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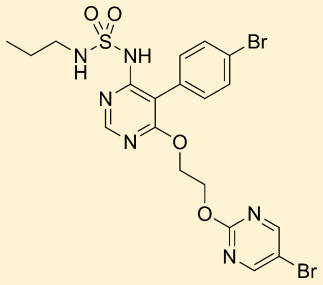
The Discovery of *N*-[5-(4-Bromophenyl)-6-[2-[(5-bromo-2-pyrimidinyl)oxy]ethoxy]-4-pyrimidinyl]-*N*′-propylsulfamide (Macitentan), an Orally Active, Potent Dual Endothelin Receptor Antagonist

Martin H. Bolli,* Christoph Boss, Christoph Binkert, Stephan Buchmann, Daniel Bur, Patrick Hess, Marc Iglarz, Solange Meyer, Josiane Rein, Markus Rey, Alexander Treiber, Martine Clozel, Walter Fischli, and Thomas Weller

Drug Discovery Chemistry, Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, CH-4123 Allschwil, Switzerland

Supporting Information

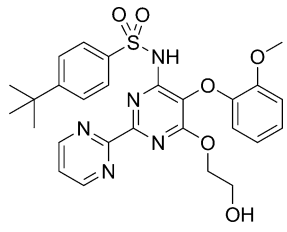
ABSTRACT: Starting from the structure of bosentan (**1**), we embarked on a medicinal chemistry program aiming at the identification of novel potent dual endothelin receptor antagonists with high oral efficacy. This led to the discovery of a novel series of alkyl sulfamide substituted pyrimidines. Among these, compound **17** (macitentan, ACT-064992) emerged as particularly interesting as it is a potent inhibitor of ET_A with significant affinity for the ET_B receptor and shows excellent pharmacokinetic properties and high in vivo efficacy in hypertensive Dahl salt-sensitive rats. Compound **17** successfully completed a long-term phase III clinical trial for pulmonary arterial hypertension.



INTRODUCTION

Over the past decade, our understanding of the function of endothelin (ET) has evolved from describing it as a purely vascular factor regulating blood pressure[1−4] to recognizing it as a hormone influencing many cellular processes such as proliferation, apoptosis, and migration leading to tissue hypertrophy, remodeling, fibrosis, and inflammation.[5−7] The three structurally closely related peptides, named endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3), are among the most potent vasoconstrictors known in humans. They convey their action through binding to two G-protein coupled receptors called ET_A and ET_B. Among the three isopeptides, ET-1 appears to be the key player in the cardiovascular system. ET-2 and ET-3 are specifically expressed in the gastrointestinal tract and the brain, respectively.[8,9] On the vascular side, ET_A receptors are mainly expressed on smooth muscle cells, mediating vasoconstriction, while ET_B receptors expressed on endothelial cells and smooth muscle cells cause vasodilation and vasoconstriction, respectively. In addition, a wealth of tissue cells such as cardiomyocytes, renal cortex, and medulla cells, fibroblasts, macrophages, and neurons produce ET-1 and/or express the two ET receptors, illustrating that the ET system is not confined to the vasculature only.[9−11] Cross talk and receptor heterodimer formation has been proposed for the two ET receptors.[6,12−15] Both ET_A and ET_B receptors are involved in mediating ET-1 deleterious effects in diseases such as chronic heart failure, idiopathic pulmonary fibrosis, pulmonary arterial hypertension, or kidney disease.[6,7,10,16] Furthermore, in many pathological situations, the ET system is up-regulated and a change in the ET_A/ET_B expression ratio in favor of ET_B is observed in diseased tissue.[11,17−19] Several studies corroborate that increased activity of the ET system in tissue correlates with disease severity.[20,21]

A large number of synthetic endothelin receptor antagonists have been investigated preclinically, and several small molecule inhibitors have been studied in clinical trials for chronic heart failure, hypertension, cancer, and fibrosis.[5,22−27] Today, the dual ET_A/ET_B receptor antagonist bosentan (**1**, Figure 1)[28−30] and the ET_A selective antagonist (*S*)-2-((4,6-dimethylpyrimidin-2-yl)oxy)-3-methoxy-3,3-diphenylpropanoic acid (ambrisentan)[31−33] are approved treatments for pulmonary arterial hypertension.



1 (Bosentan)
IC_{50} ET_A 45 nM
ET_B 202 nM

Figure 1. Structure and in vitro activity of compound **1**. IC_{50} values as measured with a ^{125}I-ET-1 binding assay.

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When we initiated our medicinal chemistry program to identify a follow-up compound to sulfonamide **1** (Figure 1), we decided that compound **1** should not only serve as the structural starting point for our quest for novel endothelin antagonists but should also represent the benchmark against which we gauge our novel compounds. A candidate suitable for clinical development needs to meet the following requirements: (1) The compound has to show a significantly increased potency on the ET_A receptor when compared to compound **1**. (2) The compound should block the ET_B receptor in vivo. (3) The compound needs to demonstrate oral efficacy superior to the one of compound **1** as assessed by measuring arterial blood pressure in conscious hypertensive Dahl salt-sensitive rats.[34−36] (4) Pharmacokinetic properties assessed in animals must support once daily dosing in humans. (5) The compound should easily distribute into tissue in order to also reach ET receptors expressed in the tissue. (6) Because compound **1** inhibits the canalicular bile salt export pump,[37] a candidate compound must be devoid of bile salt transport inhibition.

In the paragraphs below, we describe our efforts aimed at identifying a compound that meets all of the above criteria.

RESULTS AND DISCUSSION

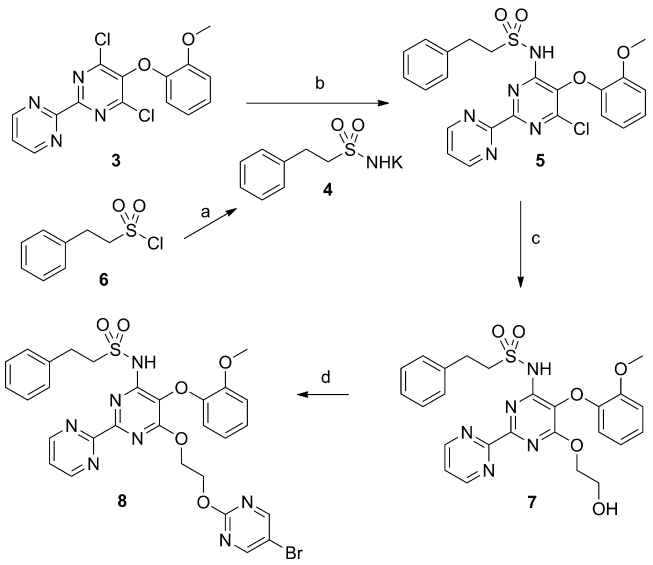
Synthesis. The following description of the synthesis of a prototypical sulfonamide (Scheme 1) and sulfamide (Scheme 2 and 3) endothelin receptor antagonist shall illustrate the general approach chosen to prepare the compounds discussed in this manuscript.

The preparation of the sulfonamide derivatives was carried out as previously described[38−42] and usually started by reacting a dichloro-pyrimidine building block[43,44] (e.g., **3**) with a sulfonamide potassium salt (e.g., **4**) in DMSO at room temperature to give the monochloro-pyrimidine intermediate (e.g., **5**). The sulfonamide potassium salts were readily accessible by treating the respective sulfonyl chloride (e.g., **6**) with ammonia, followed by potassium *tert*-butylate in methanol, while the necessary dichloro-pyrimidines were obtained as described in the literature.[40,42−46] The monochlorides such as **5** were treated with a freshly prepared solution of sodium in ethylene glycol at elevated temperatures to give the corresponding alcohols (e.g., **7**). The alcohols were dissolved in THF and deprotonated with 2.5 equiv of sodium hydride and reacted with the respective 2-chloro- or 2-(methylsulfonyl) pyrimidines at elevated temperature. As reported earlier,[47] these pyrimidines are either commercially available or prepared according to literature procedures.[48−52] Final products such as **8** were obtained in good yields as white to beige powders after crystallization from methanol.

The preparation of the various sulfamide building blocks needed for the synthesis of our sulfamide based endothelin receptor antagonists is exemplified with the propyl-sulfamide potassium salt **13** shown in Scheme 2.[40] The procedure starts by reacting chlorosulfonyl isocyanate (**9**) dissolved in dichloromethane at 0 °C with 1 equiv of *tert*-butanol to give the BOC-protected amino-sulfonyl-chloride (**10**), which was subsequently added slowly to a solution of 1 equiv of the respective amine (e.g., *n*-propylamine) in the presence of 3 equiv of triethylamine in dichloromethane at 0 °C. The mixture was stirred overnight to drive the reaction to completion and was then concentrated. The residue was dissolved in a large volume of ethyl acetate. To avoid extraction of the BOC-protected sulfamides such as **11** into water, reagents and salts were removed by washing the ethyl acetate solution very carefully with a small volume of water and brine only. The organic extract was dried, and the sulfamide **11** was isolated by evaporating the solvent. This material was dissolved in approximately 5 M HCl in dioxane to affect BOC-deprotection. After stirring the mixture at room temperature for 4−8 h, the solvent was evaporated to give sulfamide **12**. The potassium salt **13** was obtained by dissolving **12** in methanol followed by the addition of potassium *tert*-butylate and subsequent evaporation of the solvent. Purification of the potassium salt **13** was achieved by trituration in diethylether and drying under high vacuum. Overall yields of the above process were good to excellent.

