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N-Methyl-2-[4-(2-methylpropyl)phenyl]-3-(3-methoxy-5-methylpyrazin-2-ylsulfamoyl)benzamide; One of a Class of Novel Benzenesulphonamides which are Orally-Active, ET_A-Selective Endothelin Antagonists

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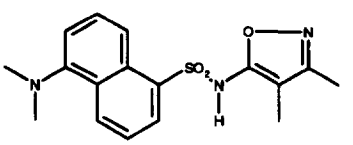
Abstract: A series of novel sulphonamides have been discovered which show high affinity and selectivity for the endothelin ET_A receptor. N-Methyl-2-[4-(2-methylpropyl)phenyl]-3-(3-methoxy-5-methylpyrazin-2-yl-sulfamoyl)benzamide (**18**) is the most widely investigated compound and is a potent antagonist *in vivo* when dosed either i.v. or orally, and has prolonged oral duration of action. © 1997 Elsevier Science Ltd.

Endothelin-1 (ET-1),[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] such as renal failure,[3] cerebral vasospasm[4] and pulmonary hypertension.[5] In mammalian tissues, two subtypes of endothelin receptor have been identified.[6] The 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[7] and smooth muscle proliferation.[8] The ET_B receptor binds ET-1, ET-2 and ET-3 with similar affinity and mediates primarily vasodilation but also vasoconstriction in certain vascular beds.[9]

Recently, the first non-peptide endothelin antagonists have been reported including a number of sulphonamides. These sulphonamides may be either ET_A-selective, such as BMS 182874,[10] or may be 'balanced' ET_A/ET_B antagonists, such as Ro 46-2005[11] and Ro 47-0203 (Bosentan).[12]



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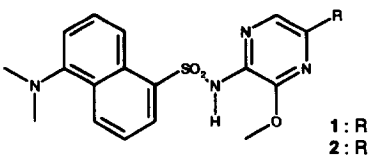

1 : R = Cl
2 : R = Br
3 : R = CH_3

We have recently described a series of ET_A-selective compounds (including **1-3**), discovered whilst preparing N-(5-(dimethylamino)-1-naphthalenesulphonamides using robotic synthesis.[13] Activity has also been reported in related sulphonamides, in which the dansyl moiety has been replaced by either 2-biphenyl or 2-(4-isobutylphenyl)phenyl group.[14] The work reported here concerns the introduction of carboxamide groups into the 3 position of N-(pyrazinyl)-2-arylbenzenesulphonamides. This work was undertaken in the hope that the newly introduced amide group might occupy the same region of space as was filled by the dialkylamino group in

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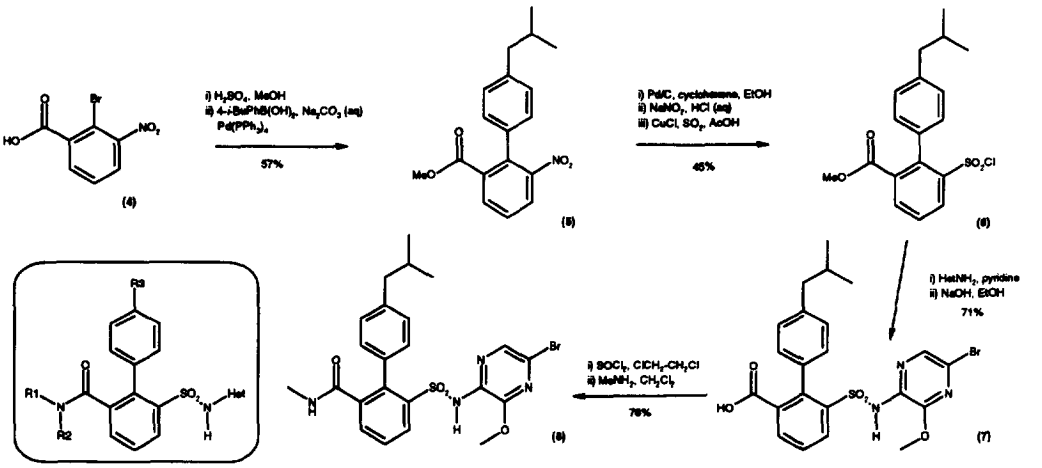
compounds **1-3**, thereby possibly providing compounds with enhanced affinity and / or selectivity for the ET_A receptor over the activity seen with compounds **1-3** or **16/17**. The general route used to prepare such compounds is illustrated for the preparation of compound **8**, as shown in scheme 1, starting from 2-bromo-3-nitrobenzoic acid (**4**):-



Scheme 1

The compounds listed in Table 1 were evaluated in a radioligand binding assay involving displacement of [^{125}I]ET-1 from membranes prepared from MEL cells transfected with cloned human ET_A receptors.[15] A similar assay was used to measure affinity for the human ET_B receptor but the data are not shown as no compound showed significant binding (>50% inhibition at 10μM).[6] Data for the analogous biphenylsulphonamides, lacking the C3 carboxamide substituent (compounds 16 and 17) are also shown.

