent functional antagonist **33**, a solid state structure was obtained (Figure 1). This structure suggests some hypotheses as to what makes the 1,5-naphthalene substitution pattern superior to other substitution patterns. Examination of the solid state structure shows that there are two severe steric interactions, one between the peri-hydrogen at C-8 and the sulfonamide group and the other between the peri-hydrogen at C-4 and the dimethylamino group.

The peri-hydrogen at C-8 projects between the sulfonamide nitrogen and one of the sulfonyl oxygen atoms (distance from H8 to sulfonyl oxygen is 2.34 Å). This interaction is severe enough to both bend the sulfonamide S atom out of the plane (the $S-C^1-C^9-C^8$ torsion, normally 0°, is 13°) and to cause the other sulfonyl oxygen to nearly eclipse the hydrogen on C-2 ($O-S-C^1-C^2$ torsion = 8°; distance from H2 to sulfonyl oxygen is 2.35 Å). This forced staggering locks the sulfonamidate anion in a orientation in space ($N-S-C^1-C^9$ torsion angle is 70°) which may be preferred by the receptor. This conformational locking is absent in benzenesulfonamides lacking an ortho substituent. In these cases the $N-S-C^{aryl}-C^{aryl}$ torsion of the sulfonamide can assume any angle between 60° and 90° (data not shown). These conformational preferences are independent of the substituent on the sulfonamide nitrogen.

The similar peri-interaction between the C-4 hydrogen and the C-5 substituent (distance from H4 to the 5-nitrogen is 2.51 Å) causes the nitrogen atom of the dimethylamino group to bend out of plane to a similar extent as seen for the sulfonamide S atom. These two interactions also substantially warp the naphthalene nucleus from planarity. Importantly, the peri interaction with H4 causes the N atom to pyramidalize and twist so that the lone pair carried on the nitrogen is isolated from conjugation with the aromatic nucleus. The pyramidalization from a classical planar anilinetype nitrogen is extensive as the nitrogen is as pyramidal as that found in ammonia. Because of this isolation and hybridization, the lone pair becomes an excellent H-bond acceptor. Again, the conformational locking presumably fixes in space the orientation of the dimethylamino group in a highly favorable manner. While the other naphthalene substitution patterns posess one or the other (or neither) of these important interactions, only the 1,5-pattern contains them both.

One of the most potent naphthalenesulfonamide antagonists, **33**, was selected for further characterization. We have already reported that **33** is an extremely ET_A selective receptor antagonist, with a K_i value greater than 200 μM in an ET_B-containing tissue, namely rat cerebellar membranes.[24] As noted above, the activity of **33** has been more extensively explored in cell-based assays, tissue-based assays, and *in vivo* assays, and these results are being reported elsewhere.[52]

Conclusions. Extensive structure–activity studies, beginning with the random screening hit **7**, led to the discovery of *N*-isoxazolylnaphthalenesulfonamides as ET_A receptor ligands. Further optimization led to the discovery of 5-amino-*N*-(3,4-dimethyl-isoxazolyl)-1-naphthalenesulfonamides as a potent class of ET_A receptor antagonists. Features of this class of molecule which contribute to receptor affinity include a 1,5-naphthalene substitution pattern, a 5-amino substituent, a relativey acidic sulfonamide NH, and a dimethylisoxazole.

Experimental Section

Radioligand Binding Assays. Receptor binding assays (A10 rat thoracic aorta smooth muscle cell membranes) were performed in VSM-A10 cell membranes as previously described[45] or in intact VSM-A10 cells as follows. VSM-A10 cells were cultured in Dulbecco's modified Eagles medium supplemented with 20% bovine calf serum in Falcon T-175 flasks. Cells from one confluent flask were detached with trypsin and used to seed a 1050 cm^2 roller bottle. Medium was removed and replaced with 200 mL of fresh medium every 3 days, as well as 24 h prior to use. Cells were used when confluence was reached. On the day of the assay, the cells were removed from the roller bottle by scraping, collected by centrifugation, and resuspended in phosphate buffered saline (PBS) supplemented with 0.1% glucose and 0.1% bovine serum albumin (BSA), hereafter referred to as PBG. A suspension of 3.5 mg of cells in 90 μL was placed into each well of a microtiter plate, and 10 μL of [^{125}I]ET-1 (2200 Ci/mmol, NEN) at 0.4 nM was added. In addition, each well contained 100 μL of ET-1, a synthetic compound, or vehicle. The cells were incubated for 3 h at 4 °C. The cells were washed twice with ice-cold PBG and collected onto a glass fiber filtermat using a cell harvester (Tomtec; Orange, CT). The filtermat was washed two additional times with ice-cold PBS supplemented with 0.1% BSA to remove unbound ligand. Radioactivity bound to the cells was quantified by scintillation counting (Betaplate, LKB). Nonspecific binding, defined in the presence of a high concentration (1 μM) of unlabeled ET-1, was subtracted from the total binding to yield specific binding, hereafter referred to simply as binding. Competition binding data were analyzed by iterative curve fitting to a one or two site binding model. Inhibition constants (K_i) were calculated from IC_{50} values.[53]