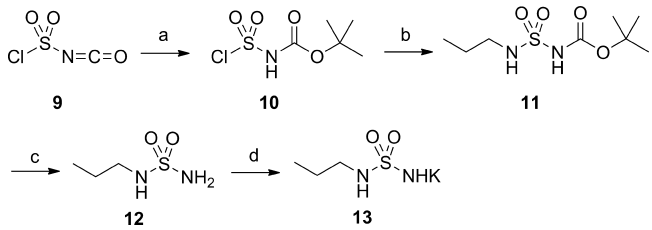
Synthesis of the sulfamide based endothelin receptor antagonists followed the preparation of the corresponding sulfonamide analogues. As an example, the preparation of sulfamide **17** is outlined in Scheme 3. Hence, reacting a dichloro-pyrimidine building block (e.g., **14**[42]) with a sulfamide

Scheme 1. General Synthesis of Sulfonamide Endothelin Receptor Antagonists Exemplified with 8[a]



[a]Reagents and conditions: (a) (i) **6**, NH_3 in MeOH, rt, 16 h, (ii) MeOH, KOtBu, rt 3 h, 83−89% over 2 steps; (b) **3**, DMSO, rt, then **4**, rt, 16 h, 89%; (c) ethylene glycol, Na, 2 h, then **5**, 70−90 °C, 18 h, 73−91%; (d) **7**, THF, NaH, 1 h, then 2-chloro-5-bromopyrimidine, 75 °C, 2 h, 43%.

Scheme 2. Synthesis of Sulfamide Potassium Salt Building Blocks[a]



[a]Reagents and conditions: (a) *tert*-BuOH, DCM, 0 °C to rt, 1 h; (b) add mixture prepared in (a) to propylamine, NEt_3, DCM, 0 °C then 16 h rt; (c) 5 M HCl in dioxane, rt, 4−8 h; (d) MeOH, KOtBu, rt, 3 h, 46−98% over 4 steps.

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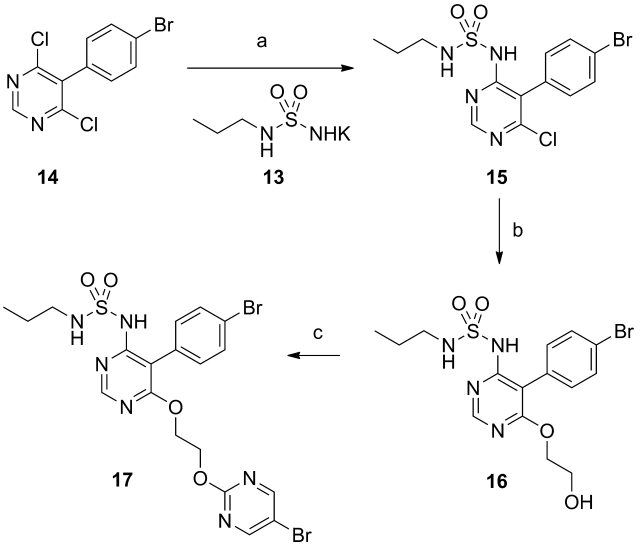
Scheme 3. General Synthesis of Sulfamide Endothelin Receptor Antagonists Exemplified with 17[a]



[a]Reagents and conditions: (a) **14**, DMSO, rt, **13**, rt, 24−72 h, 83−93% (b) ethylene glycol, DME, KOtBu, 10 min, then **15**, 100 °C, 18−24h, 86−89%; (c) **16**, THF, NaH, 0.5−1 h, then 2-chloro-5-bromopyrimidine, 60−75 °C, 2 h, 88%.

potassium salt such as **13** in DMSO at room temperature for 24−48 h gave the monochloro-pyrimidine intermediates (e.g., **15**). The ethylene-glycol side chain was introduced by applying similar conditions as described for the synthesis of the sulfonamides. Alternatively, the monochloride (e.g., **15**) was added to a mixture of 3 equiv of potassium *tert*-butylate and about 40 equiv of ethylene glycol in dimethoxyethane. These mixtures were usually heated to 90−100 °C for 24−70 h to furnish the corresponding alcohols (e.g., **16**) in yields of 70−90%. The final step, the attachment of the substituted pyrimidine, was performed as described for the sulfonamide derivatives in Scheme 1. For the preparation of compound **17**, 2-chloro-5-bromo-pyrimidine was used. In general, the target compounds could be purified easily by recrystallization from methanol.

In Vitro SAR Discussion. Inspired by the structure of Tanabe's clinical development compound *N*-(6-(2-((5-bromopyrimidin-2-yl)oxy)ethoxy)-5-(*p*-tolyl)pyrimidin-4-yl)-4-(1-hydroxy-2-methylpropan-2-yl)benzenesulfonamide (T-0201, **2**)[53] (Figure 2), we were curious to learn how the introduction of a pyrimidine to the ethylene glycol moiety of compound **1** would

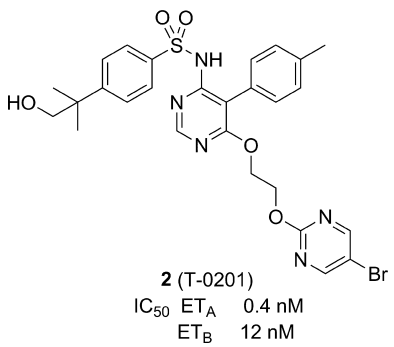
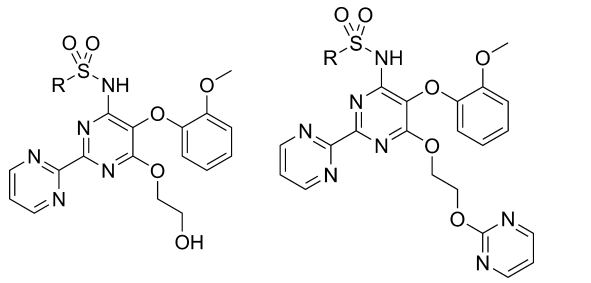


Figure 2. Structure and in vitro activity of compound **2**. IC_{50} values as measured with a ^{125}I-ET-1 binding assay.

influence its affinity for the two endothelin receptors. In a first study illustrated by the compounds listed in Table 1, we

Table 1. Effect of Additional Pyrimidine at Ethylene Glycol[a]



R	compd	IC_{50} ET_A [nM]	IC_{50} ET_B [nM]	compd	IC_{50} ET_A [nM]	IC_{50} ET_B [nM]
4-*tert*-butyl phenyl	**1**	45.0	202	**18**	4.3	76
phenethyl	**19**	25.7	1131	**20**	8.0	465
methyl	**21**	4534	9506	**22**	739	1765

[a]IC_{50} values as determined in binding assays using membranes of Chinese hamster ovary (CHO) cells containing either the recombinant ET_A or ET_B receptor, respectively. Data are given as geometric means of at least three independent measurements.

observed that such an additional pyrimidine ring improves the affinity for ET_A and ET_B significantly. Compound **1** derivative **18**, for instance, is about 10 times more potent on ET_A and about three times more active on ET_B than compound **1** itself. A similar trend was observed with the pair of phenylethane sulfonamides **19** and **20**. Interestingly, also the weakly active methane sulfonamide derivative **21** profits from the introduction of a pyrimidine ring, and the resulting compound **22** is almost 10 times more potent on both ET_A and ET_B than compound **21**. We therefore had a closer look at the structure−activity relationship (SAR) of this additional pyrimidine moiety. While various substituents were tolerated in the 5-position of the pyrimidine, substituents in positions 4 and/or 6 clearly hampered the compound's affinity for the ET receptors. This is illustrated with some examples of the phenylethane sulfonamide series compiled in Table 2. A comparable SAR, however, was observed with other scaffolds. Compared to the unsubstituted pyrimidine derivative **20**, the 5-methyl analogue **23** is less potent on both receptors. On the other hand, a 5-chloro (**24**), 5-bromo (**8**), 5-methoxy (**25**), 5-methylthio (**26**), and a 5-trifluoromethyl (**27**) substituent all led to a similarly improved affinity for ET_A. While the bromo-, methoxy-, and trifluoromethyl substituent improved the potency on ET_B only slightly, a 5-methylthio group increased the compound's affinity for this receptor significantly. The 5-cyclopropyl substitutent in **28** reproduced the profile of its unsubstituted analogue **20**. As illustrated with compound **29**, a 4,6-disubstitution pattern yielded only poorly active compounds.

On the one hand attaching a 5-bromo or a 5-methylthio-pyrimidine to the ethylene glycol moiety brought us closer to our goal of identifying endothelin antagonists more potent than compound **1**. On the other hand, these new compounds contain five aromatic rings and have a molecular weight well above 600 g/mol and therefore certainly do not qualify as lean molecules. In view of this, we carefully scrutinized the three substituents attached to positions 2, 4, and 5 of the core pyrimidine for possibilities to reduce the molecule's size. For these studies, we kept the 5-bromo-pyrimidine residue at the

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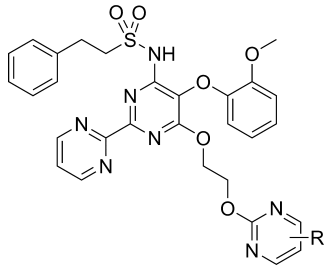
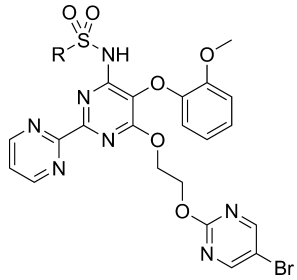
Table 2. Optimization of Pyrimidine[a]



compd	R	IC_{50} ET_A [nM]	IC_{50} ET_B [nM]
20	hydrogen	8.0	465
23	5-methyl	17.8	1501
24	5-chloro	2.8	461
8	5-bromo	2.2	216
25	5-methoxy	3.2	202
26	5-methylthio	1.1	42
27	5-trifluoromethyl	3.0	145
28	5-cyclopropyl	7.7	424
29	4,6-dimethoxy	4775	>10000

[a]IC_{50} values as determined in binding assays using membranes of Chinese hamster ovary (CHO) cells containing either the recombinant ET_A or ET_B receptor, respectively. Data are given as geometric means of at least three independent measurements.

