New Non-Peptide Endothelin-A Receptor Antagonists: Synthesis, Biological Properties, and Structure−Activity Relationships of 5-(Dimethylamino)-*N*-pyridyl-, -*N*-pyrimidinyl-, -*N*-pyridazinyl-, and -*N*-pyrazinyl-1-naphthalenesulfonamides

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Use of automated synthesis led to the discovery of several 6-membered nitrogen heterocycles as replacements for the *N*-isoxazolyl substituent present in the 1-naphthalenesulfonamide endothelin-A (ET_A) antagonist 5-(dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulfonamide (BMS 182874). In each of these heterocycles, a small substituent such as halogen para to the position of attachment to the sulfonamide nitrogen atom was found to be advantageous for ET_A receptor affinity. Of these heterocycles, 2-pyrazines offered the greatest scope for improving receptor affinity. Optimization of the substituents at the 3- and 5-positions in the pyrazine ring led to potent, ET_A-selective compounds such as 5-(dimethylamino)-*N*-(5-chloro-3-methoxy-2-pyrazinyl)-1-naphthalenesulfonamide (**7m**, ET_A pIC_{50} 8.1). When dosed orally at 10 mg/kg to conscious, normotensive rats infused with big ET-1, compounds such as **7m** showed significant inhibition of the pressor response with a duration of effect lasting for the 5-h course of the experiment.

Following the initial isolation of the vasoconstrictor peptide endothelin-1 (ET-1) from endothelial cells,[1] ET-1 and the closely-related isopeptides endothelin-2 (ET-2) and endothelin-3 (ET-3) have been implicated in a number of disease states.[2−5] Thus, blockade of the endothelin receptor may be of use in treating conditions such as renal failure,[6] cerebral vasospasm,[7] and pulmonary hypertension.[8]

In mammalian tissues, two subtypes of endothelin receptor have been identified.[9,10] The endothelin-A (ET_A) receptor binds ET-1 and ET-2 with greater affinity than ET-3 and is found mainly in vascular smooth muscle, where it mediates vasoconstriction[11−13] and smooth muscle proliferation.[14] The endothelin-B (ET_B) receptor binds ET-1, ET-2, and ET-3 with equal affinity and mediates both vasoconstriction and vasodilatation in certain vascular beds.[11,15,16] The pharmacological significance of the ET_B subtype in mammalian tissues is the subject of continuing debate.[17−20]

The endothelin antagonists reported initially were peptidic in nature. The cyclic pentapeptide BQ-123[21] and the tripeptide FR139317[22] are ET_A-selective, whereas the tripeptide BQ-788[23] is ET_B-selective, and hexapeptides such as PD 142893[24] and PD 145065[25] display antagonism at both ET_A and ET_B receptors. Of these compounds, the prototype ET_A-selective antagonist BQ-123 has been shown to be effective in animal models, most notably by hypertension[26] and acute renal failure.[27]

More recently, the first non-peptide endothelin antagonists have been described. These include the sulfonamides Ro 46-2005,[28] Ro 47-0203 (Bosentan),[29] and BMS 182874,[30,31] the 1,3-diarylindan-2-carboxylic acid SB 209670,[32] the related diaryl pyrrolidine A-127722,[33] and the butenolide PD 156707.[34] Of these compounds, BMS 182874, A-127722, and PD 156707 are ET_A-selective, whereas the remainder are mixed ET_A/ET_B antagonists. All of these non-peptide antagonists have been shown to be effective in endothelin-dependent disease models.

It appears that these non-peptide antagonists were derived by optimization of leads found through either directed[32] or high-throughput[28,30,34] screening of pharmaceutical-company compound collections. Our own screening program produced no attractive starting points for optimization, and we therefore turned our attention to non-peptide endothelin antagonists recently described in the literature.

Of the non-peptide antagonists reported at the time of starting the work described in this paper, the ET_A-selective BMS 182874 (**1**)[30,31] seemed a particularly attractive starting point for several reasons. Firstly, we sought an ET_A-selective antagonist to maintain ET_B-mediated vasodilatation and because the contractile response to endothelin in vascular tissue is thought to be predominantly ET_A-mediated.[35] Secondly, as described below, in our biological test cascade **1** showed antihypertensive activity after oral dosing at 10 mg/kg in rats challenged with big ET-1. Thirdly, a pharmacokinetic study in rats showed **1** to be rapidly absorbed through the lower intestine. Finally, sulfonamides such as **1** are simply prepared from the appropriate naphthalenesulfonyl chloride and aminoisoxazole, and we envisaged that using automated synthesis[36] a wide range of analogs of **1** could rapidly be made from readily-available aryl or heteroaryl sulfonyl chlorides and amino heterocycles.

Of these two structural parameters, varying the amino heterocycle part of the structure led to compounds with the more interesting biological properties.

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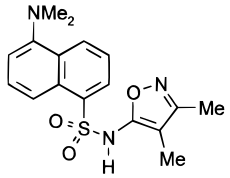
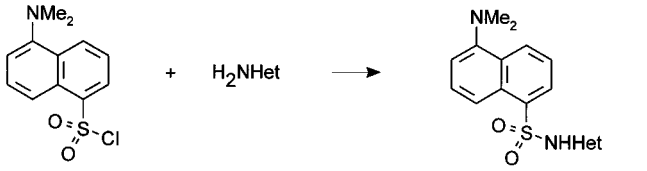


1

Figure 1. Structure of BMS 182874 (**1**).

Scheme 1



2 **3** **4a-l, 5a-d, 6a-d,h,i, 7a-h, 7j-n**

In this paper, we describe how this approach resulted in the discovery of several 6-membered nitrogen heterocycles as replacements for the isoxazole moiety in **1** and led to a series of *N*-heteroaryl 1-naphthalenesulfonamides that are potent, selective endothelin ET_A antagonists both in vitro and in vivo.

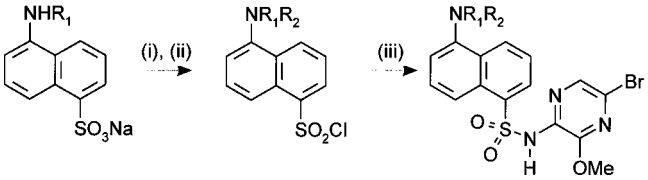
Chemistry

The naphthalenesulfonamides **4**−**7** and **11** prepared during the course of this work are listed in Tables 1−5. The majority of these compounds (**4a**−**1, 5a**−**d, 6a**−**d,h,i, 7a**−**h,j**−**n**) were obtained as shown in Scheme 1 by reaction of 5-(dimethylamino)-1-naphthalenesulfonyl chloride (**2**, dansyl chloride) with the appropriate amino heterocycle **3**.

