Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 951–954

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Bis-sulfonamides as Endothelin Receptor Antagonists

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Received 25 October 2002; revised 2 December 2002; accepted 3 December 2002

**Abstract**—Modification of the structure of bosentan **1**, the first marketed endothelin receptor antagonist (Tracleer™), by introduction of a second sulfonamide function at the alkoxy side chain, led to bis-sulfonamides **2**. This allowed to prepare dual $ET_A/ET_B$ as well as $ET_B$ receptor selective antagonists, which could serve as tools to investigate the pharmacological consequences of selective $ET_B$ receptor blockade.


The endothelins (ET-1, ET-2 and ET-3) are very potent endogenous vasoconstrictors and have been intensively investigated for their biological effects.[1,2] Together with the recently discovered urotensin II,[3] ET-1 is one of the most potent vasoconstrictors identified in humans. The endothelin peptides interact with two membrane bound G-protein coupled receptors, termed $ET_A$ and $ET_B$,[4,5] to exert their biological effects. $ET_A$ receptors, which selectively bind ET-1, are located on vascular smooth muscle cells and mediate vasoconstriction and proliferative responses. $ET_B$ receptors which bind all three endothelins, are located on endothelial cells, vascular smooth muscle cells and fibroblasts where they mediate vasodilation, vasoconstriction and fibrosis, respectively. The endothelin system has been implicated in the pathogenesis of a broad range of diseases characterized by vasoconstriction, including pulmonary arterial hypertension (PAH),[6] congestive heart failure,[7] acute and chronic renal failure,[8] and angina.[9] Recent results from clinical trials with the dual $ET_A/ET_B$-antagonist bosentan **1** (Tracleer™) clearly showed the beneficial effects of endothelin system blockade for patients suffering from PAH. A broad variety of non-peptide ET receptor antagonists have been described[10] and used as pharmacological tools to enhance the understanding of the physiological relevance of the endothelin system.[11] The choice of dual antagonists such as bosentan (**1**) for development in PAH is based on the upregulation of $ET_B$ receptors in pathological situations and their importance in mediating the detrimental effects of ET such as vasoconstriction, fibrosis, cell proliferation and aldosterone release. However, no results of large clinical trials with $ET_A$ selective antagonists in cardiovascular indications are yet available. In the treatment of melanoma it has been indicated that selective blockade of the $ET_B$ receptor subtype can have beneficial effects.[12]

With bosentan **1** as the lead structure (Fig. 1) we found new derivatives (**2**) exhibiting closely related structural features but containing an additional sulfonamide functionality. The introduction of the second sulfonamide unit leads to a selectivity pattern of compounds **2** compared to bosentan **1** and its analogues[14] and allows a certain tailoring of receptor subtype selectivity, especially for the $ET_B$ receptor. Therefore this class of ET receptor antagonists could serve as a source of pharmacological tools to investigate the physiological effects of a selective $ET_B$ receptor blockade with regard to future novel therapeutic applications.

The general synthetic pathway to the bis-sulfonamide derivatives is outlined in Scheme 1.[13] Preparation of the

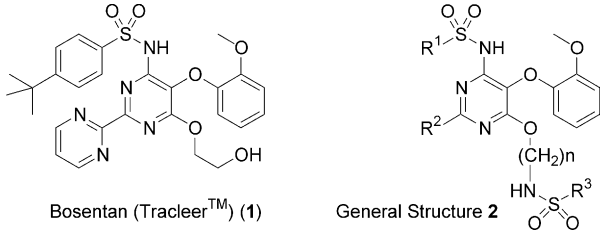


**Figure 1.**

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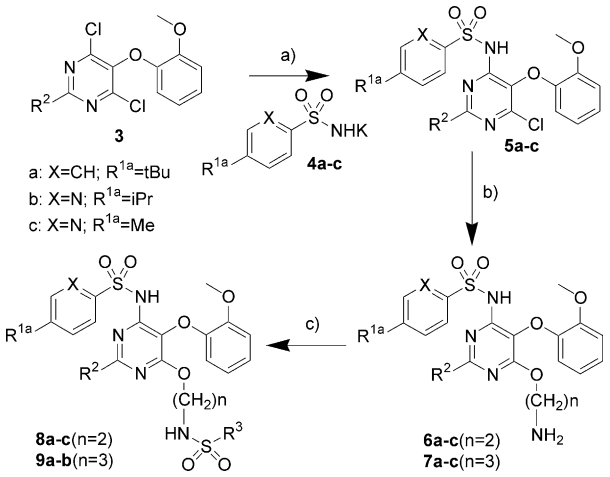
doi:10.1016/S0960-894X(02)01083-1