Table 3. From Sulfonamides to Sulfamides[a]



compd	R	IC_{50} ET_A [nM]	IC_{50} ET_B [nM]
30	4-*tert*-butyl phenyl	1.5	19.3
31	phenyl	10.4	341
32	benzyl	73	3479
8	phenethyl	2.2	216
33	3-phenyl-propyl	6.7	377
34	methyl	113	1146
35	ethyl	14	460
36	*n*-propyl	9.4	566
37	*n*-butyl	4.3	552
38	benzyl-NH−	1.9	147
39	methyl-NH−	7.9	194
40	ethyl-NH−	9.9	655
41	*n*-propyl-NH−	3.8	322
42	*n*-butyl-NH−	1.7	167
43	*N*-benzyl-methyl-N-	7.0	457
44	morpholino	187	1474

[a]IC_{50} values as determined in binding assays using membranes of Chinese hamster ovary (CHO) cells containing either the recombinant ET_A or ET_B receptor, respectively. Data are given as geometric means of at least three independent measurements. Note that compounds **8** and **30**−**37** represent sulfonamides, while compounds **38**−**44** represent sulfamides.

ethylene glycol constant. The compounds listed in Table 3 document the SAR of the sulfonamide part in position 4. Tables 4 and 5 illustrate the influence of the substituents in position 2 and 5, respectively.

In a first step, we removed the *tert*-butyl group attached to the benzene sulfonamide moiety. This resulted in a 10-fold loss of affinity for both ET receptors. When a CH_2 group was introduced as linker between the phenyl ring and the sulfur to give compound **32**, a further loss of affinity was observed. Interestingly, moving the phenyl two CH_2 units away from the sulfonamide moiety (compound **8**) restored the compound's affinity completely for the ET_A receptor and partially for the ET_B receptor. The affinity profile of phenyl-propane sulfonamide **33** is very similar to the one of the ethane analogue **32**. These observations combined with our aim to reduce molecular weight prompted us to study compounds lacking a phenyl ring in the sulfonamide part. Not unexpectedly though, the methane sulfonamide derivative **34** showed only moderate potency on the ET_A receptor. Much to our delight, however, the ethane sulfonamide **35** was almost as potent as the much bulkier phenylethane sulfonamide **8**. As shown with compounds **36** and **37**, the affinity for ET_A improves steadily with increasing length of the alkyl chain. No such trend was observed with the ET_B receptor. In a next step, we prepared a number of compounds wherein the sulfonamide moiety was replaced by a sulfamide group. As illustrated with compounds **38**−**43**, these novel sulfamide derivatives represent potent ET receptor antagonists. Only the morpholine derivative **44** shows some loss of affinity toward the two receptors. Compared to the corresponding sulfonamides, the sulfamides have almost identical affinity for the ET_A receptor (e.g., compare **8** with **38**, **35** with **39**, **36** with **40**, **37** with **41**). In contrast to the sulfonamide series, however, the IC_{50} value for the ET_B receptor improves with increasing length of the sulfamide alkyl chain. More importantly, the sulfamides show a trend toward improved potency on ET_B. This observation clearly moved the sulfamides into the focus of our interest. For the following studies, we selected sulfamide **38** as a reference compound. Hence, while keeping the benzyl sulfamide moiety constant, we prepared a series of analogues with different substituents in position 2 of the core pyrimidine. Several examples are compiled in Table 4. The pyrazine (**45**) and morpholine (**47**) derivatives showed an affinity profile almost identical to the one of the pyrimidine analogue **38**. The pyridine derivative **46**, on the other hand, lost potency, in particular on the ET_B receptor. As illustrated with compounds **48** and **49**, the loss in affinity is clearly more pronounced for the 2-cyclopropyl (**48**) and the 2-methoxy (**49**) substituted pyrimidine scaffolds. The 2-methoxy−ethoxy substituent in compound **50** led to a small loss in affinity for both receptors when compared to **38**. Interestingly, complete removal of the substituent in position 2 of the core pyrimidine (**51**) reproduced the affinity profile of compound **50**, suggesting that the contribution of the 2-substituent to the binding affinity is small. This encouraged us to further evaluate the sulfamide series with scaffolds lacking a substituent in position 2 of the core pyrimidine. We therefore prepared a number of 2-unsubstituted pyrimidine derivatives incorporating a selection of 5-substituents that were known to lead to potent ET receptor antagonists.[53−58] Some examples are compiled in Table 5. As shown with compounds **52** and **53**, a 3-methoxyphenoxy rather than a 2-methoxyphenoxy substituent led to an increase in potency on ET_A but left the affinity for ET_B unchanged. An unsubstituted phenyl ring directly bound to the core pyrimidine as in **54** had no impact on ET_A affinity

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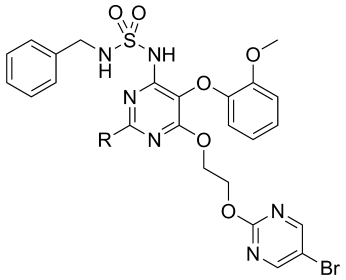
Table 4. Optimization of Scaffold: 2-Substituent



compd	R	IC_{50} ET_A[a] [nM]	IC_{50} ET_B[a] [nM]
38	2-pyrimidinyl	1.9	147
45	2-pyrazinyl	2.1	166
46	4-pyridyl	3.4	561
47	4-morpholinyl	1.4	192
48	cyclopropyl	14	1082
49	methoxy	40	2490
50	2-methoxy-ethoxy	6.9	554
51	hydrogen	6.9	525

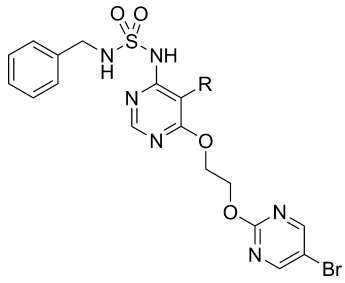
[a]IC_{50} values as determined in binding assays using membranes of Chinese hamster ovary (CHO) cells containing either the recombinant ET_A or ET_B receptor, respectively. Data are given as geometric means of at least three independent measurements.

but caused a significant loss in the activity on ET_B. Introducing a methyl group (**55**), or a chlorine (**56**) or bromine (**57**) atom to the para position of the phenyl ring improved the compounds' affinity for both receptors significantly. Introducing even larger alkyl groups, as shown with compounds **58** and **59**, did not improve the compound's affinity any further. In fact, both compounds showed a clearly reduced potential to block the ET_B receptor.

In brief, the SAR studies discussed so far have shown that: (1) Introduction of a 5-bromo- or 5-methylthiopyrimidine to the ethylene glycol improves the compound's affinity for both ET receptors significantly (Table 1 and 2). (2) The bulky 4-*tert*-butyl benzene sulfonamide in compound **1** can be replaced by a benzyl or alkyl sulfamide moiety (Table 3). (3) Sulfamide derivatives are more potent on ET_B when compared to the corresponding sulfonamide analogues (Table 3). (4) A 2-substituent at the core pyrimidine is not mandatory to obtain potent ET antagonists (Table 4). (5) A 3-methoxyphenoxy, 4-methylphenyl, 4-chlorophenyl, or 4-bromophenyl residue attached to position 5 of the core pyrimidine leads to potent ET receptor antagonists (Table 5).

In Vivo Efficacy Assessment. In a next step, we assessed in vivo the oral efficacy of some of the most potent examples listed in Table 5. For this purpose, we measured the blood pressure lowering effect following oral administration by gavage of 3 mg/kg of each compound in conscious hypertensive Dahl salt-sensitive rats equipped with telemetry.[59−61] In this model, efficient blockade of the ET receptors results in a significant reduction of mean arterial blood pressure.[35] The pharmacological response was quantified by calculating the area between the curves (ABC) of the blood pressure recordings 24 h before and after treatment. As this experiment allowed for rapid and reliable assessment of the in vivo efficacy, we did not measure pharmacokinetic properties prior to pharmacological testing. In all experiments, the heart rate remained unaffected (data not shown). At an oral dose of 3 mg/kg, compound **1** maximally reduced mean arterial blood pressure by −7 ± 4 mmHg. The duration of action was about 15 h, which translated into an ABC of −88 mmHg·h (n = 8) (Table 5). A maximal reduction of mean arterial blood pressure by −30 ± 3 mmHg was achieved with a dose of 100 mg/kg. At this dose, compound **1** was active for about 72 h and displayed an ABC of −896 mmHg·h (n = 5).