Three sets of reaction conditions were used to effect sulfonamide formation. Initially, we required conditions for automated synthesis from dansyl chloride and a range of commercially-available, 5- and 6-membered amino heterocycles, chosen on the basis of structural diversity, lack of other functionality that might interfere under the reaction conditions, and a molecular weight of less than 200. Use of stoichiometric amounts of dansyl chloride, amino heterocycle, and pyridine and a catalytic amount of 4-(dimethylamino)pyridine in dichloromethane (method A) proved effective for automation on a Zymate XP robot.[36] Of 40 amino heterocycles tried under these conditions, 25 cleanly gave the corresponding dansyl sulfonamides, the products being isolated directly by either filtration or bond elute chromatography. The remaining amino heterocycles did not react cleanly with dansyl chloride, presumably due to either poor solubility or low reactivity of the amino group, or both.

As described below, application of automated synthesis gave two pyridine derivatives (**4b,i**) that showed endothelin antagonist activity in vitro. To improve on the activity of these compounds, a range of analogs incorporating various 6-membered nitrogen heterocycles was prepared using conventional laboratory synthesis. The initial 2- and 3-pyridyl analogs **4c**−**g** were obtained using essentially the procedure employed for robotic synthesis (method B). For preparation of analogs **5a**−**d, 6a**−**d,h,i,** and **7a**−**h,j**−**n** derived from less reactive amino diazines, more forcing conditions were needed, involving either reaction in pyridine at 70−80 °C

Scheme 2[a]



8a; R_1 = Z
8b; R_1 = Ac

9a; R_1 = Z, R_2 = Me
9b; R_1 = Ac, R_2 = H
9c; R_1 = Ac, R_2 = Me

(iv) → **10a**; R_1 = Z, R_2 = Me; **11a**; R_1 = H, R_2 = Me
(v) → **10b**; R_1 = Ac, R_2 = H; **11b**; R_1 = Et, R_2 = H
(v) → **10c**; R_1 = Ac, R_2 = Me; **11c**; R_1 = Et, R_2 = Me

[a] Reagents: (i) NaH/MeI/DMF (**9a,c**); (ii) $POCl_3$/MeCN/sulfolane/70 °C; (iii) NaH/2-amino-5-bromo-3-methoxypyrazine/DME; (iv) TMSI/MeCN; (v) B_2H_6/THF.

(method C) or prior deprotonation of the amino heterocycle using sodium hydride as base (method D).

The majority of amino heterocycles needed for this work were commercially available or were prepared by published methods.[37−49] Characterization data for the remaining, novel aminohalopyrazines **3a**−**e** synthesized during the course of this work are given in Table 6. A small number of compounds synthesized for biological testing (**6e**−**g, 7i**) were obtained by manipulation of preformed *N*-heteroaryl 1-naphthalenesulfoanmides as detailed in the Experimental Section.

The compounds **11a**−**d** listed in Table 5 were prepared to investigate the effect of varying the 5-substituent in the naphthalene ring. The unsubstituted 1-naphthalenesulfonamide **11d** was prepared (method B) by reaction of 1-naphthalenesulfonyl chloride with 2-amino-5-bromo-3-methoxypyrazine,[48] while the remaining compounds **11a**−**c** were obtained as outlined in Scheme 2, starting from the acylated 5-aminonaphthalene-1-sulfonic acids **8a**,[50] **b**.[51]

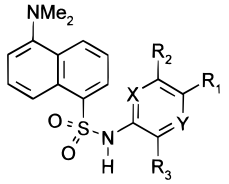
Endothelin Receptor Affinity

The compounds listed in Tables 1−5 were evaluated as endothelin antagonists in a radioligand binding assay involving displacement of [^{125}I]ET-1 from membranes prepared from cloned human ET_A receptors[9] expressed in mouse erythroleukemic (MEL) cells. The pIC_{50} for **1** in this assay is included in Table 1 for comparison. A similar assay was used to measure affinity for the human ET_B receptor[10] (data not shown).

Of the 25 initial analogs of **1** prepared by automated synthesis from dansyl chloride and a variety of amino heterocycles, only the 2- and 3-pyridyl derivatives **4b,i** (Table 1) showed affinity for the ET_A receptor, although at a lower level than the prototype **1**. To explore further the activity of the para-substituted 2-pyridyl compound **4b**, the corresponding halopyridines **4c**−**e** were prepared and found to show similar affinity. Whereas further substitution at the 6-position resulted in lower affinity (compound **4f**), introduction of a methyl group at the 3-position led to increased affinity (compound **4g**). For 3-pyridyl derivatives, replacement of chlorine by bromine increased affinity (compound **4j**).

The importance of the substituent at the position para to the position of attachment to the sulfonamide nitrogen atom was shown by the reduced activity of the parent 2- and 3-pyridyl compounds **4a,h**, possibly

Table 1. Characterization Data and ET_A Receptor Affinity for *N*-Pyridyl- and *N*-Phenyl-1-naphthalenesulfonamides **4a**−**l**

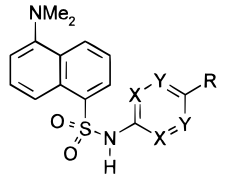


4a-l

no.	X	Y	R_1	R_2	R_3	method[a]	mp, °C	formula[b]	% inhibtn at 10 μM[c]	pIC_{50} (n)[c]
1									98	7.3 ± 0.1 (7)
4a	N	CH	H	H	H	A	235−237	$C_{17}H_{17}N_3O_2S$	<30	
4b	N	CH	Me	H	H	A	209−211	$C_{18}H_{19}N_3O_2S$	79	5.8 (1)
4c	N	CH	Cl	H	H	B	160−162	$C_{17}H_{16}ClN_3O_2S$	95	5.7 ± 0.1 (6)
4d	N	CH	Br	H	H	B	176−177	$C_{17}H_{16}BrN_3O_2S$	91	5.8 (1)
4e	N	CH	I	H	H	C	211−212	$C_{17}H_{16}IN_3O_2S$[d]	84	5.3 (1)
4f	N	CH	Br	Me	H	B	155−156	$C_{18}H_{18}BrN_3O_2S$[e]	54	5.0 (1)
4g	N	CH	Br	H	Me	B	foam	$C_{18}H_{18}BrN_3O_2S$[f]	94	6.5 ± 0.0 (2)
4h	CH	N	H	H	H	A	230−232	$C_{17}H_{17}N_3O_2S\cdot HCl$	<30	
4i	CH	N	Cl	H	H	A	145−146	$C_{17}H_{16}ClN_3O_2S$	81	5.9 (1)
4j	CH	N	Br	H	H	B	164−165	$C_{17}H_{16}BrN_3O_2S$	95	6.6 ± 0.1 (2)
4k	CH	CH	Cl	H	H	B	foam	$C_{18}H_{17}ClN_2O_2S$[g]	30	5.1 (1)
4l	CH	CH	Br	H	H	B	foam	$C_{18}H_{17}BrN_2O_2S$	72	5.5 (1)

[a] Method A: **2**/DMAP/CH_2Cl_2 (XP Zymate robot). Method B: **2**/DMAP/CH_2Cl_2. Method C: **2**/DMAP/pyridine/70−80 °C. Method D: **2**/NaH/DME. [b] Analyses for C, H, N were correct within ±0.4% unless otherwise stated. [c] pIC_{50} for inhibition of specific binding of [^{125}I]ET-1 to human ET_A receptor (n = no. of replicate determinations). [d] H, N; C: calcd, 45.6; found, 45.0. [e] H, N; C: calcd, 51.4; found, 50.4.[f] H, N; C: calcd, 51.4; found, 49.7. [g] H, N; C: calcd, 59.9; found, 59.0.