**Scheme 1.** Synthesis of the bis-sulfonamides **8a–c/9a–b**: (a) **4a–c**, DMSO, rt, 12–24 h; (b) NaH, HO–$(CH_2)n$-$NH_2$, THF/DMF, 0 °C to rt; (c) $R^3$-$SO_2$–Cl, $CH_2Cl_2$, base, rt, 5–12 h.

dichloro-pyrimidine precursors **3** was accomplished via a classical pyrimidine synthesis as described in the literature.[14] Compounds **3** were reacted with the sulfonamide potassium salts **4a–c**[14] in DMSO at room temperature to give the mono-chloro precursors **5a–c** which could be further transformed to compounds **6a–c** ($n=2$)/**7a–c** ($n=3$) by reaction with the selectively *O*-deprotonated corresponding aminoalcohol. Preparation of the final bis-sulfonamide compounds **8a–c** ($n=2$)/**9a–b** ($n=3$) was achieved by reaction of **6a–c** ($n=2$)/**7a–c** ($n=3$) with sulfonyl chlorides in dichloromethane or chloroform in the presence of a base like Hünig's base, triethylamine, or DBU. All compounds were characterized by TLC, LC–MS and $^1H$ NMR.

$IC_{50}$ values were determined in a radio-ligand binding assay against $^{125}I$-ET-1 using CHO cells stably expressing human $ET_A$ or $ET_B$ receptors.[17] Of selected compounds in vitro functional inhibitory potencies were determined: potency ($pA_2$) for prevention of ET-1 induced constriction of rat aortic rings ($ET_A$ receptors) and of sarafotoxin S6c induced constriction of rat tracheal rings ($ET_B$ receptors).[17] The results are displayed in Tables 1–3. The compounds are sorted into three groups according to their sulfonamide substituent in position 4 of the central pyrimidine ring.

In the first group, bearing a 4-*tert*-butylphenylsulfonamide unit in position 4 of the pyrimidine core (Table 1), compounds containing a cyclopropyl group in position 2 of the central pyrimidine (e.g., **10**, **13**, **15** and **17**) were usually more potent than the corresponding compounds having a 2-pyrimidinyl substituent in this position (**11**, **12**, **14**, **16** and **18**). We also prepared compounds with a

**Table 1.** 4-*t*-Butylphenylsulfonamides: inhibition of [$^{125}I$]-ET-1 binding to membranes of CHO-cells expressing the human $ET_A$ or $ET_B$ receptor ($IC_{50}$) and functional inhibitory potency ($pA_2$) preventing the ET-1 induced contraction of rat aortic rings

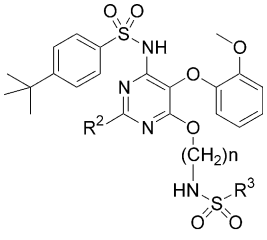
| | Compd | $R^2$ | $R^3$ | $n$ | $IC_{50}$ (nM) | | $pA_2$ | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $ET_A$ | $ET_B$ | $ET_A$ | $ET_B$ |
|  | **10** | Cyclopropyl | Ethyl | 2 | 119 | 323 | | |
| | **11** | 2-Pyrimidinyl | Ethyl | 2 | 487 | 873 | | |
| | **12** | 2-Pyrimidinyl | 2-Thienyl | 2 | 64 | 16 | 6.08 | 7.15 |
| | **13** | Cyclopropyl | 4-Tolyl | 2 | 4900 | 299 | | |
| | **14** | 2-Pyrimidinyl | 4-Tolyl | 2 | 8390 | 420 | | |
| | **15** | Cyclopropyl | Ethyl | 3 | 48 | 33 | 6.52 | |
| | **16** | 2-Pyrimidinyl | Ethyl | 3 | 1591 | 101 | | |
| | **17** | Cyclopropyl | 2-Thienyl | 3 | 14 | 2 | 6.16 | 7.95 |
| | **18** | 2-Pyrimidinyl | 2-Thienyl | 3 | 86 | 4 | | |
| **8a**(n=2) / **9a**(n=3) | | | | | | | | |

**Table 2.** 5-iso-Propylpyridylsulfonamides: inhibition of [$^{125}I$]-ET-1 binding to membranes of CHO-cells expressing the human $ET_A$ or $ET_B$ receptor ($IC_{50}$) and functional inhibitory potency ($pA_2$) preventing the ET-1 induced contraction of rat aortic rings