Table 5. Optimization of Scaffold: 5-Substituent



compd	R	IC_{50} ET_A[a] [nM][a]	IC_{50} ET_B[a] [nM][a]	max ΔMAP[b] [mmHg]	ABC[c] [mm Hg·h] (n)
1	*d*	45	202	−7 ± 4	−88 (8)
52	3-methoxyphenoxy	1.0	457	−10 ± 4	−245 (5)
53	2-chloro-5-methoxyphenoxy	1.4	712	−24 ± 5	−639 (5)
54	phenyl	6.8	5067	nd	nd
55	4-methylphenyl	0.7	441	nd	nd
56	4-chlorophenyl	1.4	506	−18 ± 6	−593 (6)
57	4-bromophenyl	1.6	461	−14 ± 3	−445 (6)
58	4-ethylphenyl	2.3	1264	nd	nd
59	4-isopropyl-phenyl	3.6	2474	nd	nd

[a]IC_{50} values as determined in binding assays using membranes of Chinese hamster ovary (CHO) cells containing either the recombinant ET_A or ET_B receptor, respectively. Data are given as geometric means of at least three independent measurements. [b]Maximal mean arterial blood pressure reduction as extracted from the moving average over 6 h. [c]Area between curve (ABC) as calculated from the blood pressure recordings 24 h before and after administration of 3 mg/kg of the compound to conscious hypertensive Dahl salt-sensitive rats, max. ΔMAP = maximal reduction in mean arterial blood pressure; n = number of animals in the experiment, nd = not determined. [d]For the structure of compound **1**, see Figure 1.

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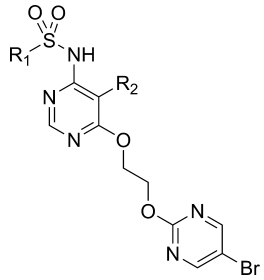
Table 6. Optimization of the Sulfamide Moiety[a]



compd	R_1	R_2	IC_{50} ET_A[b] [nM]	IC_{50} ET_B[b] [nM]	max ΔMAP[c] [mmHg]	ABC[d] [mm Hg·h] (*n*)
57	benzyl-NH−	4-bromophenyl	1.6	461	−14 ± 3	−445 (6)
60	methyl-NH−	4-bromophenyl	1.7	812	−21 ± 6	−1045 (5)
61	ethyl-NH−	4-bromophenyl	0.8	830	−20 ± 10	−598 (6)
17	*n*-propyl-NH−	4-bromophenyl	0.5	391	−25 ± 6	−922 (6)
62	*n*-butyl-NH−	4-bromophenyl	0.3	158	−16 ± 4	−443 (5)
63	cyclopropyl-NH−	4-bromophenyl	4.4	1474	nd	nd
64	*N*-benzyl-methyl-N−	4-bromophenyl	4.3	5208	nd	nd
65	morpholino	4-bromophenyl	2.8	2829	nd	nd
66	*n*-propyl-NH−	4-chlorophenyl	1.2	784	nd	nd
67	*n*-propyl-NH−	3-methoxyphenoxy	1.9	468	−15 ± 3	−391 (6)
68	*n*-propyl-NH−	6-chloro-3-methoxyphenoxy	0.4	208	−21 ± 5	−500 (6)
69	*n*-propyl	4-bromophenyl	2.2	3058	−25 ± 4	−1116 (6)
70	*n*-butyl	4-bromophenyl	1.1	2424	−22 ± 3	−734 (5)

[a]Note that compounds **17**, **57**, and **60**−**68** represent sulfamides, while compounds **69** and **70** are sulfonamides. [b]IC_{50} values as determined in binding assays using membranes of Chinese hamster ovary (CHO) cells containing either the recombinant ET_A or ET_B receptor, respectively. Data are given as geometric means of at least three independent measurements. [c]Maximal mean arterial blood pressure reduction as extracted from the moving average over 6 h. [d]Area between curve (ABC) as calculated from the blood pressure recordings 24 h before and after administration of 3 mg/kg of the compound to conscious hypertensive Dahl salt-sensitive rats; max ΔMAP = maximal reduction in mean arterial blood pressure; *n* = number of animals in the experiment; nd = not determined.

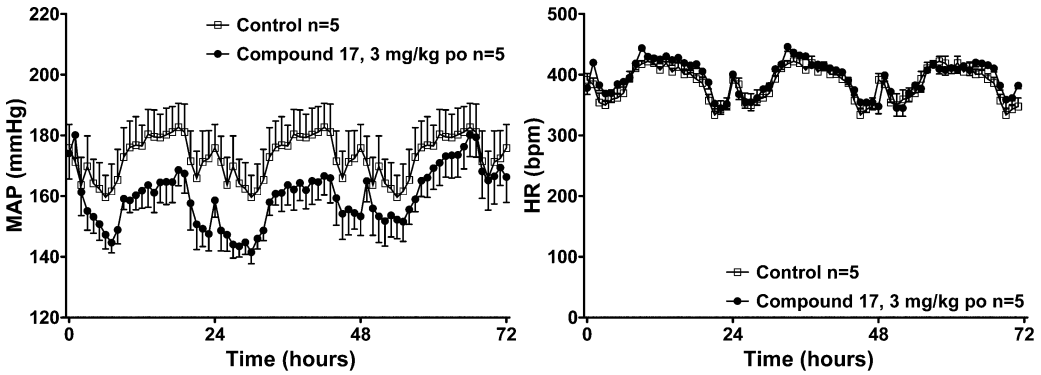


Figure 3. Mean arterial blood pressure (MAP, left) and heart rate (HR, right) recordings in conscious hypertensive Dahl salt-sensitive rats before (control) and after oral administration of 3 mg/kg of compound **17**. Results are expressed as mean ± SEM.

As apparent from the in vivo data given in Table 5, compounds incorporating a 3-methoxyphenoxy (**52**), 2-chloro-5-methoxyphenoxy (**53**), a 4-chlorophenyl (**56**), or a 4-bromophenyl (**57**) substituent at position 5 of the core pyrimidine clearly exceeded the in vivo activity of compound **1**. At this point, we decided to evaluate sulfamides with a 4-bromophenyl substituted pyrimidine scaffold in more detail. Several examples prepared for this study are listed in Table 6. For the ET_A receptor, the 4-bromophenyl derivatives reproduce the relative SAR observed with the corresponding 2-methoxyphenoxy analogues (Table 3) on a higher potency level (e.g., **39** vs **60**, **40** vs **61**, **44** vs **65**), and several compounds reach affinities below 1 nM. With respect to ET_B, the relative SAR between the 4-bromophenyl derivatives and the 2-methoxyphenoxy-analogues is again similar. However, this time the bromo-phenyl derivatives in Table 6 are often less potent when compared to their analogues in Table 3 (e.g., **38** vs **57**, **43** vs **64**). Notable exceptions are compounds **17** and **62**, which both retain their ET_B affinity.

When we tested the sulfamides of Table 6 in our rat model of hypertension, compounds incorporating a rather small alkyl sulfamide showed excellent in vivo activity (e.g., **60**, **61**, **17**). The propyl-sulfamide **17** emerged as a particularly interesting compound as it combined high in vivo efficacy with high potency on ET_B. We therefore extended our study to the three propyl sulfamides incorporating a 4-chlorophenyl (**66**), a 3-methoxyphenoxy (**67**), or a 6-chloro-3-methoxyphenoxy (**68**) substituent in position 5 of the core pyrimidine (Table 6). The in vitro activity of compound **66** was inferior to the one of compound **17** as it was clearly less potent on ET_B. While the 3-

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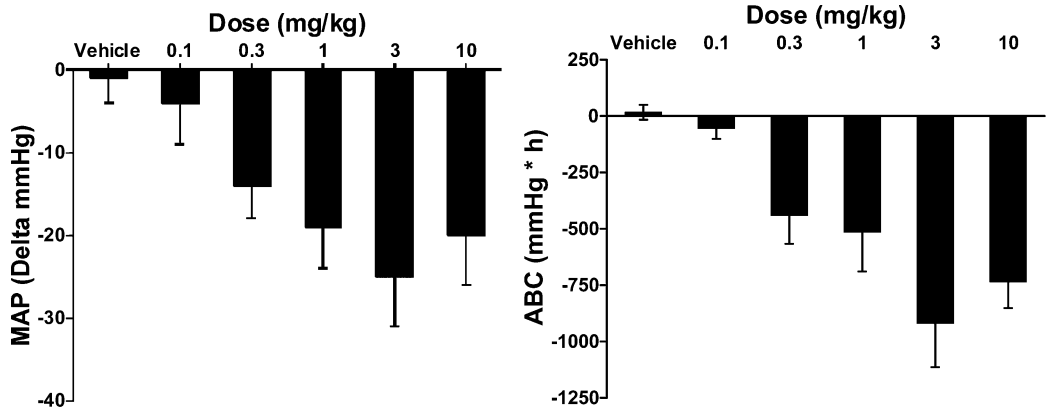

Figure 4. Dose–response curve of mean arterial blood pressure (MAP) reduction and area between curves (ABC) in hypertensive Dahl salt-sensitive rats after oral administration of compound **17** in 5% arabic gum. Results are expressed as mean ± SEM.

methoxyphenoxy derivative **67** was only slightly less potent than **17**, the chloro substituted analogue **68** was more potent on ET_B. However, both compounds **67** and **68** showed a clearly lower activity in vivo. A comparison of sulfamide **17** with sulfonamides **69** and **70** confirmed our earlier finding that the sulfamides present a significantly higher affinity for the ET_B receptor. In our context, the sulfonamides **69** and **70** are therefore clearly less attractive despite their high in vivo activity in the Dahl salt-sensitive rat model of hypertension (Table 6).