Table 2. Characterization Data and ET_A Receptor Affinity for *N*-Pyrimidyl-1-naphthalenesulfonamides **5a**−**d**



5a-d

no.	X	Y	R	method[a]	mp, °C	formula[b]	% inhibtn at 10 μM[c]	pIC_{50} (n)[c]
5a	N	CH	H	C	235−237	$C_{16}H_{16}N_4O_2S$	54	5.2 (1)
5b	N	CH	Cl	C	189	$C_{16}H_{15}ClN_4O_2S$	84	5.8 (1)
5c	N	CH	Br	C	197−199	$C_{16}H_{15}BrN_4O_2S$[d]	80	5.7 (1)
5d	CH	N	Br	C	177−179	$C_{16}H_{15}BrN_4O_2S$	88	6.2 ± 0.1 (3)

[a−c] See Table 1. [d] H, N; C: calcd, 47.2; found, 46.2.

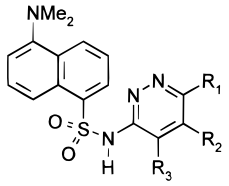
consistent with a specific role for the substituent in binding to the human ET_A receptor. To determine the importance of the ring nitrogen atom, para-substituted aniline derivatives **4k,l** were prepared and found to show only weak activity. A variety of aniline derivatives with a wide range of substituents was subsequently prepared by robotic synthesis (data not shown), but none showed increased affinity.

To investigate the effect of introducing a second ring nitrogen atom and to give access to substitution patterns that might be difficult to introduce in a precursor 2- or 3-aminopyridine, the diazine derivatives listed in Tables 2−4 were prepared. Like the corresponding pyridines, the parent pyrimidine and pyridazine derivatives **5a** and **6a** showed reduced affinity, whereas substitution with halogen or methyl at the para position gave a range of compounds with moderate affinity (**5b,c, 6b**−**d**). The unsubstituted pyrazine **7a** showed modest affinity, and again this was enhanced by halogen substitution at the para position (**7c,d**).

The effect of further substitution was examined in the pyridazine and pyrazine series of antagonists. In the pyridazine series (Table 3), manipulation of the 6-substituent led to compounds with electron-withdrawing (**6e**) and -releasing (**6f,g**) substituents at the position para to the sulfonamide nitrogen atom. Of these compounds, only **6f**, in which a methoxy substituent was introduced at the 6-position, was comparable in affinity to the corresponding halopyridazines **6c,d**. Further ring substitution either maintained (**6i**) or reduced (**6h**) receptor affinity.

Based on the reference 5-bromopyrazine derivative **7d**, a more detailed study of the effect of substitution was carried out at the 3-position in the pyrazine series of antagonists (Table 4). Introduction of alkyl, cyano, and hydroxyl groups (**7e**−**i**) all resulted in diminished affinity, but introduction of a methoxy group gave compound **7j** with comparable affinity to the prototype antagonist **1**. The importance of the 3-methoxy group in binding of **7j** to the human ET_A receptor was evident from the reduced activity seen on increasing the bulk of the alkoxy substituent (**7k,l**). Replacement of the 5-bromo substituent with a chloro or methyl substituent led to compounds **7m,n** with optimized ET_A receptor affinity. Like the prototype **1**, at a concentration of 10 μM none of the compounds **7j,m,n** showed measurable

Table 3. Characterization Data and ET_A Receptor Affinity for *N*-Pyridazinyl-1-naphthalenesulfonamides **6a**−**i**

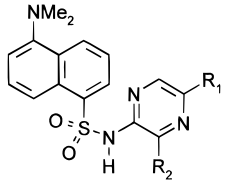


6a-i

no.	R_1	R_2	R_3	method[a]	mp, °C	formula[b]	% inhibtn at 10 μM[c]	pIC$_{50}$ (*n*)[c]
6a	H	H	H	C	117−120	$C_{16}H_{16}N_4O_2S$[d]	<30	
6b	Me	H	H	C	113−115	$C_{17}H_{18}N_4O_2S$[e]	68	5.8 (1)
6c	Cl	H	H	C	153−154	$C_{16}H_{15}ClN_4O_2S$	70	6.7 ± 0.2 (3)
6d	Br	H	H	C	151−153	$C_{16}H_{15}BrN_4O_2S$[f]	95	6.6 (1)
6e	CN	H	H	*g*	160−165	$C_{17}H_{15}N_5O_2S\cdot 0.25Me_2NCHO$	<30	
6f	OMe	H	H	*h*	125−127	$C_{17}H_{18}N_4O_3S$	92	6.5 (1)
6g	OEt	H	H	*h*	194−198	$C_{18}H_{20}N_4O_3S$	64	5.4 (1)
6h	Cl	Me	H	D	110−112	$C_{17}H_{17}ClN_4O_2S$[i]	64	5.3 (1)
6i	Cl	H	Me	D	182−186	$C_{17}H_{17}ClN_4O_2S$[j]	89	6.4 ± 0.2 (3)

[a−c] See Table 1. [d] C, H; N: calcd, 17.1; found, 15.9. [e] H; C: calcd, 59.6; found, 58.8. N: calcd, 16.4; found, 15.5. [f] H; C: calcd, 47.2; found, 47.7. N: calcd, 13.8; found, 12.6. [g] Prepared via corresponding 6-iodopyridazine (see the Experimental Section). [h] Prepared from **6a** (see the Experimental Section). [i] H; C: calcd, 54.2; found, 53.3. N: calcd, 14.9; found, 14.2. [j] C, H; N: calcd, 14.9; found, 13.9.