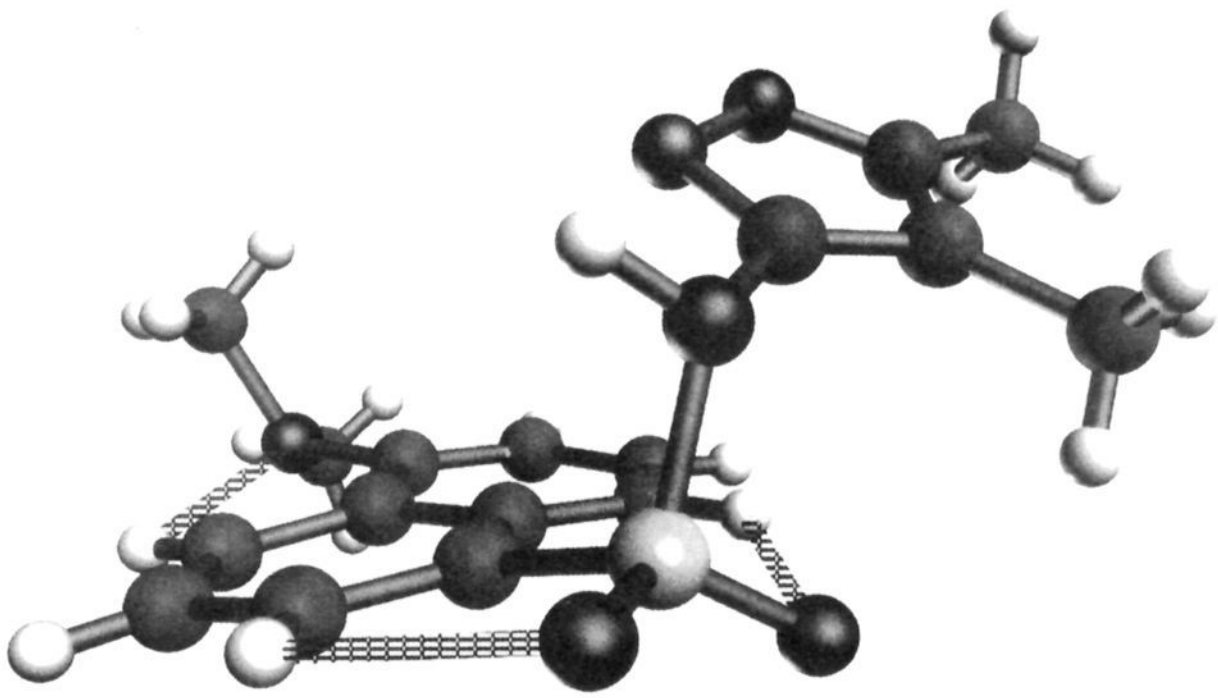


Figure 1. Solid state structure of **33**. Close approaches are indicated by dashed lines and are discussed in the text.

In Vitro Functional Assay. Functional assays (rabbit carotid artery rings) were performed as previously described.[45] K_B values were determined in experiments in which at least 3 different concentrations of test compound were studied. Apparent K_B values ($K_{B_{app}}$) were calculated when only one antagonist concentration was used. The ability of the compounds to inhibit the increase in intracellular free calcium ion concentration elicited by 3 nM ET-1 was determined in A10 cells as described elsewhere for rat aortic smooth muscle cells.[54] Results from these experiments are reported as IC_{50} values.