In brief, the *n*-propylsulfamide derivative **17**, later named macitentan,[62] fulfilled the requirements we set forth at the beginning of our discovery program. Sulfamide **17** is highly potent on the ET_A receptor with significant affinity for the ET_B receptor and maximally reduces mean arterial blood pressure for at least 60 h at an oral dose of 3 mg/kg (Figure 3). Following a dose–response experiment, maximal reduction of mean arterial blood pressure by −25 ± 6 mmHg was reached with a dose of 3 mg/kg (Figure 4). No change in heart rate was observed over the range of 1–100 mg/kg in conscious Dahl salt-sensitive rats (Figure 3, data not shown). In a multiple dose experiment, Dahl salt-sensitive rats were treated for five consecutive days with 1 mg/kg/day of **17** (Figure 5). Mean arterial blood pressure was stably reduced by −20 mmHg over the whole treatment period and returned to baseline about 48 h after the last dose was given. No signs of tachyphylaxis were observed, and no rebound effect manifested at the end of the study as blood pressure values returned to baseline.

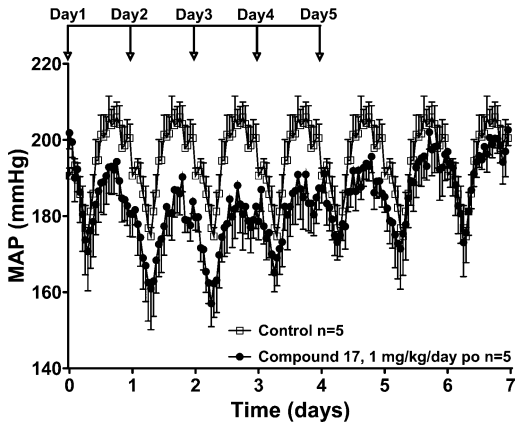

Figure 5. Mean arterial blood pressure (MAP) recordings of a repeated dose experiment administering 1 mg/kg/day of compound **17** over five consecutive days to hypertensive Dahl salt-sensitive rats equipped with telemetry (ABC = 1842 mmHg·h). Results are expressed as mean ± SEM.

In view of the excellent pharmacodynamic behavior of compound **17**, it came as no surprise that **17** also showed favorable pharmacokinetic properties (Table 7). In an iv experiment with male Wistar rats, the compound showed a low plasma clearance of 6.7 mL/min·kg translating into a half-life of 5.1 h. The volume of distribution reached 1.8 L/kg, indicating that the compound is distributing well into tissues. At an oral dose of 10 mg/kg, compound **17** reached a peak plasma concentration of 1670 ng/mL at 6 h after administration. Oral bioavailability was 69%. A similar pharmacokinetic behavior was found in the dog, emphasizing the compound's attractiveness for further development. In the dog, compound **17** was rapidly (T_{max} = 2 h) and almost completely (F = 87%) absorbed and reached plasma concentrations close to 5000 ng/mL after oral administration of 10 mg/kg. Clearance and half-life are comparable in rat and dog.

In the course of our PK studies we discovered that compound **17** is metabolized to the depropylated sulfamide **71** (Figure 6). This compound represents a major metabolite reaching plasma exposure levels 4–5 times higher than that of **17**. Sulfamide **71** binds to the ET_A and ET_B receptors with IC_{50} values of 3.4 and 987 nM, respectively and therefore clearly adds to the pharmacological activity of the parent compound **17**.[62] As we learned later, metabolite **71** is also formed in humans.[63] Interestingly, oral administration of 10 mg/kg of the butyl analogue **62** to Wistar rats also led to significant plasma concentrations (C_{max} = 4100 ng/mL at 6 h after administration) of sulfamide **71**.

In 10.9% of the patients treated with compound **1**, a transient, dose dependent increase in serum liver aminotransferase levels of >3 times the upper limit of normal is observed.[64−66] Mechanistically, this observation has been attributed to the ability of compound **1** to inhibit bile salt transport in hepatocytes, eventually leading to intrahepatic cholestasis.[37] Minimizing the interaction of a potential follow-up compound with hepatic bile acid transport has therefore been a further goal of our optimization efforts. Several compounds have been assessed for their potential to interact with bile salt transport in a rat model,[37] and compound **17** showed no sign of bile salt transport inhibition in this model (data not shown).

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Table 7. PK Parameters of 17 in Male Wistar Rats and Male Beagle Dogs

species	iv dose[a] [mg/kg]	AUC_{0-inf} [ng h/mL]	CL [mL/min·kg]	Vss [L/kg]	$t_{1/2}$ [h]	oral dose[b] [mg/kg]	AUC_{0-inf} [ng h/mL]	C_{max} [ng/mL]	T_{max} [h]	F [%]
Wistar rat (n = 5)	1	2680 ± 730	6.7 ± 2.1	1.8 ± 0.9	5.1 ± 1.9	10	18500 ± 8100	1670 ± 371	6.0	69
beagle dog (n = 3)	1	3170 ± 520	5.4 ± 1.0	0.9 ± 0.2	4.1 ± 0.4	10	27500 ± 6500	4590 ± 2540	2.0	87

[a]Wistar rat: solution in 50 mM aqueous tris(hydroxymethyl)methylamine (Tris) pH 8.3 buffer containing 45% PEG400; beagle dog: solution in 25% *N*-methylpyrrolidone (NMP), 25% PEG400, 50% 50 mM aqueous Tris pH 8.3 buffer. [b]Wistar rat: suspension in 7.5% aqueous modified gelatin; beagle dog: solution in PEG400.

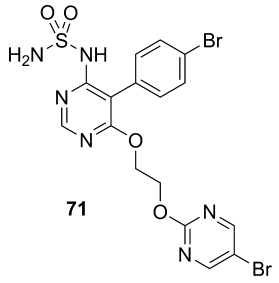

Figure 6. Stucture of sulfamide **71**, a major metabolite of compound **17** in Wistar rats and Beagle dogs.

CONCLUSIONS

In conclusion, starting from the structure of compound **1**, we have discovered a novel series of highly potent ET receptor antagonists. While these novel compounds are still based on a pyrimidine core, the *tert*-butyl benzene sulfonamide part of compound **1** has been replaced by a series of simple alkyl sulfamide moieties. When compared to the corresponding sulfonamides, these novel sulfamides are characterized by an enhanced affinity for the ET_B receptor, rendering them a particularly interesting class of novel ET receptor antagonists. In addition, many of these sulfamides show high oral efficacy in reducing arterial blood pressure in conscious hypertensive Dahl salt-sensitive rats. In vivo dealkylation of the alkyl sulfamide moiety in compounds **17** and **62**, for instance, to the pharmacologically active sulfamide **71** is another interesting feature of the sulfamide class as it leads to sustained pharmacological activity.

Sulfamide **17** emerged as a highly attractive candidate for further studies and can be characterized as a potent ET_A receptor antagonist with significant affinity for the ET_B receptor. In rat and dog models, the compound is well absorbed, distributes well into tissue, and is slowly cleared, suggesting that it is suitable for once daily dosing in humans. Recently published data on a phase I clinical trial with compound **17** confirm these expectations.[63] In hypertensive Dahl salt-sensitive rats, **17** efficiently and dose dependently reduced the arterial blood pressure. More detailed studies with sulfamide **17** on its tissue penetration and ability to block the ET_A and ET_B receptor in vivo have been described in a separate publication.[62] In a rat model, compound **17** did not increase plasma bile salt concentrations. Compound **17** successfully completed a phase III clinical trial for pulmonary arterial hypertension recently.[67] A phase IIIb clinical trial for digital ulcers[68,69] and a phase I/Ib trial for recurrent glioblastoma[70] are currently ongoing.