Table 4. Characterization Data and ET_A Receptor Affinity for *N*-Pyrazinyl-1-naphthalenesulfonamides **7a**−**n**



7a-n

no.	R_1	R_2	method[a]	mp, °C	formula[b]	% inhibtn at 10 μM[c]	pIC$_{50}$ (*n*)[c]
7a	H	H	B	206−207	$C_{16}H_{16}N_4O_2S$[d]	58	6.0 ± 0 (2)
7b	Me	H	D	153−154	$C_{17}H_{18}N_4O_2S$[e]	91	5.9 (1)
7c	Cl	H	D	112−113	$C_{16}H_{15}ClN_4O_2S$	97	6.8 (1)
7d	Br	H	C	161−163	$C_{16}H_{15}BrN_4O_2S$	96	6.9 ± 0.2 (2)
7e	Br	Me	D	171−172	$C_{17}H_{17}BrN_4O_2S$	96	6.2 (1)
7f	Br	Et	D	171−172	$C_{118}H_{19}BrN_4O_2S$	68	5.6 (1)
7g	Br	Pr	D	144−146	$C_{19}H_{21}BrN_4O_2S$	<30	
7h	Br	CN	D	180−182	$C_{17}H_{14}BrN_5O_2S$[f]	<30	
7i	Br	OH	*g*	123−126	$C_{16}H_{15}BrN_4O_3$[h]	81	5.3 (1)
7j	Br	OMe	C	166−167	$C_{17}H_{17}BrN_4O_3S$	93	7.2 ± 0.2 (7)
7k	Br	OEt	D	179−181	$C_{18}H_{19}BrN_4O_3S$	92	6.3 (1)
7l	Br	OBn	C	145−147	$C_{23}H_{21}BrN_4O_3S$	<30	
7m	Cl	OMe	D	137−138	$C_{17}H_{17}ClN_4O_3S$	97	8.0 ± 0.1 (6)
7n	Me	OMe	D	126−127	$C_{18}H_{20}N_4O_3S$	98	8.1 ± 0.1 (10)

[a−c] See Table 1. [d] H; C: calcd, 58.5; found, 59.5. N: calcd, 17.1; found, 16.1. [e] H, N; C: calcd, 59.6; found, 58.9. [f] H; C: calcd, 47.2; found, 45.6. N: calcd, 16.2; found, 15.5. [g] Prepared from **7j** (see the Experimental Section). [h] C, N; H: calcd, 3.6; found, 4.4.

affinity for the ET_B receptor, nor did any of the other ET_A antagonists listed in Tables 1−5.

In a guinea pig portal vein preparation, compound **7m** showed functional antagonism of the ET_A-induced contractile response with a pA_2 value of 7.20 ± 0.05 (n = 6). The corresponding value in this assay for the prototype **1** was 6.20 ± 0.06 (n = 3).

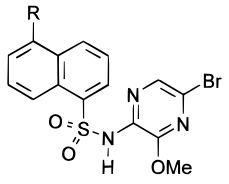
The key role of the acidic sulfonamide NH in binding of compounds related to **1** to the ET_A receptor has been reported previously.[31] pK_a measurements on representative examples of the above *N*-heteroaryl 1-naphthalenesulfonamides showed the acidity of these compounds to be similar to that of **1** (e.g., pK_a 5.0 for **7m** vs 4.5 for **1**).

Finally, with **7j** as reference compound a limited investigation was carried out to study the effect of varying the 5-(dimethylamino) substituent in the naphthalene ring (Table 5). While fine tuning of the *N*-alkyl groups gave compounds **11a**−**c** with similar affinity for the ET_A receptor to the 5-(dimethylamino) compound **7j**, removal of the substituent resulted in ca. 100-fold loss of affinity (**11d** vs **7j**) and highlighted the importance of the 5-substituent in binding to the ET_A receptor, similarly to previously-reported structure−activity relationships for the prototype **1**.[31]

Pharmacological Evaluation

Selected compounds listed in Tables 1−5 were evaluated for endothelin antagonism in vivo by determining their mean dose ratios for inhibition of the pressor response induced by infusion of big ET-1 in conscious, normotensive rats. Data for **7m** as a representative member of this series of compounds are shown in Table 7, together with the corresponding data for **1** as a comparison.

Table 5. Characterization Data and ET_A Receptor Affinity for *N*-Pyrazinyl-1-naphthalenesulfonamides **11a−d**

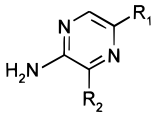


11a-d

no.	R	mp, °C	formula[a]	% inhibtn at 10 μM[b]	pIC_{50} (*n*)[b]
11a	NHMe	171−173	$C_{16}H_{15}BrN_4O_3S$[c]	100	7.8 ± 0.2 (2)
11b	NHEt	216−218	$C_{17}H_{17}BrN_4O_3S$	95	8.6 ± 0.1 (3)
11c	N(Me)Et	foam	$C_{18}H_{19}BrN_4O_3S$	99	7.9 (1)
11d	H	169−171	$C_{15}H_{12}BrN_3O_3S$	83	5.7 (1)

[a,b] See Table 1. [c] H; C: calcd, 45.4; found, 46.1. N: calcd, 13.2; found, 12.1.

Table 6. Characterization Data for Aminopyrazine Intermediates **3a−e**



3a-e

no.	R_1	R_2	mp, °C	formula[a]
3a	Br	Et	75−76	$C_6H_8BrN_3$
3b	Br	Pr	52−54	$C_7H_{10}BrN_3$
3c	Br	OBn	89−90	$C_{11}H_{10}BrN_3O$
3d	Cl	OMe	132−133	$C_5H_6ClN_3O$
3e	Me	OMe	74−75	$C_6H_9N_3O$

[a] See Table 1.

As can be seen from Table 7, following intravenous administration at a dose of 10 mg/kg, compound **7m** showed a greater effect in this animal model than the prototype **1**. After oral administration at the same dose, **7m** demonstrated a significant effect on the pressor response for the 5-h time course of the experiment. The magnitude and duration of effect seen for this compound exceeded the effect of **1** at the same oral dose. Further work using *N*-heteroaryl sulfonamides such as **7m** as a starting point and leading to potent, selective ET_A antagonists with improved pharmacological profiles will be described in subsequent publications.

Summary

Use of automated synthesis to explore variation of the *N*-isoxazolyl substituent in the 1-naphthalenesulfonamide endothelin ET_A antagonist BMS 182874 (**1**) led to the discovery of several 6-membered nitrogen heterocycles as replacements for the isoxazole moiety. Like the prototype **1**, 1-naphthalenesulfonamides incorporating these heterocycles showed good ET_A selectivity.

In each of these heterocycles, a small substituent such as halogen para to the position of attachment to the sulfonamide nitrogen atom was found to be advantageous for receptor affinity. Of these heterocycles, 2-pyrazines offered the greatest scope for improving affinity. Optimization of the substituents at the 3- and 5-positions in the pyrazine ring led to ET_A-selective compounds such as **7m** (pIC_{50} 8.1).

When dosed orally at 10 mg/kg to conscious, normotensive rats infused with big ET-1, compounds such as **7m** showed significant inhibition of the pressor response with a duration of effect lasting for the 5-h course of the experiment. Further work leading to more potent, ET_A-selective antagonists with improved pharmacological profiles will be described in subsequent publications.

Experimental Section

All operations were carried out at room temperature unless otherwise stated. All evaporations were carried out at below 50 °C using a rotary evaporator. Flash chromatography was performed on silica gel (Merck Kieselgel, Art. 9385). Bond elute chromatography was performed on silica gel using 20-g Mega Bond Elut cartridges (Varian, part no. 1225-6042). Melting points were taken on a Buchi apparatus in glass capillary tubes and are uncorrected. Automated synthesis was carried out on a Zymate XP robot (Zymark Corp., Hopkinton, MA 01748). ^{1}H NMR spectra were recorded on a Bruker WM200, WM250, or WM400 instrument and are reported as δ values (parts per million) relative to Me_4Si as internal standard. Chemical ionization mass spectra (CIMS) were recorded on a VG 70-250 SE spectrometer. Positive or negative fast atom bombardment spectra (FABMS) were determined on a VG ZAB 2-SE spectrometer.