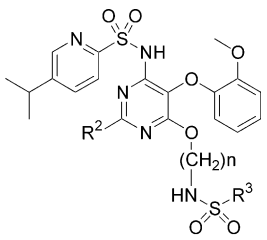
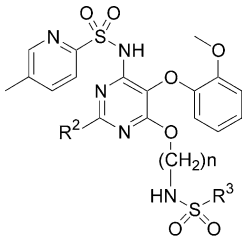
| | Compd | $R^2$ | $R^3$ | $n$ | $IC_{50}$ (nM) | | $pA_2$ | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $ET_A$ | $ET_B$ | $ET_A$ | $ET_B$ |
|  | **19** | Cyclopropyl | Ethyl | 2 | 50 | 397 | 6.46 | 6.57 |
| | **20** | Cyclopropyl | Propyl | 2 | 80 | 84 | 6.54 | 6.44 |
| | **21** | Cyclopropyl | 4-Tolyl | 2 | 1050 | 81 | | |
| | **22** | Trimethoxyphenyl | Propyl | 2 | 261 | 24 | 5.50 | 7.48 |
| | **23** | Trimethoxyphenyl | 2-Thienyl | 2 | 1317 | 7 | | 7.41 |
| | **24** | Trimethoxyphenyl | 4-Tolyl | 2 | 5115 | 4 | < 5 | 7.46 |
| | **25** | Morpholinyl | Ethyl | 2 | 19 | 122 | 7.02 | 6.36 |
| | **26** | Morpholinyl | Propyl | 2 | 140 | 103 | 6.08 | 6.62 |
| | **27** | Morpholinyl | 2-Thienyl | 2 | 587 | 100 | | |
| | **28** | Morpholinyl | 4-Tolyl | 2 | 1385 | 9 | | 7.3 |
| | **29** | Cyclopropyl | Ethyl | 3 | 45 | 103 | 6.98 | 6.57 |
| | **30** | Cyclopropyl | Propyl | 3 | 82 | 15 | 6.61 | 7.16 |
| | **31** | Cyclopropyl | 2-Thienyl | 3 | 22 | 4 | 6.92 | 8.04 |
| | **32** | Cyclopropyl | 4-Tolyl | 3 | 77 | 19 | 6.51 | 7.31 |
| | **33** | Morpholinyl | 4-Tolyl | 3 | 116 | 24 | 5.32 | 6.97 |
| **8b**(n=2) / **9b**(n=3) | | | | | | | | |

**Table 3.** 5-Methyl-2-pyridylsulfonamides: inhibition of [$^{125}$I]-ET-1 binding to membranes of CHO-cells expressing the human $ET_A$ or $ET_B$ receptor ($IC_{50}$) and functional inhibitory potency ($pA_2$) preventing the ET-1 induced contraction of rat aortic rings

| | Compd | $R^2$ | $R^3$ | $n$ | $IC_{50}$ (nM) | | $pA_2$ | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $ET_A$ | $ET_B$ | $ET_A$ | $ET_B$ |
|  | **34** | Cyclopropyl | Ethyl | 2 | 582 | 5660 | | |
| | **35** | Cyclopropyl | Propyl | 2 | 1320 | 4670 | | |
| | **36** | Cyclopropyl | 2-Thienyl | 2 | 1850 | 8240 | | |
| | **37** | 4-Pyridyl | Ethyl | 2 | 185 | 5402 | | |
| | **38** | 4-Pyridyl | 2-Thienyl | 2 | 2838 | 3676 | | |
| | **39** | 4-Pyridyl | 4-Tolyl | 2 | 2193 | 1537 | | |
| | **40** | Trimethoxyphenyl | 2-Thienyl | 2 | 2770 | 351 | | |
| | **41** | Piperonyl | 2-Thienyl | 2 | 2190 | 2110 | | |
| | **42** | Morpholinyl | Propyl | 2 | 51 | 931 | 6.61 | 5.58 |
| | **43** | Morpholinyl | 2-Thienyl | 2 | 724 | 447 | | |

**8c**(n=2)

morpholinyl-, piperazinyl-, piperonyl-, methyl- or methylsulfanyl-group or a hydrogen atom in position 2 (not depicted in Table 1) which were substantially less potent in receptor binding assays. With respect to the substituent at the second sulfonamide unit and the length $n$ of the spacer, it can be seen that $R^3$ = ethyl gives compounds with moderate to good activity with a slight preference for the $ET_A$ receptor if $n = 2$ (e.g., **10** and **11**). This trend is inversed by the introduction of a longer spacer $n = 3$ (e.g., **15** and **16**) and more pronounced in case $R^2$ = 2-pyrimidinyl although the receptor affinity is in general better with $R^2$ = cyclopropyl. Replacement of the ethyl group by a 2-thienyl group in position $R^3$ gives rise to more potent receptor antagonists (e.g., **12**) already slightly selective for the $ET_B$ receptor with $n = 2$. Introduction of the longer spacer ($n = 3$) in combination with $R^3$ = 2-thienyl yields compounds **17**, with a 7-fold selectivity for the $ET_B$ receptor, and **18**, exhibiting 24-fold selectivity for the $ET_B$ receptor, with $IC_{50}$ values in the low nM range. With respect to functional inhibitory potency, the $ET_B$-selectivity of compound **17** is close to 200, as reflected in the $pA_2$ values. Increasing the size of the substituent $R^3$ does not increase the $ET_B$-selectivity of the compounds but leads to a substantial loss of activity towards both receptors as reflected by comparison of compounds **12** versus **14** and **10** versus **13**. Introduction of a methyl-, trifluoromethyl-, isopropyl-, 4-methoxyphenyl-, 4-bromophenyl- or 2-pyridyl group as $R^3$ always gives less active ET receptor antagonists (not depicted in Table 1).