EXPERIMENTAL SECTION

Chemistry. All reagents and solvents were used as purchased from commercial sources (Sigma-Aldrich Switzerland, Lancaster Synthesis GmbH, Germany, Acros Organics USA). Moisture sensitive reactions were carried out under an argon atmosphere. Progress of the reactions was followed either by thin-layer chromatography (TLC) analysis (Merck, 0.2 mm silica gel 60 F_{254} on glass plates) or by LC-MS. LC-MS: UltiMate 3000 RS pump, UltiMate 3000 column compartment (40 °C), UltiMate 3000 RS DAD, MSQplus (all Dionex, Switzerland), Sedex 85 ELSD (Sedere, VWR Germany). Column: Zorbax SB-AQ, 5 μm, 120 Å, 4.6 mm × 50 mm (Agilent). Gradient: 5−95% acetonitrile in water containing 0.04% of trifluoroacetic acid, within 1 min. Flow: 4.5 mL/min. Or LC-MS*: Waters Micromass; ZMD-platform with ESI-probe with Alliance 2790 HT. Colum: Gromsil ODS4, 3 μm, 120 Å; 2 mm × 30 mm. Gradient: 0−100% acetonitrile in water containing 0.05% formic acid, within 6 min. Flow: 0.45 mL/min; t_R is given in min. Purity of the target compounds was confirmed on two additional columns: on a Waters Atlantis T3, 5 μm, 4.6 mm × 30 mm, eluting with a gradient of 5−95% of acetonitrile in water containing 0.04% of trifluoroacetic acid; and on a Waters XBridge C18, OBD, 5 μm, 4.6 mm × 50 mm (Waters, Switzerland), eluting with a gradient of 5−95% of acetonitrile in water containing 13 mM of NH_3. According to these three LC-MS analyses, all compounds showed a purity >95% (UV at 230 nm), and about 80% of the compounds showed a purity >98%. Purity and identity was further confirmed by NMR spectroscopy. HR-LC-MS: analytical pump, Waters Acquity Binary, Solvent Manager. MS: SYNAPT G2MS, source temperature 150 °C, desolvatation temperature 400 °C, desolvatation gas flow 400 L/h. Cone gas flow: 10 L/h. Extraction cone: 4 RF. lens: 0.1 V. Sampling cone: 30. Capillary: 1.5 kV. High resolution mode. Gain: 1.0. MS function: 0.2 s per scan, 120−1000 amu in full scan, centroid mode. Lock spray: leucine enkephalin 2 ng/mL (556.2771 Da) scan time 0.2 s with interval of 10 s and average of five scans. DAD: Acquity UPLC PDA detector. Column: Acquity UPLC BEH C18 1.7 μm 2.1 mm × 50 mm from Waters, thermostatted in the Acquity UPLC column manager at 60 °C. Eluents: water + 0.05% formic acid; B, acetonitrile + 0.05% formic acid. Gradient: 2−98% B over 3.0 min. Flow: 0.6 mL/min. Detection: UV 214 nm and MS, t_R is given in min. Prep HPLC: Waters XBridge Prep C18, 5 μm, OBD, 19 mm × 50 mm, gradient of acetonitrile in water containing 0.4% of formic acid, flow 75 mL/min. Melting points were determined by differential scanning calorimetry (DSC) using a DSC822e/400 instrument and the HSS7 sensor from Mettler Toledo, Switzerland. The sample is weighed in a 40 μL aluminum crucible which is open under a flow of 15 mL/min of nitrogen during the experiment. The scan goes from 20 to 320 °C at a rate of 5 K/min. The peak temperature of the melting endotherm was determined with the STARe software and was assigned to the melting point (mp). NMR spectroscopy: NMR spectra were recorded on a Varian Mercury 300VX spectrometer (^{1}H 300 MHz, ^{13}C 75 MHz) or Bruker Avance II, 400 MHz UltraShield, ^{1}H (400 MHz), ^{13}C (100 MHz); chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintuplet), h (hextet), hept (heptuplet) or m (multiplet). X-ray diffraction: Crystals of **17** were mounted on a Bruker Nonius diffractometer equipped with a CCD detector and reflections were measured using monochromatic Mo Kα radiation. The structure was solved by direct methods using SIR92, refinement was performed with CRYSTALS. Full matrix least-squares refinement was performed with anisotropic temperature factors for all atoms except hydrogen which were included at calculated positions with isotropic temperature factors. Coordinates,

anisotropic temperature factors, bond lengths, and angles will be deposited with the Cambridge Crystallographic Data Centre.

Endothelin Receptor Inhibition. Binding assays were performed in 96-well plates with CHO membranes containing the recombinant ET_A or ET_B receptors in 200 μL of 50 mM Tris-HCl, 25 mM $MnCl_2$, 1 mM EDTA pH 7.4, 0.5% BSA, 2.5% DMSO. The wells containing 0.5 μg/well of ET_A membranes or 0.2 μg/well of ET_B membranes were incubated for 2 h at RT with 15 pM [^{125}I]ET-1 (10000 cpm) in the presence or absence of test substances. Minimum and maximum binding was derived from samples with and without 1 μM of unlabeled ET-1, respectively. After the 2 h incubation period, the membranes were filtered through GF/C filterplates (MultiScreen MAHFC 1H60 from Millipore), which had been pre-equilibrated with binding buffer, using cold 50 mM Tris HCl, 0.1% BSA pH 7.5. After drying of the filterplates, 25 μL of scintillation cocktail (MicroScint 20, Perkin-Elmer) were added to each well and the filterplates were counted in a microplate counter (TopCount, Canberra Packard S.A. Zürich, Switzerland). IC_{50} values were calculated as the concentration of antagonist inhibiting 50% of the specific binding of ET-1. Two reference compounds were tested: BQ-123, a specific ET_A antagonist, and sarafotoxin 6c (Bachem, Switzerland), a specific ET_B antagonist. For these reference inhibitors, the following IC_{50} values were found (geometric mean values): ET_A mb, 20.5 nM (n = 13) for BQ-123 and >100 nM (n = 10) for Sarafotoxin; ET_B mb, 0.208 nM (n = 12) for Sarafotoxin and >1000 nM (n = 12) for BQ-123.

Telemetric in Vivo Studies.[59−61] In vivo efficacy of the compounds was assessed by oral administration of 3 mg/kg of the compound suspended in 5% arabic gum to female hypertensive Dahl salt-sensitive rats equipped with a telemetric system recording the arterial blood pressure and heart rate. For both of these parameters, each rat served as its own control by using the data of the last 24 h before treatment. An area between curve (ABC) was calculated between the control and the treatment period to assess the compound's efficacy. Maximal mean arterial blood pressure reduction was extracted from the moving average over 6 h of the blood pressure recordings.

Pharmacokinetics in the Rat. The pharmacokinetic profile was determined in the male Wistar rat (n = 5). For this purpose, compound **17** was formulated as a solution in 50 mM aqueous tris(hydroxymethyl)methylamine (Tris) pH 8.3 buffer containing 45% PEG400 for intravenous administration at a dose of 1.0 mg/kg, and as a microsuspension in 7.5% aqueous modified gelatin for oral dosing at 10 mg/kg. Blood samples were taken at regular time intervals over a period of 24 h from a preimplanted catheter, and plasma was generated by centrifugation at 4000 rpm at 4 °C. Plasma concentrations were determined using a specific and sensitive LC-MS/MS method with a limit of quantification of 1.5 ng/mL.

Pharmacokinetics in the Dog. The pharmacokinetic profile of compound **17** was determined in male beagle dogs (n = 3). The compound was administered orally at a dose of 10 mg/kg as a solution in PEG400 and intravenously at a dose of 1 mg/kg as a solution in 25% *N*-methylpyrrolidone (NMP), 25% PEG400, 50% 50 mM aqueous Tris pH 8.3 buffer.

Potassium (Phenethylsulfonyl)amide (4).[71] To a vigorously stirred solution of *N*-chlorosuccinimide (38.6 g, 289 mmol) in dichloromethane (300 mL) and water (150 mL), 2-phenyl-ethyl-mercaptane (10.0 g, 72.3 mmol) was added. The mixture became slightly warm (30 °C). The resulting suspension was stirred at rt for 3 h. The solvent was decanted, and the remaining slurry was extracted twice with dichloromethane (2 × 200 mL). The organic extracts were washed twice with satd aq $NaHCO_3$ solution and water, combined, dried over $MgSO_4$, filtered, and concentrated. The residue was suspended in heptane:ethyl acetate 10:1, filtered, and washed with additional ethyl acetate/hexane. The filtrate was concentrated, and the crude product was purified by colum chromatography on silica gel eluting with heptane:ethyl acetate 10:1 to give 2-phenyl-ethane sulfonylchloride (8.25 g, 56%) as a pale-beige oil; R_f (silica gel, heptane:ethyl acetate 10:1) 0.32. This material (8.25 g, 40.3 mmol) was dissolved in THF (50 mL), and a solution of satd aq NH_3 (10 mL) was slowly added at −20 °C. The mixture was stirred at rt for 16 h before it was neutralized with 25% aq HCl. The organic solvent was evaporated, and the remaining residue was diluted with water (100 mL) and extracted four times with ethyl acetate (4 × 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give 2-phenyl-ethane sulfonamide (6.21 g, 83%) as a pale-yellow solid. LC-MS: t_R = 0.58 min, $[M + H]^+$ = not detectable. To a solution of this material (6.21 g, 33.5 mmol) in methanol (60 mL), potassium *tert*-butylate (3.76 g, 33.5 mmol) was added. The mixture was stirred until a clear solution formed. The mixture was concentrated and the remaining residue was triturated with diethyl ether, filtered, and dried to give **4** (6.67 g, 89%) as a white powder. ^{1}H NMR (DMSO-d_6): δ 7.18−7.32 (m, 5 H), 3.05−3.11 (m, 2 H), 2.93−2.99 (m, 2 H). ^{13}C NMR (DMSO-d_6): δ 140.1, 129.0, 128.8, 126.7, 56.8, 30.9.

4,6-Dichloro-5-(2-methoxyphenoxy)-2,2′-bipyrimidine (3). Prepared as described in the literature.[39,41] LC-MS: t_R = 0.79 min, $[M + H]^+$ = 349.04. ^{1}H NMR ($CDCl_3$): δ 9.05 (d, J = 4.9 Hz, 2 H), 7.47 (t, J = 4.9 Hz, 1 H), 7.13 (td, J_t = 8.0 Hz, J_d = 1.5 Hz, 1 H), 7.00 (dd, J_1 = 8.3 Hz, J_2 = 1.5 Hz, 1 H), 6.90 (td, J_t = 8.0 Hz, J_d = 1.4 Hz, 1 H), 6.79 (dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz, 1 H), 3.85 (s, 3 H). ^{13}C NMR ($CDCl_3$): δ 160.2, 158.2, 156.6, 155.5, 149.3, 145.0, 144.6, 125.0, 121.8, 121.0, 116.9, 113.0, 56.2.