2-Amino-5-bromo-3-ethylpyrazine (3a). A solution of Br_2 (0.21 mL, 640 mg, 4.0 mmol) in $CHCl_3$ (40 mL) was added dropwise over 1 h to a stirred solution of 2-amino-3-ethylpyrazine[52] (492 mg, 4.0 mmol) and pyridine (0.32 mL, 316 mg, 4.0 mmol) in $CHCl_3$ (100 mL) shielded from light. The solution was stirred for a further 30 min and then washed with water (50 mL) and dried ($MgSO_4$). The solvent was removed by evaporation, and the residue was purified by flash chromatography, eluting with 25% EtOAc/isohexane to give **3a** (660 mg, 82%): mp 75−76 °C (after recrystallization from isohexane); ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H), 2.55 (q, 2H), 6.4 (s, 2H), 7.9 (s, 1H). Anal. ($C_6H_8BrN_3$) C, H, N.

A similar procedure starting from 2-amino-3-propylpyrazine (obtained analogously to the preparation of 2-amino-3-ethylpyrazine[52] from 2-chloro-3-propylpyrazine[53]) gave **3b**.

2-Amino-3-(benzyloxy)-5-bromopyrazine (3c). NaH (oil free; 192 mg, 8.0 mmol) was added to a solution of benzyl alcohol (864 mg, 8.0 mmol) in THF (10 mL), and the mixture was stirred for 30 min. 2-Amino-3,5-dibromopyrazine[47] (1.01 g, 4.0 mmol) was added, and the solution was heated under reflux for 4 h. Water (50 mL) was added, and the mixture

Table 7. Effects of Compounds **1** (BMS 182874) and **7m** after Intravenous and Oral Dosing to Rats Infused with Big ET-1

			oral MDR[c]			
no.	pIC_{50}[a]	iv MDR[b]	0.5 h	1 h	3 h	5 h
vehicle		1.03 (0.60−1.75)	1.09 (0.98−1.22)	1.03 (0.94−1.13)	1.05 (0.89−1.24)	1.28 (1.13−1.44)
1	7.3	2.68** (2.21−3.26)	3.37*** (2.38−4.77)	2.28** (1.45−3.56)	2.18** (1.38−3.46)	1.55 (0.91−2.61)
7m	8.0	8.87*** (3.65−21.55)	3.91*** (2.92−5.24)	4.42*** (3.29−5.94)	2.54*** (1.64−3.90)	2.07*** (1.55−2.76)

[a] See Table 1. [b] Mean dose ratio for inhibition of pressor response 5 min after intravenous administration of compound at 10 mg/kg to pithed rats ($n = 4$); 95% confidence limits are in parentheses. [c] Mean dose ratio for inhibition of pressor response at time points shown after oral administration of compound at 10 mg/kg to conscious, normotensive rats ($n = 6$); 95% confidence limits are in parentheses. **$p < 0.01$, ***$p < 0.001$ vs vehicle-treated group (unpaired Student's *t*-test).

was extracted with EtOAc (2 × 25 mL). The extracts were washed with water (25 mL) and saturated NaCl (25 mL) and dried ($MgSO_4$). Volatile material was removed by evaporation, and the residue was purified by flash chromatography, eluting with 35% EtOAc/isohexane. The appropriate fractions were combined and concentrated, and the residue was triturated with isohexane to give **3c** (570 mg, 51%): mp 89−90 °C; ^{1}H NMR (DMSO-d_6) δ 5.4 (s, 2H), 6.5 (br s, 2H), 7.3−7.45 (m, 3H), 7.5−7.6 (m, 2H), 7.65 (s, 1H). Anal. ($C_{11}H_{10}BrN_3O$) C, H, N.

2-Amino-5-chloro-3-methoxypyrazine (3d). Reaction of 2-amino-5-chloropyrazine[44] with bromine by an analogous procedure to that used for the preparation of **3a** gave in 74% yield 2-amino-3-bromo-5-chloropyrazine: mp 102−105 °C (after trituration with isohexane); ^{1}H NMR (DMSO-d_6) δ 7.0 (s, 2H), 8.1 (s, 1H).

2-Amino-3-bromo-5-chloropyrazine (78.0 g, 0.374 mol) was added to a solution of sodium methoxide (30.3 g, 0.56 mol) in methanol (1.66 L), and the solution was heated under reflux for 6 h. The solvent was removed by evaporation, and water (1 L) was added to the residue. The mixture was extracted with CH_2Cl_2 (3 × 1 L), and the extracts were washed with water (1 L) and dried ($MgSO_4$). The solvent was removed by evaporation, and the residue was triturated with isohexane to give **3d** (56.5 g, 95%): mp 132−133 °C; ^{1}H NMR (DMSO-d_6) δ 3.9 (s, 3H), 6.45 (br s, 2H), 7.5 (s, 1H). Anal. ($C_6H_9N_3O$) C, H, N.

2-Amino-3-methoxy-5-methylpyrazine (3e). Reaction of 2-amino-3-bromo-5-methylpyrazine[46] with sodium methoxide by an analogous procedure to that used for the preparation of **3d** gave **3e** in 78% yield: mp 74−75 °C (after trituration with isohexane); ^{1}H NMR (DMSO-d_6) δ 2.55 (s, 3H), 3.95 (s, 3H), 4.75 (s, 2H), 7.4 (s, 1H). Anal. ($C_5H_6ClN_3O$) C, H, N.

All other amino heterocycles used as precursors of 1-naphthalenesulfonamides were commercially available or were prepared by published methods: 2-amino-5-iodopyridine,[37] 5-amino-2-bromopyrimidine,[38] 3-aminopyridazine,[39] 3-amino-6-methylpyridazine,[40] 3-amino-6-bromopyridazine,[39] 3-amino-6-chloro-4-methylpyridazine,[41] 3-amino-6-chloro-5-methylpyridazine,[42] 2-amino-5-methylpyrazine,[43] 2-amino-5-chloropyrazine,[44] 2-amino-5-bromopyrazine,[45] 2-amino-5-bromo-3-methylpyrazine,[46] 2-amino-5-bromo-3-cyanopyrazine,[47] 2-amino-5-bromo-3-methoxypyrazine,[48] and 2-amino-5-bromo-3-ethoxypyrazine.[49]