General Chemical Procedures. Aminonaphthalenesulfonic acids were obtained from Aldrich/Bader, Pfaltz and Bauer, or TCI America. Aminoisoxazoles were obtained from Maybridge Chemical Co., Lancaster Synthesis, or Fluka. Melting points were recorded on a Thomas-Hoover capillary apparatus and are reported uncorrected. IR spectra were recorded on a Mattson Sirius 100 spectrometer. Proton NMR (^{1}H NMR) and carbon NMR (^{13}C NMR) spectra were obtained on JOEL FX-270 or GX-400 spectrometers and are reported relative to tetramethylsilane (TMS) reference. Analytical and preparative HPLC were performed on YMC columns (A-302, S-5, 120A ODS, 4.6 × 150 mm; SH-345-15, S-15, 120A ODS, 20 × 500 mm) with acetonitrile:water gradients containing 0.1% trifluoroacetic acid. Chromatography was performed under flash conditions using EM Science silica 0.040−0.063 mm particle size. THF was distilled from Na/benzophenone. Solutions were dried with magnesium sulfate unless otherwise noted. Experimentals for the preparation of **64**, **66**, **68**, **71**, and **72** were described in the supplementary material from ref 24.

***N*-[5-[[(3,4-Dimethyl-5-isoxazolyl)amino]sulfonyl]-2-naphthalenyl]acetamide (35).** To a suspension of 6-amino-1-naphthalenesulfonic acid (10.0 g, 44.8 mmol) in water (10 mL) was added 5 N NaOH (9 mL, 45 mmol). The mixture was warmed to effect complete solution, and the solvent was evaporated to provide the sodium salt of 6-amino-1-naphthalenesulfonic acid (11.3 g, 100%) as a white solid. A portion of this material (10.0 g, 40.8 mmol) was suspended in acetic anhydride (100 mL). The mixture was heated at 95 °C for 4 h, cooled to room temperature, and evaporated to provide the sodium salt of 6-acetylamino-1-naphthalenesulfonic acid (11.2 g, 87%) as a white powder. A solution of this material (1.00 g, 3.48 mmol) in chlorosulfonic acid (5.0 mL, 75.2 mmol) was stirred at room temperature under argon for 2.5 h and added dropwise to about 400 mL of crushed ice, and the mixture was stirred until the ice melted. A fine precipitate formed which was filtered, washed with copious amounts of water (400 mL), and dried to yield 6-(acetylamino)-1-naphthalenesulfonyl chloride (0.85 g, 86%). A solution of this material (0.70 g, 2.47 mmol) in pyridine (3 mL) was added dropwise to a solution of 3,4-dimethyl-5-isoxazolamine (0.36 g, 3.2 mmol) and 4-(dimethylamino)pyridine (0.057 g, 0.47 mmol) in pyridine (3 mL). The mixture was heated at 70 °C for 6 h, cooled to room temperature, and added dropwise to water (100 mL). The solution was acidified to pH 3 with 6 N HCl, and the resulting white precipitate was collected by filtration and dried (0.71 g, 80%). Recrystallization of 0.20 g of the solid from methanol/water afforded **35** as light brown crystals (0.14 g): mp 232−236 °C dec; ^{1}H NMR (DMSO-d_6) δ 10.34 (s, 1 H), 8.49 (m, 2 H), 8.18 (d, 1 H, J = 8 Hz), 7.98 (d, 1 H, J = 8 Hz), 7.75 (m, 1 H), 7.59 (t, 1 H, J = 8 Hz), 2.14 (s, 3 H), 2.03 (s, 3 H), 1.47 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 168.9, 161.3, 155.1, 137.7, 134.9, 134.6, 134.2, 127.5, 125.0, 124.8, 123.7, 121.6, 115.7, 105.3, 24.1, 10.2, 5.7; IR (KBr) 1661, 1624, 1609, 1547, 1505, 1464, 1418, 1375, 1348, 1331, 1161, 1134 cm^{-1}; MS (M + H)$^+$ 360.