***N*-(6-Chloro-5-(2-methoxyphenoxy)-[2,2′-bipyrimidin]-4-yl)-2-phenylethane-sulfonamide (5).** A solution of **3** (2.20 g, 6.30 mmol) and **4** (3.10 g, 13.86 mmol) in DMF (50 mL) was stirred at rt for 16 h. About half of the solvent was removed under reduced pressure before the mixture was diluted with diethyl ether (50 mL). A 10% aqueous solution of citric acid (50 mL) was added. The beige suspension that formed was filtered, the collected solid was washed with water (30 mL) and diethyl ether (30 mL), and dried under high vacuum at 50 °C to give **5** (2.79 g, 89%) as a beige powder. LC-MS: t_R = 0.89 min, $[M + H]^+$ = 497.81. ^{1}H NMR (DMSO-d_6): δ 8.99 (d, J = 4.9 Hz, 2 H), 7.64 (t, J = 4.9 Hz, 1 H), 7.20−7.26 (m, 4 H), 7.14−7.20 (m, 1 H), 7.10 (d, J = 8.0 Hz, 1 H), 7.02 (t, J = 7.3 Hz, 1 H), 6.83 (t, J = 7.5 Hz, 1 H), 6.61 (s br, 1 H), 3.83 (s, 3 H), 2.82−2.97 (m, 2 H).

***N*-(6-(2-Hydroxyethoxy)-5-(2-methoxyphenoxy)-[2,2′-bipyrimidin]-4-yl)-2-phenyl-ethanesulfonamide (7).** At rt, potassium *tert*-butoxide (6.88 g, 6.03 mmol) was added portionwise to a solution of **5** (304 mg, 0.60 mmol) in ethylene glycol (10 mL) and DME (2 mL). The reaction mixture was heated to reflux for 17 h and then cooled to rt before being poured into 10% aq citric acid solution (25 mL). The mixture was extracted twice with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with water (2 × 50 mL) and brine (50 mL), dried over Na_2SO_4, filtered, and evaporated in vacuo to give sufficiently pure **7** as an off-white solid to be used without further purification. LC-MS: t_R = 0.89 min, $[M + H]^+$ = 524.05. ^{1}H NMR ($CDCl_3$): δ: 3.21−3.28 (m, 2 H), 3.91−3.95 (m, 2 H), 3.98 (s, 3 H), 4.29−4.38 (m, 2 H), 4.66−4.70 (m, 2 H), 6.95 (t, J = 7.8 Hz, 1 H), 7.03 (d, J = 7.3 Hz, 1 H), 7.13−7.30 (m, 7 H), 7.42 (t, J = 4.8 Hz, 1 H), 8.48 (s br, 1 H), 8.93−8.99 (m, 2 H).

***N*-(6-(2-((5-Bromopyrimidin-2-yl)oxy)ethoxy)-5-(2-methoxyphenoxy)-[2,2′-bipyrimidin]-4-yl)-2-phenylethanesulfonamide (8).** To a suspension of NaH (29 mg, 0.72 mmol, 60% in mineral oil) in THF (2 mL) and DMF (2 mL), alcohol **7** (150 mg, 0.29 mmol) was added. The mixture was stirred at rt for 5 min before 2-chloro-5-bromopyrimidine (138 mg, 0.72 mmol) was added. The mixture was stirred at 70 °C for 4 h before another portion of 2-chloro-5-bromopyrimidine (138 mg, 0.72 mmol) and NaH (29 mg, 0.72 mmol) was added. Stirring was continued at 70 °C for 22 h before another portion of 2-chloro-5-bromopyrimidine (138 mg, 0.72 mmol) and NaH (29 mg, 0.72 mmol) was added. Stirring was continued for further 3 h before the mixture was cooled to rt and poured into 10% aq citric acid solution (75 mL). The mixture was extracted twice with ethyl acetate (2 × 50 mL). The combined extracts were washed with 10% aq citric acid solution (50 mL) and concentrated. The crude product was purified on prep TLC plates (silica gel, 0.5 mm) using ethyl acetate:methanol:7 N NH_3 in methanol 8:2:1 to give **8** (84 mg, 43%) as a pale yellow solid; R_f (silica gel, ethyl acetate:methanol:7 N NH_3 in methanol 8:2:1) 0.68. LC-MS: t_R = 0.93 min, $[M + H]^+$ = 680.01. ^{1}H NMR ($CDCl_3$) (2 conformers 1:2): δ 8.88−8.96 (m, 2 H), 8.49 (s, 1.33 H), 8.43 (s, 0.67 H), 7.50 (t, J = 5.0 Hz, 0.33 H), 7.35−

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7.42 (m, 0.67 H), 7.16−7.27 (m, 3.33 H), 7.01−7.15 (m, 2 H), 6.88−6.98 (m, 1.34 H), 6.79−6.87 (m, 1.67 H), 6.72−6.78 (m, 0.33 H), 5.02−5.07 (m, 0.67 H), 4.96−5.02 (m, 1.33 H), 4.72−4.77 (m, 0.67 H), 4.66−4.71 (m, 1.33 H), 4.18−4.34 (m, 1.33 H), 3.96 (s, 2 H), 3.90 (s, 1 H), 3.11−3.25 (m, 1.33 H), 2.91−3.00 (m, 0.67 H), 2.66−2.73 (m, 0.67 H). ^{13}C NMR ($CDCl_3$) (2 conformers): δ 163.48, 163.47, 161.9, 161.8, 159.5, 159.4, 157.7, 157.6, 157.5, 155.9, 150.5, 149.7, 149.4, 146.7, 145.8, 138.6, 137.7, 128.58, 128.55, 128.4, 128.2, 127.3, 126.7, 126.2, 125.2, 122.9, 122.5, 121.5, 121.2, 120.8, 115.3, 113.0, 112.4, 112.1, 112.0, 66.1, 66.0, 65.1, 64.9, 56.5, 56.0, 55.8, 55.5, 29.7, 29.6.

Potassium (*N*-Propylsulfamoyl)amide (13). Chlorosulfonyl isocyanate (**9**, 28.3 g, 0.2 mol) was dissolved in DCM (100 mL) and cooled to 0 °C before a solution of *tert*-BuOH (18.8 mL; 0.2 mol) in DCM (100 mL) was added over 30 min. Stirring was continued without cooling for additional 30 min. The mixture was then carefully added over 1 h to a solution of *n*-propylamine (16.5 mL, 0.2 mol) and triethylamine (42 mL, 0.3 mol) in DCM (400 mL) at 0 °C. Stirring was continued without cooling for additional 16 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in EtOAc (1 L) and washed with water (100 mL) and brine (40 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give **11**. ^{1}H NMR ($CDCl_3$): δ 0.97 (t, J = 7 Hz, 3 H), 1.51 (s, 9 H), 1.62 (m, 2 H), 3.04 (q, J = 6 Hz, 2 H), 5.12 (t br, J = 5 Hz, 1 H), 7.17 (s br, 1 H). ^{13}C NMR ($CDCl_3$): δ 11.6, 22.8, 28.4, 46.0, 84.1, 150.3. The residue was dissolved in dioxane (100 mL) followed by the addition of HCl in dioxane (4M; 250 mL). Stirring was continued for 4 h at rt. The solvent was evaporated under reduced pressure, and the residue was dried under HV to give sulfamide **12**. ^{1}H NMR (DMSO-d_6; 300 MHz; Varian): δ 0.87 (t, 3H), 1.43 (m, 2H), 2.81 (q, 2H), 3.09 (m, 1H; NH), 6.42 (s, 2H; NH_2). ^{13}C NMR($CDCl_3$): δ 11.6, 23.1, 45.7. The residue was dissolved in MeOH (200 mL), and KOtBu (22.4 g; 0.2 mol) was added. The solvent was removed under reduced pressure. The residue was taken up in diethylether (500 mL), and the precipitate was filtered off, washed with cold ether, and dried at HV to give potassium (*N*-propylsulamoyl)amide (**13**) (32.4 g, 92%) as an off-white powder.

5-(4-Bromophenyl)-4,6-dichloropyrimidine (14).[42] To a solution of 4-bromophenylacetic acid (73.9 g, 0.34 mol) in methanol (400 mL), thionyl chloride (49.05 g, 30 mL, 0.41 mol) was added dropwise while the temperature of the reaction mixture was kept at 0−5 °C. Upon complete addition, the cooling was removed and the mixture was allowed to warm to rt. Stirring was continued for 3 h before the solvent was removed in vacuo. The yellow oil was dissolved in benzene and evaporated. The residue was dissolved in ethyl acetate and washed with brine, 2 N aq Na_2CO_3, and again brine. The organic extract was dried over $MgSO_4$ and evaporated and dried under high vacuum at 85 °C for 30 min to give 4-bromophenylacetic acid methyl ester (74.9 g, 95%) as a yellow oil. LC-MS: t_R = 0.93 min, $[M + H]^+$ = not detectable. ^{1}H NMR (DMSO-d_6): δ 3.60 (s, 3H), 3.67 (s, 2H), 7.22 (d, J = 8.5, 2H), 7.50 (d, J = 8.5, 2H). ^{13}C NMR ($CDCl_3$): δ 40.9, 52.5, 121.4, 131.2, 131.9, 133.1, 171.6.