Procedure for Preparation of 5-(Dimethylamino)-1-naphthalenesulfonamides Using Zymate XP Robot (Method A). A 0.33 M stock solution of 5-(dimethylamino)-1-naphthalenesulfonyl chloride and pyridine in CH_2Cl_2 was prepared, and 4-(dimethylamino)pyridine (0.05 equiv) was added. Samples of 40 commercially-available amino heterocycles (1 mmol) were placed in tubes in a rack on a Zymate XP robot, and 3-mL aliquots of the stock solution were added to each tube via the robot arm. The tubes were checked visually, and insoluble amino heterocycles were noted. The tubes were agitated overnight. Insoluble solids were filtered off and checked against starting amino heterocycles by TLC. In tubes where no precipitate was formed, the solution was analyzed by TLC and any product formed isolated by bond elute chromatography, eluting with MeOH/CH_2Cl_2 in the range 0−5% as appropriate. Of the 40 commercially-available amino heterocycles used, 25 gave products in yields in the range 10−90%. Compounds were tested for endothelin antagonist affinity in vitro without full characterization. Compounds showing activity (**4b,i**) and representative inactive compounds (e.g., **4a,h**) were then characterized and generally found to give satisfactory analytical data, e.g., **4i**: mp 145−146 °C; ^{1}H NMR (DMSO-d_6) δ 2.85 (s, 6H), 7.3 (dd, 2H), 7.45 (dd, 1H), 7.45−7.6 (m, 3H), 8.05 (d, 1H), 8.25 (dd, 1H), 8.35 (d, 1H), 8.5 (d, 1H); FABMS *m/e* 362 $(M + H)^+$. Anal. ($C_{17}H_{16}ClN_3O_2S$) C, H, N.

5-(Dimethylamino)-*N*-(5-chloro-2-pyridyl)-1-naphthalenesulfonamide (4c) (Method B). A solution of 5-(dimethylamino)-1-naphthalenesulfonyl chloride (1.35 g, 5.0 mmol), 2-amino-5-chloropyridine (0.64 g, 5.0 mmol), pyridine (0.40 g, 5.0 mmol), and 4-(dimethylamino)pyridine (20 mg) in CH_2Cl_2 (20 mL) was left to stand for 3 days. The solution was split into two equal portions, and each portion was purified by bond elute chromatography, eluting with CH_2Cl_2. The appropriate fractions were concentrated, and the residue was triturated with ether to give **4c** (0.77 g, 43%): mp 160−162 °C; ^{1}H NMR (DMSO-d_6) δ 2.85 (s, 6H), 7.05 (d, 1H), 7.3 (d, 1H), 7.6−7.8 (m, 3H), 8.1 (d, 1H), 8.3 (dd, 1H), 8.4 (d, 1H), 8.5 (d, 1H); FABMS *m/e* 362 $(M + H)^+$. Anal. ($C_{17}H_{16}ClN_3O_2S$) C, H, N.

5-(Dimethylamino)-*N*-(5-bromo-3-methoxy-2-pyrazinyl)-1-naphthalenesulfonamide (7j) (Method C). 5-(Dimethylamino)-1-naphthalenesulfonyl chloride (15.2 g, 56.0 mmol) and 2-amino-5-bromo-3-methoxypyrazine[48] (9.5 g, 47.0 mmol) were mixed intimately and added to pyridine (150 mL) at 0 °C. The solution was kept at 0 °C for 1 h and then heated at 80 °C for 18 h. Volatile material was removed by evaporation, and CH_2Cl_2 (250 mL) was added to the residue. Insoluble material was removed by filtration, and the filtrate was washed with water (100 mL) and saturated NaCl (100 mL). The solution was treated with charcoal and dried ($MgSO_4$). The solvent was removed by evaporation, and the residue was purified by flash chromatography, eluting with 20% EtOAc/isohexane. The resulting solid was triturated with ether and recrystallized from EtOH to give **4j** (4.7 g, 23%): mp 166−167 °C; ^{1}H NMR (DMSO-d_6) δ 2.8 (s, 6H), 3.9 (s, 3H), 7.25 (d, 1H), 7.55−7.7 (m, 2H), 7.8 (s, 1H), 8.3 (d, 1H), 8.4−8.5 (m, 2H), 11.5 (br s, 1H); CIMS *m/e* 437 $(M + H)^+$. Anal. ($C_{17}H_{17}BrN_4O_3S$) C, H, N.

5-(Dimethylamino)-*N*-(5-chloro-3-methoxy-2-pyrazinyl)-1-naphthalenesulfonamide (7m) (Method D). NaH (60% dispersion in oil; 2.38 g, 59.6 mmol) was freed from oil and suspended in DME (20 mL) under Ar. A solution of **3d** (3.78 g, 23.8 mmol) in DME (80 mL) was added, and the mixture was stirred for 30 min. A solution of 5-(dimethylamino)-1-naphthalenesulfonyl chloride (6.43 g, 23.8 mmol) in DME (60 mL) was added over 30 min, and the mixture was stirred for 19 h. Aqueous citric acid (40%; 200 mL) was added, and the mixture was extracted with EtOAc (3 × 200 mL). The extracts were washed with water (200 mL), dried ($MgSO_4$), and concentrated. The residue was recrystallized from EtOH to give **4m** (4.26 g, 46%): mp 137−138 °C; ^{1}H NMR (DMSO-d_6) δ 2.8 (s, 6H), 3.9 (s, 3H), 7.25 (d, 1H), 7.55−7.7 (m, 2H), 7.75 (s, 1H), 8.3 (d, 1H), 8.4 (d, 1H), 8.5 (d, 1H), 11.5 (br s, 1H); FABMS *m/e* 393 $(M + H)^+$. Anal. ($C_{17}H_{17}ClN_4O_3S$) C, H, N.

***N*-(6-Cyano-3-pyridazinyl)-5-(dimethylamino)-1-naphthalenesulfonamide (6e).** Reaction of 3-amino-6-iodopyridazine[54] with 5-(dimethylamino)-1-naphthalenesulfonyl chloride according to method C, but carrying out the reaction at 20 °C, gave in 55% yield 5-(dimethylamino)-*N*-(6-iodo-3-pyridazinyl)-1-naphthalenesulfonamide: mp 177−178 °C; ^{1}H NMR (DMSO-d_6) δ 2.8 (s, 6H), 7.2 (d, 1H), 7.55−7.7 (m, 3H), 7.9 (d, 1H), 8.3 (d, 1H), 8.4 (d, 1H), 8.9 (d, 1H).

A mixture of 5-(dimethylamino)-*N*-(6-iodo-3-pyridazinyl)-1-naphthalenesulfonamide (227 mg, 0.5 mmol), KCN (130 mg, 2.0 mmol), and CuI (10 mg) in DMF (2 mL) was heated at 160 °C for 4 h. The solvent was removed by evaporation, and water (10 mL) was added to the residue. Aqueous citric acid (8%) was added to pH 5, and the precipitated solid was filtered off. The solid was purified by bond elute chromatography, eluting with MeOH/CH_2Cl_2 on a gradient from 0 to 7%, to give **6e** (60 mg, 34%): mp 160−165 °C; ^{1}H NMR (DMSO-d_6) δ 2.8 (s, 6H), 7.2 (d, 1H), 7.55−7.7 (m, 3H), 8.0 (d, 1H), 8.4 (d, 2H), 8.5 (d, 1H), 13.7 (br s, 1H); FABMS *m/e* 353 $(M + H)^+$. Anal. ($C_{17}H_{15}N_5O_2S \cdot 0.25Me_2NCHO$) C, H; N: calcd, 19.2; found, 18.6.