6-Amino-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulfonamide (34). A solution of **35** (0.84 g, 2.4 mmol) in 5 N NaOH (5 mL) and methanol (1 mL) was heated at 70 °C overnight. After cooling to room temperature, the mixture was acidified to pH 3 with 6 N HCl, forming a precipitate which was filtered and dried under vacuum to yield 0.65 g (88%). Recrystallization of 0.20 g from methanol/water afforded **34** as brown crystals (0.12 g), mp 179−180 °C. ^{1}H NMR (DMSO-d_6) δ 8.27 (d, J = 9 Hz, 1 H), 7.84 (d, J = 9 Hz, 1 H), 7.68 (m, 1 H), 7.38 (m, 1 H), 7.12 (dd, J ~8−9, 2−3 Hz, 1 H), 6.96 (d, J = 2 Hz, 1 H), 2.03 (s, 3 H), 1.48 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 161.2, 155.3, 147.3, 136.2, 134.9, 132.0, 125.0, 124.3, 123.9, 120.0, 106.7, 105.0, 10.2, 5.7; IR (KBr) 1657, 1634, 1514, 1427, 1337, 1165, 1136, 712, 594, 583, 538 cm^{-1}; MS (M + H)$^+$ 318.

6-(Dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulfonamide (36). To a solution of 3 M H_2SO_4 (0.33 mL, 0.98 mmol) and 13.3 M formaldehyde (0.44 mL, 5.9 mmol) in THF (6 mL) at 0 °C was added dropwise a solution of **34** (0.31 g, 0.97 mmol) and sodium cyanoborohydride (0.44 g, 7.0 mmol) in tetrahydrofuran (6 mL). The mixture was stirred at 0 °C for 5 h and was made basic with 2 N NaOH (6 mL). The THF was removed under vacuum, and the solution was brought to pH 3.5 with 6 N HCl. The mixture was stirred for several hours, and the precipitate was collected by filtration, dried, chromatographed (2% methanol/methylene chloride), and recrystallized from ethanol/water to provide **36** as yellow crystals (25 mg, 15%): mp 182−183 °C; ^{1}H NMR (CD_3OD) δ 8.54 (d, J = 9 Hz, 1 H), 8.04 (d, J = 8 Hz, 1 H), 7.90 (m, 1 H), 7.46 (m, 2 H), 7.17 (d, J = 2 Hz, 1 H), 3.19 (s, 6 H), 2.16 (s, 3 H), 1.62 (s, 3 H); ^{13}C NMR (CD_3OD) δ 163.1, 157.0, 150.3, 137.8, 136.0, 134.4, 126.4, 126.2, 125.3, 122.0, 119.0, 107.9, 107.2, 40.6, 10.5, 6.1; IR (KBr) 1620, 1514, 1427, 1375, 1329, 1163, 1136, 602, 586 cm^{-1}; MS (M + H)$^+$ 346.

5-(Dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulfonamide (33). Dansyl chloride (50.0 g, 185 mmol) was continuously added via a screw-thread solid addition funnel over a 25 min period to a solution of 3,4-dimethyl-5-isoxazolamine (27.0 g, 241 mmol) and 4-(dimethylamino)-pyridine (4.53 g, 37.1 mmol) in dry pyridine (250 mL) which was held in a 75 °C bath. The mixture was stirred for 21 h and cooled to room temperature, and most of the pyridine was

evaporated. The residue was poured into water (800 mL), and the pH of the mixture was brought to 9.5 with 2 N NaOH. The dark brown homogeneous solution was stirred for 1 h and filtered through Celite, and the filtrate was brought to pH 4 with 6 N HCl. A thick brown gum formed which was mechanically agitated over a 3 h period during which time it became a solid mass. The mixture was stirred for 3 d, and the free-flowing solid was collected by filtration, washed with water (3 × 200 mL), and dried to provide 55.6 g of a brown-green solid. A solution of this solid in methylene chloride (200 mL) was loaded onto a silica gel plug (75 mm dia, 150 g) packed with methylene chloride, and the plug was eluted with 1/1 ethyl acetate/methylene chloride. The eluate was evaporated to provide 53.1 g of a yellow solid which was recrystallized from ethanol/water to yield 50.0 g (78%) of **33**: mp 134–135 °C; ^{1}H NMR (DMSO-d_6) δ 11.3 (s, 1 H), 8.52 (d, J = 9 Hz, 1 H), 8.27 (d, J = 8 Hz, 1 H), 8.15 (dd, J = 8, 1 Hz, 1 H), 7.60–7.67 (m, 2 H), 7.28 (d, J = 8 Hz, 1 H), 2.83 (s, 6 H), 2.03 (s, 3 H), 1.51 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 161.3, 155.2, 151.5, 135.4, 130.6, 129.2, 129.0, 128.2, 123.6, 118.5, 115.3, 105.0, 45.0, 10.2, 5.7; IR (KBr) 3003, 2828, 2785, 1657, 1476, 1348, 1167, 1150, 791, 623 cm^{-1}; MS (M + H)$^+$ 346.

