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DISCOVERY OF RO 48-5695: A POTENT MIXED ENDOTHELIN RECEPTOR ANTAGONIST OPTIMIZED FROM BOSENTAN

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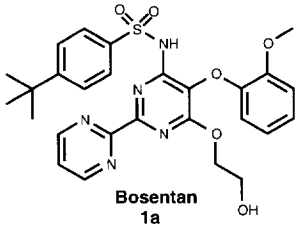
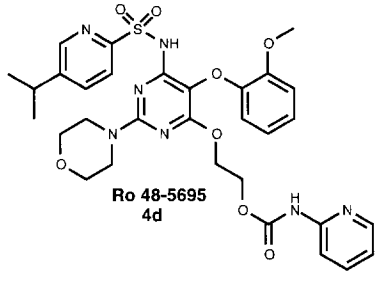
Abstract: Implementation of a pyridylcarbamoyl group and an isopropylpyridylsulfonamide substituent as key components in the scaffold of Bosentan resulted in the identification of the potent orally active endothelin receptor antagonist Ro 48-5695. It shows affinities for ET_A and ET_B receptors in the low nanomolar range and high functional antagonistic potency *in vitro*. © 1997 Elsevier Science Ltd.

Introduction

Since the discovery of the endothelins as a new class of vasoconstrictive and mitogenic peptides in 1988,[1] endothelin-1 (ET-1) has attracted considerable scientific interest for its extremely potent and long-lasting vasoconstrictor effect and its binding to G protein-coupled receptors, pharmacologically and structurally described as ET_A and ET_B receptor subtypes.[2] We have elucidated the pathophysiological significance of the endothelin regulatory system in different experimental models,[3] and in humans[4] under conditions of systemic vasoconstriction, using the first orally active nonpeptidic endothelin receptor antagonist Ro 46-2005[3] and the improved derivative Bosentan **1a**,[5] which is in clinical development. Both belong to a distinct class of functionalized arylsulfonamido pyrimidines.[6] Since then, several other structurally different nonpeptidic endothelin receptor antagonists have been discovered showing mixed or ET_A receptor-selective profiles.[7]





Rationale

Due to recent evidence showing the contribution not only of ET_A but also of ET_B[8] receptors in mediating vasoconstriction, efforts were directed to synthesize potent orally active antagonists with a balanced receptor subtype selectivity profile. Optimization programmes were based on the Bosentan scaffold and guided by a 3-D model of antagonist-receptor interaction.[9] Work was focussed on: (i) functionalization of the hydroxy-