5-(Dimethylamino)-*N*-(6-methoxy-3-pyridazinyl)-1-naphthalenesulfonamide (6f). A mixture of compound **6c** (362 mg, 1.0 mmol) and sodium methoxide (162 mg, 3.0 mmol) in *N*-methylpyrrolidone (1 mL) was heated under reflux for 4 h. The solvent was removed by evaporation under high vacuum, and the residue was purified by flash chromatography, eluting with 1% MeOH/CH_2Cl_2, to give **6f** (140 mg, 33%): mp 125−127 °C; ^{1}H NMR (DMSO-d_6) δ 2.9 (s, 6H), 3.8 (s, 3H), 7.2−7.3 (2d, 2H), 7.5−7.7 (m, 2H), 7.8 (d, 1H), 8.3 (d, 1H), 8.4−8.6 (m, 2H); CIMS *m/e* 359 $(M + H)^+$. Anal. ($C_{17}H_{18}N_4O_3S$) C, H, N.

5-(Dimethylamino)-*N*-(5-bromo-3-hydroxy-2-pyrazinyl)-1-naphthalenesulfonamide (7i). A solution of **7j** (218 mg, 0.5 mmol) and sodium thiomethoxide (105 mg, 1.5 mmol) in

DMF (5 mL) was heated at 130 °C for 4 h. Aqueous citric acid (8%; 20 mL) was added, and the mixture was extracted with EtOAc (2 × 20 mL). The extracts were washed with water (3 × 20 mL), dried ($MgSO_4$), and concentrated. The residue was purified by bond elute chromatography, eluting with MeOH/CH_2Cl_2 on a gradient from 0 to 2%, to give **7i** (69 mg, 33%): mp 123−126 °C; ^{1}H NMR (DMSO-d_6) δ 2.8 (s, 6H), 7.2 (d, 1H), 7.35−7.45 (br, 1H), 7.55−7.7 (m, 2H), 8.3 (dd, 1H), 8.5 (t, 2H); FABMS *m/e* 423 $(M + H)^+$. Anal. ($C_{16}H_{15}BrN_4O_3S$) C, N; H: calcd, 3.6; found, 4.4.

5-[(Benzyloxycarbonyl)methylamino]-1-naphthalenesulfonyl Chloride (9a). NaH (60% dispersion in oil; 2.89 g, 72.4 mmol) was added over 5 min to a solution of sodium 5-[(benzyloxycarbonyl)amino]-1-naphthalenesulfonate (**8a**)[50] (11.0 g, 29.0 mmol) in DMF (110 mL). The mixture was stirred for 30 min, and then iodomethane (4.15 g, 29.2 mmol) was added dropwise over 30 min. The mixture was stirred for 20 h and then added to water (300 mL). Volatile material was removed by evaporation under high vacuum, and the residue was dissolved in water (100 mL). The solution was washed with EtOAc (100 mL) and acidified to pH 3 with concentrated HCl. The mixture was extracted with CH_2Cl_2 (3 × 100 mL), and the extracts were dried ($MgSO_4$). The solvent was removed by evaporation, and the residue was purified by flash chromatography, eluting with AcOH/MeOH/CH_2Cl_2 (0.1:20:80), to give 5-[(benzyloxycarbonyl)methylamino]-1-naphthalenesulfonic acid (6.5 g, 60%) as a foam: ^{1}H NMR (DMSO-d_6 + CD_3CO_2D) δ 3.3 (s, 3H), 5.0 (s, 2H), 7.05 (br s, 1H), 7.25 (br s, 2H), 7.4−7.6 (m, 4H), 7.8 (d, 1H), 7.95 (s, 1H), 8.05 (d, 1H), 8.9 (d, 1H).

$POCl_3$ (0.73 mL) was added to a solution of 5-[(benzyloxycarbonyl)methylamino]-1-naphthalenesulfonic acid (741 mg, 2.0 mmol) in MeCN (2 mL) and sulfolane (2 mL), and the solution was heated at 70 °C for 2 h under Ar. The solution was added to ice−water (30 mL) and extracted with EtOAc (2 × 20 mL). The extracts were washed with water (20 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography, eluting with 30% EtOAc/isohexane, to give **9a** (510 mg, 66%) as a foam: ^{1}H NMR (DMSO-d_6) δ 3.3 (s, 3H), 4.9−5.3 (m, 2H), 6.9−7.6 (m, 8H), 7.75 (d, 1H), 8.0 (dd, 1H), 8.85 (dd, 1H); FABMS *m/e* 390 $(M + H)^+$. Anal. ($C_{19}H_{16}ClNO_4S$) C, H, N.

5-[(Benzyloxycarbonyl)methylamino]-*N*-(5-bromo-3-methoxy-2-pyrazinyl)-1-naphthalenesulfonamide (10a). Reaction of **9a** with 2-amino-5-bromo-3-methoxypyrazine[48] according to method D and purifying the crude product by bond elute chromatography (40% EtOAc/isohexane) gave **12a** as a foam in 50% yield: ^{1}H NMR (DMSO-d_6) δ 3.3 (s, 3H), 3.9 (s, 3H), 4.9−5.3 (m, 2H), 6.9−7.6 (m, 9H), 8.1 (d, 1H), 8.35 (dd, 1H), 8.6 (d, 1H).

5-(Methylamino)-*N*-(5-bromo-3-methoxy-2-pyrazinyl)-1-naphthalenesulfonamide (11a). Iodotrimethylsilane (0.20 mL, 282 mg, 1.41 mmol) was added over 1 min to a solution of **10a** (330 mg, 0.60 mmol) in MeCN (6 mL), and the solution was stirred for 30 min. MeOH (10 mL) was added, and the solution was concentrated. The residue was purified by bond elute chromatography, eluting with EtOAc/isohexane on a gradient from 0 to 100%, to give **11a** (30 mg, 12%): mp 171−173 °C (after trituration with 50% ether/isohexane); ^{1}H NMR (DMSO-d_6) δ 2.85 (s, 3H), 3.9 (s, 3H), 6.55 (d, 1H), 7.4−7.5 (m, 2H), 7.7 (s, 1H), 7.95 (d, 1H), 8.25 (dd, 1H), 8.45 (d, 1H), 11.3 (br s, 1H); FABMS *m/e* 425 $(M + H)^+$. Anal. ($C_{16}H_{15}BrN_4O_3S$) H; C: calcd, 45.4; found, 46.1. N: calcd, 13.2; found, 12.1.