***N*-(3,4-Dimethyl-5-isoxazolyl)-5-(ethylamino)-1-naphthalenesulfonamide (52).** To a solution of borane (1.0 M in THF, 1.9 mL, 1.9 mmol) in THF (13 mL) at 0 °C was added **32** (0.24 g, 0.62 mmol). The mixture was stirred at 0 °C for 15 min, at ambient temperature for 1.25 h, and at reflux for 2 h. The mixture was evaporated, water was slowly added to the residue, and the mixture was acidified to pH 4.5 with 1 N HCl and extracted with methylene chloride (2 × 75 mL). The combined organic phases were dried and evaporated, and the residue was chromatographed (20% ethyl acetate/methylene chloride) to afford 0.12 g (58%) of **52** as a greenish yellow solid: mp 75–85 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, 3H, J = 7.0 Hz), 1.61 (s, 3H), 2.01 (s, 3H), 3.22 (q, 2H, J = 7.0 Hz), 6.62 (d, 1H, J = 7.6 Hz), 7.28 (t, 1H, J = 8.0 Hz), 7.41 (t, 1H, J = 8.7 Hz), 7.82 (d, 1H, J = 7.6 Hz), 8.00 (d, 1H, J = 8.8 Hz), 8.05 (d, 1H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 6.0, 10.5, 13.9, 14.3, 38.5, 105.4, 106.2, 112.6, 122.3, 124.0, 126.8, 129.1, 129.3, 129.5, 134.6, 144.4, 155.1, 161.6. IR (KBr) 3432, 1653, 1576, 1462, 1424, 1329, 1163, 777, 590 cm^{-1}; MS (M + H)$^+$ 346.

***N*-(3,4-Dimethyl-5-isoxazolyl)-5-(2-oxo-1-pyrrolidinyl)-1-naphthalenesulfonamide (56).** To a slurry of cesium carbonate (290 mg, 0.90 mmol) in dry DMF (5 mL) at 60 °C was added a solution of *N*-(3,4-dimethyl-5-isoxazolyl)-5-[1-(4-bromo-1-oxobutyl)amino]-1-naphthalenesulfonamide (210 mg, 0.45 mmol; prepared from **31** and 4-bromobutyryl chloride) in 5 mL of dry DMF dropwise over 30 min. The mixture was stirred for 90 min and evaporated, and the residue was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (2×), acidified to pH 3 with 6 N HCl, and extracted with dichloromethane (3×). The combined dichloromethane phases were washed with brine, dried, and evaporated. The residue was crystallized from ethyl acetate/hexanes and the crystalline solid was triturated with hexanes to afford 124 mg (73%) of **56** as a tan solid: mp 183–187 °C; ^{1}H NMR ($CDCl_3$) δ 1.69 (s, 3H), 2.08 (s, 3H), 2.32 (q, 2H, J = 7.0 Hz), 2.7 (t, 2H, J = 8.4 Hz), 3.86 (t, 2H, J = 7.0 Hz), 7.4 (m, 3H), 7.9 (d, 1H, J = 8.7 Hz), 8.17 (d, 1H, J = 8.51 Hz), 8.5 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ 6.6, 10.8, 19.3, 31.6, 52.2, 124.9, 125.0, 125.7, 128.1, 129.2, 129.5, 130.1, 130.5, 135.9, 155.1, 163.0; IR (KBr) 2959, 1651, 1616, 1344, 1165, 1152, 797 cm^{-1}; MS (M + H)$^+$ 386.

4-[[1-[[(3,4-Dimethyl-5-isoxazolyl)amino]sulfonyl]-naphthalen-5-yl]amino]butanoic Acid (63). A solution of **56** (200 mg, 0.52 mmol) in methanol (5 mL) and aqueous 4 N NaOH (15 mL) was heated at 70 °C for 52 h. The solution was cooled to room temperature and acidified to pH 3 with 6 N HCl, and the resulting yellow precipitate was collected by filtration, rinsed with water, dried under vacuum, and chromatographed (10% methanol/dichloromethane) to afford 60 mg (29%) of **63** as a yellow solid: mp 128–132 °C; ^{1}H NMR (CD_3OD) δ 1.50 (s, 3H), 2.02 (s, 3H), 2.04 (q, 2H, J = 7.0 Hz), 2.4 (t, 2H, J = 7.2 Hz), 3.34 (t, 2H, J = 6.9 Hz), 6.73 (d, 1H, J = 7.8 Hz), 7.4 (m, 2H), 7.9 (d, 1H, J = 8.6 Hz), 8.12 (d, 1H, J = 7.4 Hz), 8.36 (d, 1H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 4.6, 9.0, 23.7, 31.7, 42.9, 104.3, 112.04, 116.5, 121.6, 123.5, 127.3, 128.8, 128.9, 129.0, 135.0, 136.3, 144.9, 162.0; IR (KBr) 2960, 1663, 1610, 1320, 1161, 797 cm^{-1}; MS (M + H)$^+$ 404.