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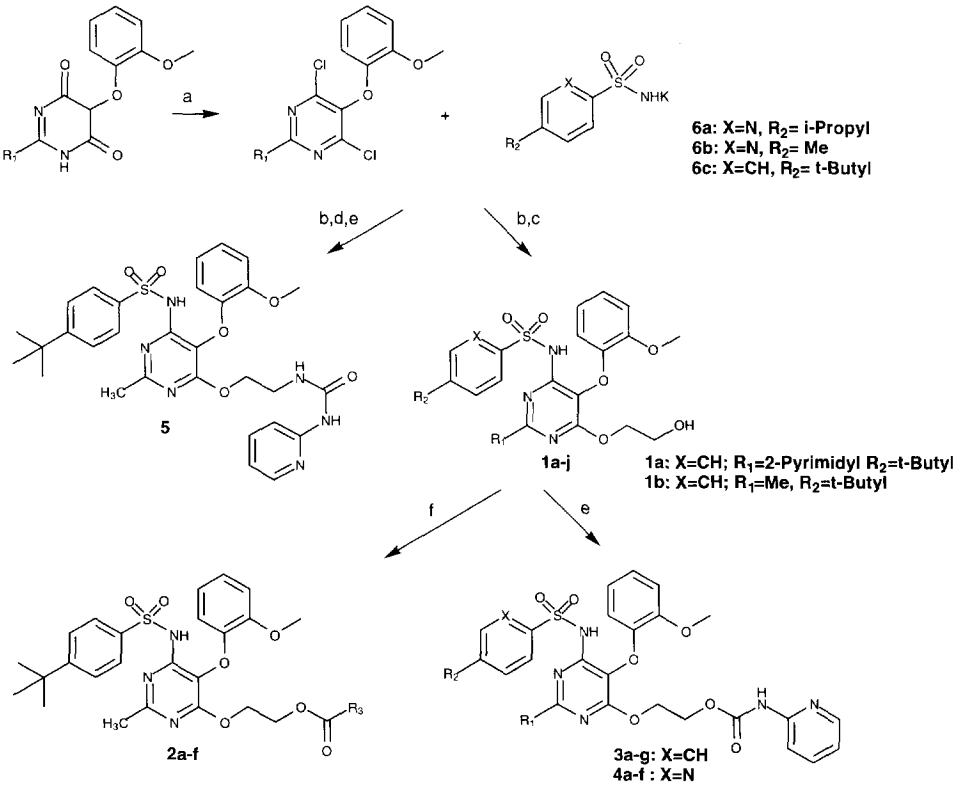
ethoxy substituent incorporated in the 6-position of the central pyrimidine ring of Bosentan - to identify novel substructures with additional binding sites to the receptors; (ii) modification of the size and lipophilicity of the substituents in the 2- and 4-positions of the central pyrimidine - to refine potency and selectivity profiles. The different stages of chemical optimization which led to the identification of Ro 48-5695 (**4d**) are described, together with *in vitro* activity profiles for key compounds.

Chemistry

The general synthesis of the trifunctionalized (hetero)arylsulfonamido pyrimidines **2a-f**, **3a-g**, **4a-f** and **5** of Table 1-3 is outlined in Scheme 1. Preparation of the intermediates **1a-j** was accomplished essentially as already described for Bosentan (**1a**)[5] and followed a classical approach of pyrimidine synthesis: accordingly, appropriate carboxamidines were condensed with (2-methoxyphenoxy)malonic acid diethyl ester to yield the corresponding 2-substituted tetrahydropyrimidine-4,6-diones which were further converted to the respective 4,6-dichloro derivatives on treatment with $POCl_3/PCl_5$, Scheme 1.

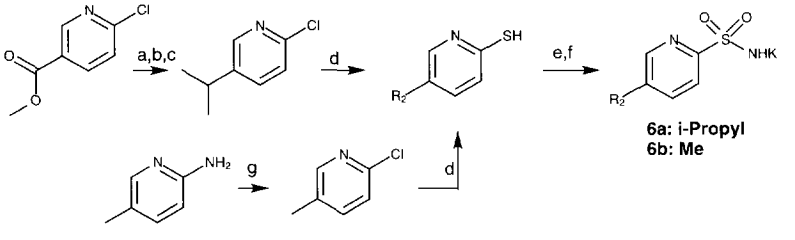
Scheme 1 Synthesis of the trifunctionalized (hetero)arylsulfonamido pyrimidines



Reagents: (a) $POCl_3/PCl_5$, reflux; (b) DMSO, rt; (c) Na / $HOCH_2CH_2OH$,100°C; (d) Na / $HOCH_2CH_2NH_2$,100°C; (e) pyridine-2-carbonyl-azide (3 eq), DMAP (0.3 eq), toluene,100°C, (f) carboxylic ester formation: BOP,TEA, CH_3CN; 2-, 3- and 4-pyridylcarbamates as for (e)