5-Acetamido-*N*-(5-bromo-3-methoxy-2-pyrazinyl)-1-naphthalenesulfonamide (10b). Reaction of 5-acetamido-1-naphthalenesulfonyl chloride (**9b**)[51] with 2-amino-5-bromo-3-methoxypyrazine[48] according to method C and purifying the crude product by flash chromatography, eluting with MeOH/CH_2Cl_2 on a gradient from 3 to 5%, gave **10b** in 8% yield: mp 221−225 °C; ^{1}H NMR (DMSO-d_6) δ 2.2 (s, 3H), 3.9 (s, 3H), 7.5−7.75 (m, 3H), 7.8 (s, 1H), 8.3−8.4 (m, 2H), 8.65 (d, 1H), 10.05 (s, 1H), 11.5 (br s, 1H); FABMS *m/e* 451 $(M + H)^+$. Anal. ($C_{17}H_{15}BrN_4O_4S$) C, H, N.

5-(Ethylamino)-*N*-(5-bromo-3-methoxy-2-pyrazinyl)-1-naphthalenesulfonamide (11b). Diborane (1.0 M) in THF (6.65 mL, 6.65 mmol) was added to a solution of **10b** (725 mg, 1.66 mmol) in dry THF (60 mL) at 0 °C under Ar. The solution was heated under reflux for 24 h, and then volatile material was removed by evaporation. The residue was purified by flash chromatography, eluting with 35% EtOAc/isohexane, to give **11b** (230 mg, 32%): mp 216−218 °C (after trituration with ether); ^{1}H NMR (DMSO-d_6) δ 1.3 (t, 3H), 3.25 (q, 2H), 3.9 (s, 3H), 6.6 (d, 1H), 7.4−7.6 (m, 2H), 7.75 (s, 1H), 7.95 (d, 1H), 8.25 (d, 1H), 8.5 (d, 1H), 11.4 (br s, 1H); FABMS *m/e* 437 $(M + H)^+$. Anal. ($C_{17}H_{17}BrN_4O_3S$) C, H, N.

5-(*N*-Methylacetamido)-1-naphthalenesulfonyl Chloride (9c). Use of a similar procedure to that described for the preparation of **9a**, but starting from sodium 5-acetamido-1-naphthalenesulfonate (**8b**),[51] gave **9c** as an oil in 42% overall yield: ^{1}H NMR (DMSO-d_6) δ 1.6 (s, 3H), 3.2 (s, 3H), 7.45−7.65 (m, 3H), 7.7 (dd, 1H), 8.05 (dd, 1H), 8.9 (dd, 1H); CIMS *m/e* 298 $(M + H)^+$.

5-(*N*-Methylacetamido)-*N*-(5-bromo-3-methoxy-2-pyrazinyl)-1-naphthalenesulfonamide (10c). Reaction of **9c** with 2-amino-5-bromo-3-methoxypyrazine[48] according to method D and purifying the crude product by flash chromatography, eluting with MeOH/CH_2Cl_2 on a gradient from 1 to 10%, gave **10c** as a foam in 21% yield: ^{1}H NMR (DMSO-d_6) δ 1.6 (s, 3H), 3.2 (s, 3H), 3.9 (s, 3H), 7.6−7.7 (m, 4H), 8.1 (d, 1H), 8.35−8.45 (m, 1H), 8.8−8.9 (m, 1H), 11.6 (br s, 1H); FABMS *m/e* 465 $(M + H)^+$.

5-(Ethylmethylamino)-*N*-(5-bromo-3-methoxy-2-pyrazinyl)-1-naphthalenesulfonamide (11c). Use of a similar procedure to that described for the preparation of **11b**, but starting from **10c** and purifying the crude product by bond elute chromatography (1% MeOH/CH_2Cl_2), gave **11c** as a foam in 41% yield: ^{1}H NMR (DMSO-d_6) δ 1.1 (t, 3H), 2.8 (s, 3H), 3.1 (q, 2H), 3.9 (s, 3H), 7.3 (d, 1H), 7.6−7.7 (m, 2H), 7.7 (s, 1H), 8.3−8.4 (m, 1H), 8.5 (t, 2H), 11.5 (br s, 1H); FABMS *m/e* 451 $(M + H^+)$. Anal. ($C_{18}H_{19}BrN_4O_3S$) C, H, N.

Determination of Affinity for Cloned Human Endothelin ET_A and ET_B Receptors Expressed in Mouse Erythroleukemic Cells. The cDNAs for the ET_A and ET_B receptors were obtained by polymerase chain reaction (PCR) of a human kidney cDNA library or a human placental cDNA template, respectively. The cDNAs were subcloned and expressed in MEL-C88 cells using standard published methodology.[55] MEL cell membranes (cloned human ET_A and ET_B) were incubated with [^{125}I]ET-1 (30 pM final) and competing ligands, in a final incubation volume of 225 μL. Samples were incubated for 180 min at 30 °C, and the incubation was terminated by filtering through GF/B filters using a Brandel cell harvester. [^{125}I]ET-1 binding was quantified by gamma counting.

Antagonism of ET_A-Induced Contractile Response in Guinea Pig Portal Vein. Guinea pigs of either sex and weight > 250 g (n = 3−6) were killed by cervical dislocation. A 1-cm length of portal vein was removed and put into oxygenated (95% O_2/5% CO_2) Krebs solution (118 mM NaCl, 4.75 mM KCl, 2.54 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 0.93 mM KH_2PO_4, 11 mM glucose). The portal vein was cleared of connective tissue and an approximately 4-mm length set up for isometric recording of the circular muscle by inserting two fine stainless steel wires through the lumen. The tissue was suspended in a 20-mL organ bath containing oxygenated Krebs solution at 32 °C. After an equilibration time of approximately 1.5 h, a cumulative partial dose−response curve to big ET-1 was constructed using a 3-fold concentration increment to the point where a tension increase of >100 mg was produced. Big ET-1 was washed out, and a time period of 2 h was allowed to elapse. The tissue was again washed, and a partial dose−response curve to big ET-1 in the presence of the test antagonist was determined. The dose−ratio shift was calculated and the pA_2 value determined.

Antagonism of ET_A-Induced Pressor Responses by Compounds Administered Intravenously to Pithed Rats. Male Alderley Park Wistar rats (280−320 g, n = 4) were pithed under halothane anesthesia, and arterial and venous catheters were implanted for measurement of blood pressure and administration of big ET-1, respectively. Repeated cumulative dose−response curves to big ET-1 (0.1−4.0 nmol/kg) were

constructed both prior to and 5 min after intravenous administration of test compound. The dose of big ET-1 to induce a rise in mean arterial pressure of 30 mmHg was estimated for each curve and the dose−ratio shift calculated.

Antagonism of ET_A-Induced Pressor Responses by Compounds Administered Orally to Conscious, Normotensive Rats. Male Alderley Park Wistar rats (220−260 g, $n = 6$) were prepared under Saffan (alphaxalone/alphadolone) anesthesia with indwelling arterial and venous catheters for measurement of blood pressure and administration of big ET-1, respectively. Repeated cumulative dose−response curves to big ET-1 (0.1−4.0 nmol/kg) were constructed both prior to and at time points following oral administration of test compound. The dose of big ET-1 to induce a rise in mean arterial pressure of 30 mmHg was estimated for each curve and the dose−ratio shift calculated.

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