***N*-(3,4-Dimethyl-5-isoxazolyl)-5-(1-pyrrolidinyl)-1-naphthalenesulfonamide (51).** A solution of *N*-(3,4-dimethyl-5-isoxazolyl)-5-[(4-chlorobutyl)amino]-1-naphthalenesulfonamide (2.13 g, 5.23 mmol; prepared by reductive amination of **31** and 2-(3-chloropropyl)-1,3-dioxolane) and *N*-methylmorpholine (4 mL) in DMF (25 mL) was heated at 75 °C for 4 h. The solvent was removed under vacuum, and the residue was dissolved in water. The aqueous solution was acidified to pH 3 with 6 N HCl and extracted with ethyl acetate (3×), and the combined organic phases were washed with brine, dried, and evaporated. The residue was chromatographed with ethyl acetate:hexanes (1:1) to afford 320 mg of a yellow semisolid, which was recrystallized from aqueous ethanol to afford 159 mg (7%) of **51** as a green solid: mp 170–173 °C; ^{1}H NMR ($CDCl_3$) δ 1.70 (s, 3H), 2.10 (s, 3H), 2.05 (m, 4H), 3.35 (t, 4H, J = 6.6 Hz), 7.10 (d, 1H, J = 9.1 Hz), 7.45 (t, 1H, J = 7.8 Hz), 7.55 (t, 1H, J = 9.2 Hz), 8.18 (m, 1H), 8.5 (d, 1H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 6.5, 10.8, 25.0, 53.3, 107.2, 112.8, 116.0, 122.2, 128.9, 129.0, 129.8, 130.0, 132.0, 133.0, 155.1, 161.8; IR (KBr) 3441, 2963, 1653, 1460, 1346, 1130, 793 cm^{-1}; MS (M + H)$^+$ 372.

***N*-(3,4-Dimethyl-5-isoxazolyl)-5-(1-piperidinyl)-1-naphthalenesulfonamide, Trifluoroacetate (2:1) Salt (62).** To a mixture of **31** (1.5 g, 4.7 mmol) in glacial acetic acid (40 mL) and dioxane (20 mL) at 0 °C was added a 50% solution of glutaric dialdehyde (0.85 g, 4.7 mmol). The mixture was stirred at 0 °C for 1 h, sodium cyanoborohydride (1.5 g, 24 mmol) was added in portions over 1 h, and the mixture was stirred overnight and evaporated. The residue was partitioned between water and ethyl acetate, and the aqueous layer was acidified to pH 3 with 6 N HCl and extracted with ethyl acetate (3×). The combined organic phases were washed with brine, dried, and evaporated. The residue was chromatographed with ethyl acetate:hexanes (1:1). Fractions containing product were combined and evaporated. The residue was dissolved in 80% aqueous acetonitrile containing 0.1% trifluoroacetic acid and subjected to gradient preparative HPLC (70% to 45% aqueous acetonitrile containing 0.1% trifluoroacetic acid). Fractions containing clean product were pooled and lyophilized from water to afford 48 mg (3%) of **62** as a fluffy brown lyophilate: mp 89–93 °C; ^{1}H NMR (CD_3OD) δ 1.6 (s, 3H), 1.73 (m, 2H), 1.98 (m, 4H), 2.08 (s, 3H), 3.25 (m, 4H), 7.64 (m, 2H), 8.2 (dd, 1H), 8.42 (d, 1H, J = 8.5 Hz), 8.5 (d, 1H, J = 8.5 Hz); ^{13}C NMR (CD_3OD) δ 4.8, 9.1, 23.3, 25.5, 52.6, 106.0, 117.0, 121.4, 124.0, 127.9, 128.9, 129.2, 129.5, 129.8, 135.9, 161.7; IR (KBr) 2934, 1670, 1460, 1204, 1161, 793 cm^{-1}; MS (M + H)$^+$ 386.