Table 1 - In vitro ET_A Binding Data for compounds 8-17

Compound	R_1	R_2	R_3	ET_A pIC_{50}
8	$-CH_3$	H	*i*-Bu	8.9
9	H	H	*i*-Bu	9.1
10	$-CH_2CH_2OCH_3$	H	*i*-Bu	8.8
11	-*n*-C_5H_{11}	H	*i*-Bu	6.7
12	$-CH_3$	$-CH_3$	*i*-Bu	9.0
13	H	H	H	7.4
14	$-CH_3$	H	H	7.4
15	-*n*-C_5H_{11}	H	H	8.4
16	-	-	*i*-Bu	7.4
17	-	-	H	7.5

For those compounds where R_3 is isobutyl, introduction of the carboxamide functionality at C3 generally leads to an increase in receptor affinity, although compound **11** would seem to be an exception to this general

rule. Curiously, in the series of compounds where R_3 is hydrogen, the n-pentyl amide (**15**) also stands out as exceptional, being the only compound made where an increase in affinity over that observed for the parent compound **17** could be determined. One interpretation of these data may be that the R_1 and R_3 groups compete for a common lipophilic pocket in the receptor. A range of carboxamide substituents were examined; primary, secondary and cyclic amides all showed good to excellent ET_A affinity.

Representative compounds from table 1 were tested *in vivo* after oral (conscious rat) dosing; potency was assessed against the pressor response induced by big ET-1 in Alderley Park rats. A partial dose-response curve to big ET-1 (range 0.1-4.0 nmol kg^{-1} i.v.), sufficient to increase mean arterial pressure $\geq$ 30 mmHg, was obtained prior to, then at various times after, administration of compound in 10% DMSO. Big ET-1 curves were obtained 1h after oral dosing, to conscious rats, in routine screening. Data from representative compounds are shown in table 2 where activity is quoted as the mean dose-ratio (MDR) obtained 1h after oral administration of compound.

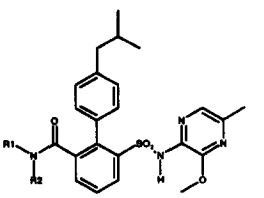
Table 2 - Oral Activity of compounds 8 and 10

Compound	R_1	R_2	R_3	$mgkg^{-1}$	MDR (1h)
8	$-CH_3$	H	*i*-Bu	3	2.7
10	$-CH_2CH_2OCH_3$	H	*i*-Bu	3	2.1

Although these data were encouraging we decided to make analogues using the other amino-pyrazines we had identified in our earlier work with compounds **1-3**.[13] The most interesting compounds found were those bearing a methyl group at the C5 position of the pyrazine. The *in vitro* and *in vivo* activities of five compounds in this series are shown in Table 3. In general the compounds show broadly similar affinities for the ET_A receptor and all are highly selective for this receptor over the ET_B receptor (data not shown). However, the compounds were considerably more active *in vivo* than the 5-bromo pyrazine analogues shown in Table 2.

Table 3 - Activity of compounds 18-22

Compound	R_1	R_2	ET_A pIC_{50}	$mgkg^{-1}$	MDR (1h)
18	$-CH_3$	H	9.3	1	3.2
19	H	H	9.0	1	2.4
20	$-CH_2CH_2OH$	H	9.2	1	4.2
21	$-CH_2CH_2OCH_3$	H	9.3	1	3.2
22	$-CH_3$	$-CH_3$	8.6	1	1.6



The analogue which proved to be of greatest interest was N-methyl-2-[4-(2-methylpropyl)phenyl]-3-(3-methoxy-5-methylpyrazin-2-ylsulfamoyl)benzamide (**18**). This compound showed a pIC_{50} of 9.3 in the ET_A receptor binding assay which was similar to the value obtained with the corresponding bromo compound, **8**

(pIC_{50} = 8.9). Activity *in vivo* after oral dosing was assessed as before, with mean dose-ratios (MDR) being obtained 30 min, 2h and 4h after administration of **18**, 2.5 $mgkg^{-1}$ (5 animals), or vehicle (12 animals).

Table 4. Oral activity of Compound 18 dosed at 2.5 $mgkg^{-1}$ in the conscious rat.

	30 min	2 hours	4 hours
Vehicle	1.1 [1.0-1.2]	1.1 [1.1-1.2]	1.0 [1.0-1.2]
18	6.2 [4.4-8.9]***	3.3 [1.6-6.6]*	4.0 [2.6-6.3]***

(*P<0.05, *** P<0.001 vs vehicle, Student's *t*-test for unpaired data).

Results are expressed as mean dose ratios [95% confidence limits] measured at a pressor response of 30 mmHg.

The results shown in table 4 demonstrate that changing the nature of the heterocycle has given **18** a much better oral profile (MDR of 4.0 at 4h when dosed at 2.5$mgkg^{-1}$ dose) when compared to that of its close analogue **8** (MDR of 2.7 at 1h when dosed at 3 $mgkg^{-1}$). Further studies on these compounds are continuing.

In summary, we have prepared a series of ET_A-selective endothelin antagonists which show improved potency over both the unsubstituted biphenylsulphonamides (**16/17**) and the dansyl sulphonamides **1-3**. N-Methyl-2-[4-(2-methylpropyl)phenyl]-3-(3-methoxy-5-methylpyrazin-2-ylsulfamoyl)benzamide (**18**) shows high *in vivo* oral potency and, when dosed at 2.5$mgkg^{-1}$ in the conscious rat, has a duration of action in excess of 4h.

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