Table 2 gives an overview on the compounds with a 5-isopropyl-2-pyridyl group as the sulfonamide substituent in position 4 of the central pyrimidine. It can be seen that the best binding affinities towards the $ET_A$ receptor (e.g., **25**, **29** and **31**) are in the same range (10 nM < $IC_{50}$ < 50 nM) as those of compounds **15** and **17** (Table 1) having a 4-*tert*-butyl-phenyl group instead of the 5-isopropyl-2-pyridyl group. Within the group of compounds with the ethyl spacer ($n = 2$), selectivity towards the $ET_B$ receptor strongly depends on the nature of the substituent in position 2 of the central pyrimidinyl unit. Increasing the size of this substituent from cyclopropyl (**19**, **20**, **21**) to morpholinyl (**25**, **26**, **27**, **28**) and finally to 3,4,5-trimethoxyphenyl (**22**, **23**, **24**) leads to highly potent and selective $ET_B$ antagonists. This is also reflected in the $pA_2$ values of compounds **22**, **23** and **24** in the functional assay using rat tracheal rings. In all three groups of compounds with $R^2$ = cyclopropyl, morpholinyl, and 3,4,5-trimethoxyphenyl and $n = 2$, the influence of $R^3$ always exhibits the same tendencies: smaller groups like ethyl allow some affinity towards the $ET_A$ receptor (e.g., **19** and **25**), whereas increasing the size of substituent $R^3$ from propyl (e.g., **22**) to 2-thienyl (e.g., **23**) or 4-tolyl (e.g., **24**) leads to a loss of affinity for the $ET_A$ receptor and simultaneously a gain of affinity for the $ET_B$ receptor. Increasing the length of the spacer between the central pyrimidine and the second sulfonamide unit by an additional $CH_2$ group leads to receptor antagonists exhibiting a flat SAR with respect to $ET_A$ affinity. In this group, $ET_B$ affinity seems to be less depending on the nature of the substituent $R^2$, for example **32** ($R^2$ = cyclopropyl) and **33** ($R^2$ = morpholinyl) both gave $IC_{50}$ values in the range of 20 nM. The group of compounds with $R^2$ = cyclopropyl and $n = 3$ (**29**, **30**, **31**, **32**) show the same SAR tendency for $ET_B$ activity as already observed in Table 1. The best compound, **31**, bearing the 2-thienyl-group, exhibits $pA_2$ values of 6.92 ($ET_A$) and 8.04 ($ET_B$) which indicate the potential within the group of bis-sulfonamide ET receptor antagonists.

In Table 3, the 5-isopropyl-2-pyridyl sulfonamide is replaced by a 5-methyl-2-pyridyl sulfonamide. The $IC_{50}$ values both towards the $ET_A$ and $ET_B$ receptor are substantially lower as of comparable derivatives depicted in Tables 1 and 2. Compounds containing a 5-methyl-2-pyridyl sulfonamide unit in position 4 of the central pyrimidine are described to be $ET_A$ selective compared to compounds with a 4-*tert*-butyl-phenyl sulfonamide in this position.[15,16] Since we had potent and selective $ET_B$ receptor antagonists, we intended to increase the activity towards the $ET_A$ receptor by the introduction of the 5-methyl-2-pyridyl sulfonamide. However, the concept valid for ET receptor antagonists with small substituents in position 6 of the central pyrimidine core described earlier was not applicable to our bis-sulfonamide ET receptor antagonist series.[10,15,16]

The introduction of a second sulfonamide group in structures related to bosentan **1** led to a new class of ET receptor antagonists: the bis-sulfonamides **2**. The newly discovered, additionally functionalized group of compounds allows the preparation of receptor antagonists which are highly active (e.g., **17**) and selective towards the $ET_B$ receptor (e.g., **23** and **24**, with a binding affinity ratio in favour of the $ET_B$ receptor of 188 and 1278, respectively). The bis-sulfonamide ET receptor antagonists can serve as tools to further investigate the pharmacological consequences of potent and selective $ET_B$ receptor blockade and help to gain additional insight in the function of the $ET_B$ receptor in pathological situations. Further investigations should lead to in-depth pharmacological profiling of the best compounds out of this series.

## Acknowledgements

The authors would like to thank Walter Schmutz, Pascal Rebmann, Josiane Rein, Celine Boukhadra and Helene Hettrich for their excellent thechnical support and Prof. Henri Ramuz for helpful discussions.

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