5-[[[5-(Dimethylamino)-1-naphthalenyl]sulfonyl]amino]-3-methyl-4-isoxazolecarboxylic Acid, Ethyl Ester (79). To a solution of ethyl bromopropiolate (15.4 g, 87 mmol) and acetaldoximine (7.94 mL, 130 mmol) in methylene chloride (50 mL) was added Clorox (277 mL, about 208 mmol) dropwise over 2.5 h. The blue-green solution was stirred for 30 min and partitioned, the aqueous phase was washed with methylene chloride, and the combined organic phases were dried and evaporated to afford 22.2 g of orange oil. Flash chromatography (10% ether/hexanes) afforded 7.44 g (36%) of a 2:1 mixture of 3-methyl-5-bromo-4-(ethoxycarbonyl)isoxazole and 3-methyl-4-bromo-5-(ethoxycarbonyl)isoxazole as a clear oil. A solution of this mixture (2.03 g, 8.67 mmol), dansylamide (2.17 g, 8.67 mmol), and cesium carbonate (5.64 g, 17.3 mmol) was heated at 77 °C in DMF (10 mL) for 3 h, and the bulk of the solvent was removed under vacuum with heating. The residue was partitioned between methylene chloride and 5% aqueous potassium hydrogen sulfate, the aqueous phase was washed with methylene chloride, and the combined organic phases were dried, evaporated, and warmed under high vacuum to afford 4.26 g of light brown oil. The oil was passed through a pad of silica with ethyl acetate to afford 3.30 g of yellow solid which was dissolved in ether and filtered. The filtrate was evaporated and subjected to chromatography (ethyl acetate) to provide 0.24 g of clean **79** as a light yellow foam and 2.2 g of impure **79** as a yellow foam. The impure material was dissolved in ether and chilled to afford 0.54 g (15%) of **79** as yellow cubes which became an amorphous solid on gentle

warming under vacuum: mp 146–148 °C; ^{1}H ($CDCl_3$) δ 1.33 (3H, t, J = 7.6 Hz), 2.25 (3H, s), 2.89 (6H, s), 4.29 (2H, q, J = 7.0 Hz), 7.22 (1H, d, J = 7.6 Hz), 7.41–7.65 (2H, m), 8.28 (1H, d, J = 8.8 Hz), 8.52 (1H, dd, J = 1.2, 7.6 Hz), 8.63 (1H, d, J = 8.8 Hz); ^{13}C ($CDCl_3$) δ 11.57, 14.17, 45.32, 61.02, 115.36, 117.89, 123.30, 129.04, 129.38, 129.76, 131.92, 132.29, 133.07, 152.19, 158.75, 164.51; IR (KBr) 1688, 1577, 1512, 1283, 1134, 1096, 785 cm^{-1}; MS (M − H)$^-$ 402.

Crystal Structure Analysis. Crystals of **33** were obtained from ethyl acetate as yellow-green prisms. Unit cell parameters: a = 8.896(1) Å, b = 22.302(3) Å, c = 8.712(1) Å, V = 1728.5(6) Å^3, space group $Pna2_1$, D_{obs} = 1.32 g cm^{-3} (flotation), D_{calc} = 1.327 for Z = 4, $C_{17}H_{19}N_3O_3S$. A total of 1716 (λ = 1.5418 Å) reflections were measured on an Enraf-Nonius CAD4 diffractometer at −31 °C with the ω–2θ variable scan technique and were corrected for Lorentz polarization factors. The structure was solved by direct methods and refined on the basis of 1568 "observed" reflections with $I \geq 3\sigma(I)$. Although hydrogen positions were evident in difference maps, only the hydrogen on N11 was introduced in its observed position. All other hydrogens were introduced in idealized positions. Least squares refinement of the coordinates and anisotropic temperature factors of the non-hydrogen atoms converged at R = 0.044 (R_w = 0.059). The function minimized in the least squares refinements was $\Sigma_w(|F_o| - |F_c|)^2$ and R is defined as $\Sigma||F_o| - |F_c||/\Sigma|F_o|$.

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Supplementary Material Available: Tables of atomic coordinates, thermal parameters, and bond distances and angles for **33** are provided (5 pages). Ordering information is given on any current masthead page.

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