NaH (24.0 g, 0.82 mol, 60% in mineral oil) was suspended twice in THF (75 mL), and the solvent was decanted. The remaining slurry was diluted with THF (500 mL) and heated to 35−40 °C. A solution of 4-bromophenylacetic acid methyl ester (74.9 g, 0.33 mol) in THF (200 mL) was carefully added dropwise. Gas evolution started after about half of the phenylacetic acid ester had been added and the mixture became slightly warm. The addition was continued at 45 °C. Upon completion of the addition, stirring was continued at 40 °C for 5 min before the mixture was allowed to cool to rt. The evolution of gas had stopped before dimethylcarbonate (117.9 g, 1.31 mol) was added dropwise. Evolution of gas started, and the mixture became warm (32 °C). Upon completion of the addition, stirring of the mixture was continued for 24 h before it was cooled to 0 °C and acidified by adding 25% aq HCl (100 mL) and diluted with water (150 mL). The organic solvent was removed under reduced pressure, and the remaining aqueous mixture was extracted three times with ethyl acetate (3 × 200 mL). The organic extracts were washed with 1 N aq HCl and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was crystallized from diethyl ether/hexane. The solid material was collected, washed with cold diethyl ether/hexane, and dried under high vacuum to give 2-(4-bromophenyl)-malonic acid dimethyl ester (74.1 g, 79%) as beige crystals; mp 76−77 °C; R_f (silica gel, heptane:ethyl acetate 1:1) 0.7, shows decomposition. LC-MS: this compound is not stable under HPLC conditions. ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 6 H), 4.61 (s, 1 H), 7.25−7.30 (m, 2 H), 7.46−7.51 (m, 2 H). ^{13}C NMR ($CDCl_3$): δ 53.4, 57.3, 122.9, 131.2, 131.7, 132.0, 168.2.

A solution of 2-(4-bromophenyl)-malonic acid dimethyl ester (11.73 g, 40.9 mmol) in methanol (100 mL) was added at 0 °C to a solution of sodium (2.83 g, 0.118 mmol) in methanol (100 mL). The mixture was stirred for 18 h at rt before formamidine hydrochloride (4.10 g, 50.9 mmol) was added. The suspension was stirred at rt for 4 h. The solvent was removed, and the residue was suspended in 10% aq citric acid (100 mL) and stirred for 10 min. The white precipitate was collected, washed with 10% aq citric acid, water, evaporated three times from cyclohexane, and dried under high vacuum at 40 °C to give 5-(4-bromophenyl)-pyrimidine-4,6-diol (9.90 g, 91%) as a pale-beige powder; R_f (silica gel, heptane:ethyl acetate 1:1) at start. LC-MS: t_R = 0.52 min, $[M + H]^+$ = 267.08. ^{1}H NMR (DMSO-d_6): 7.43−7.48 (m, 2 H), 7.50−7.55 (m, 2 H), 8.13 (s, 1 H), 12.1 (s br, 2 H). ^{13}C NMR (DMSO-d_6): δ 101.4, 119.3, 130.6, 132.9, 133.2, 148.7, 163.2.

To a suspension of 5-(4-bromophenyl)-pyrimidine-4,6-diol (43.0 g, 0.16 mmol) in $POCl_3$ (500 mL) was carefully added *N*,*N*-dimethylaniline (50 mL). The mixture was heated to 130 °C for 2 h. The dark-brown solution was evaporated, and the residue was dissolved in dichloromethane (200 mL). Water (500 mL) was slowly added, and the mixture was stirred at rt for 30 min before the organic phase was separated. The aqueous phase was extracted two more times with dichloromethane. The organic extracts were washed with water and 1 N aq HCl, treated with charcoal, dried over $MgSO_4$, filtered, and evaporated. The product was purified by crystallization from 2-propanol. The crystals were collected, washed with cold 2-propanol and dried under high vacuum to give **14** (39.8 g, 81%) as beige crystals; mp 100−101 °C; R_f (silica gel, heptane:ethyl acetate 1:1) 0.79. LC-MS: t_R = 0.92 min, $[M + H + CH_3CN]^+$ = 344.0. ^{1}H NMR ($CDCl_3$): δ 8.81 (s, 1 H), 7.68 (dt, J_d = 8.9 Hz, J_t = 2.4 Hz, 2 H), 7.22 (dt, J_d = 9.0 Hz, J_t = 2.4 Hz, 2 H). ^{13}C NMR ($CDCl_3$): δ 124.1, 131.0, 131.7, 132.3, 157.1, 161.4.

***N*-5-(4-Bromophenyl)-6-chloro-4-pyrimidinyl-*N*′-propylsulfamide (15).** To a solution of **14** (760 mg; 2.50 mmol) in DMSO (5 mL), the sulfamide potassium salt **13** (529 mg; 3.00 mmol) was added. The mixture was stirred at rt for 48 h, diluted with brine (25 mL), and extracted three times with ethyl acetate (3 × 25 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was crystallized form methanol to give **15** (706 mg, 69%) as a white solid; R_f (silica gel, heptane:ethyl acetate 1:1) 0.49. LC-MS: t_R = 0.89 min, $[M + H]^+$ = 404.98. ^{1}H NMR ($CDCl_3$): δ 0.96 (t, J = 7.6 Hz, 3 H), 1.50−1.66 (m, 2 H), 2.94−3.03 (m, 2 H), 5.52 (s br, 1 H), 6.90 (s br, 1 H), 7.16−7.21 (m, 2 H), 7.68−7.74 (m, 2 H), 8.65 (s, 1 H). ^{13}C NMR ($CDCl_3$): δ 11.6, 22.7, 46.1, 118.6, 125.0, 128.9, 131.2, 133.6, 156.6, 157.0, 159.9.

***N*-5-(4-Bromophenyl)-6-(2-hydroxyethoxy)-4-pyrimidinyl-*N*′-propylsulfamide (16).** Ethylene glycol (58.7 g, 53 mL; 946 mmol) was dissolved in dimethoxyethane (80 mL), and KOtBu (8.80 g; 78 mmol) was added. The temperature rose to 40 °C. After stirring for 10 min, monochloride **15** (10.55 g; 26 mmol) was added and stirring was continued at 100 °C for 70 h. The reaction mixture was carefully added to a 1:1 mixture of water:10% aq citric acid solution. The product was extracted with ethyl acetate (2 × 120 mL), the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduce pressure. The crude product was purified by flash column chromatography (silicagel, heptanes:ethyl acetate 1:1) to give alcohol **16** (9.65 g, 86%) as a white solid; R_f (silica gel, heptane:ethyl acetate 1:1) 0.20. LC-MS: t_R = 0.88 min, $[M + H]^+$ = 430.98. ^{1}H NMR ($CDCl_3$): δ 0.95 (t; J = 7.6 Hz, 3 H), 1.60 (h, J = 7.6 Hz, 2 H), 2.98 (t, J = 7.0 Hz, 2 H), 3.82−3.86 (m, 2 H), 4.47−4.51 (m, 2 H), 5.70 (s br, 1 H), 7.13−7.22 (m, 2 H), 7.61−7.67 (m, 2 H), 8.48 (s, 1 H). ^{13}C NMR ($CDCl_3$): δ 11.6, 22.7, 46.1, 61.9, 69.8, 104.8, 123.8, 128.1, 131.6, 133.2, 155.9, 156.4, 166.6.

***N*-[5-(4-Bromophenyl)-6-[2-[(5-bromo-2-pyrimidinyl)oxy]-ethoxy]-4-pyrimidinyl]-*N*′-propylsulfamide (17).** Sodium hydride (1.67 g, 69.6 mmol, 55% in mineral oil) was suspended twice in hexane (10 mL), and the hexane was discarded. The remaining slurry was suspended in THF (200 mL), and **16** (10.0 g, 23.2 mmol) was added in portions. The mixture was stirred for 15 min and diluted with DMF (40 mL) before 5-bromo-2-chloropyrimidine (5.38 g; 27.8 mmol) was added. The reaction mixture was heated to 60 °C, and stirring was continued for 2 h. The mixture was poured into 10% aq citric acid solution (250 mL), and the product was extracted twice with ethyl acetate (2 × 300 mL). The combined organic extracts were washed twice with water (2 × 200 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by crystallization from methanol (100 mL) to give **17** (11.99 g, 88%) as a white powder; mp 135−136 °C; R_f (silica gel, heptane:ethyl acetate 1:1) 0.44. LC-MS: t_R = 0.79 min, $[M + H]^+$ = 588.86 (major isotope). HR-LC-MS: t_R = 1.96 min; $(m + H)/z$ = 586.9711, found = 586.9714. ^{1}H NMR ($CDCl_3$): δ 8.51 (s, 2 H), 8.49 (s, 1 H), 7.58−7.63 (m, 2 H), 7.16−7.21 (m, 2 H), 6.88 (s, 1 H), 5.61 (t, J = 6.2 Hz, 1 H), 4.72−4.76 (m, 2 H), 4.62−4.66 (m, 2 H), 2.99 (q, J = 6.8 Hz, 2 H), 1.61 (h, J = 7.3 Hz, 2 H), 0.97 (t, J = 7.4 Hz, 3 H). ^{13}C NMR ($CDCl_3$): δ 11.6, 22.7, 46.1, 65.3, 65.9, 104.8, 112.4, 123.7, 128.0, 131.7, 133.0, 155.7, 156.4, 159.7, 163.5, 166.3.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details on synthesis and characterization of the sulfamide and 4,6-dichloropyrimidine building blocks and the target compounds **8** and **17**−**71**. Two X-ray single crystal structures of compound **17** crystallized from different solvents. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The corresponding data sets have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, United Kingdom, http://www.ccdc.cam.ac.uk/, under the following deposition numbers: 888522 (crystals from methanol), 888506 (crystals from EtOAc/hexane).

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +41 61 565 65 65. Fax: + 41 61 565 65 00. E-mail: martin.bolli@actelion.com.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ABC, area between curves; CHO, Chinese hamster ovary; CSD, Cambridge Structural Database; ELSD, evaporative light scattering detector; ET, endothelin; ET_A, endothelin A receptor; ET_B, endothelin B receptor; EtOAc, ethyl acetate; KOtBu, potassium *tert*-butoxide; MAP, mean arterial pressure; SEM, standard error of the mean

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