The (hetero)arylsulfonamides **6a-c** were introduced as potassium salts in DMSO followed by treatment with sodium glycolate in ethylene glycol to give pyrimidines **1a-j**. Subsequent (hetero)arylcarbamic ester formation was performed by treatment with the corresponding (heteroaryl)carbonyl azides[10] in toluene at 100°C, via *in situ* Curtius rearrangement to the respective isocyanates, to give **2d-f**, **3a-g** and **4a-f**, Table 1-3. (The carboxylic ester derivatives **2a-c**, Table 1, were prepared from **1b** and the corresponding carboxylic acids with BOP as coupling reagent in acetonitrile.) The synthetic route towards the (2-pyridyl)ureido derivative **5**, Scheme 1, was essentially as for **3a-g, 4a-f** - but with sodium in ethanolamine replacing sodium glycolate in the reaction sequence. The pyridylsulfonamides **6a,b** were prepared according to Scheme 2 starting from 6-chloronicotinic acid methyl ester and 2-amino-5-methylpyridine, respectively. After conversion to the corresponding 2-chloropyridine derivatives as shown below, the mercapto group was introduced using thiourea in refluxing hydrochloric acid. Subsequent oxidation with Cl_2 in aqueous acetic acid gave the corresponding pyridylsulfonylchlorides as crystals which were directly treated with aqueous ammonia to give the sulphonamides; formation of the potassium salts with potassium t-butylate as a base then gave **6a,b**.

Scheme 2 Preparation of the pyridylsulfonamides 6a-b



Reagents: (a) CH_3MgBr, Et_2O, reflux, 95%; (b) p-TosOH (0.05 eq), xylene, reflux, 85%; (c) H_2, Pt/C (5%), EtOH, 92%; (d) $CS(NH_2)_2$, 25%HCl, 20h reflux, 90% for R_1=i-Propyl and for R_1=Me; (e) Cl_2, aq. AcOH, 15°C, isolation of crystals then 25% aq. NH_3/THF, 5°C, 91% for R_1=i-Propyl, 87% for R_1=Me; (f) KOtBu, MeOH, rt, quant., (g) methyl nitrite, H_2O/HCl, 15°C, 82%

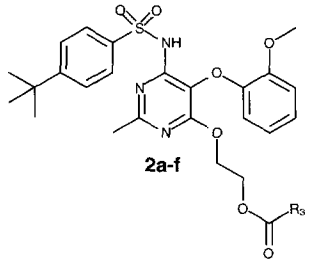
Biological *in vitro* assays

Receptor binding affinities (IC_{50} values) were determined in a radio-ligand binding assay against ^{125}I- ET-1 with membranes prepared from human placenta - for ET_B - and with recombinant human ET_A receptor expressed in baculovirus-infected Sf9 or CHO cells as described.[5] *In vitro* functional inhibitory potencies were determined in two different set-ups: (i) 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[5] (ET_B receptors);
(ii) potency (IC_{50}) to inhibit ET-1 induced intracellular calcium mobilization in HEK-293 cells, co-transfected with human recombinant ET_A or ET_B receptors and with aequorea victoria aequorin as reporter gene.[11]

Results - Structure-activity relationships

The binding affinities of selected compounds derived from **1b** by introducing different groups to the hydroxy-ethoxy moiety are shown in Table 1.

Table 1. Identification of the (2-pyridyl)carbamoyl substructure

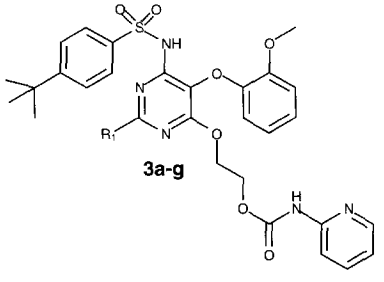


2a-f

Compd.	R_3	IC_{50} (μM) ET_A(Sf9)	ET_B
1b		1.5	0.07
5		0.08	1.24
2a	CH_2NHCH_3	1.7	0.3
2b	3-furyl	1.1	1.0
2c	3-pyridyl	0.88	0.77
2d	**(2-pyridyl)-NH**	**0.006**	**0.12**
2e	(3-pyridyl)-NH	0.9	0.7
2f	(4-pyridyl)-NH	1.9	2.8

This effort resulted in the identification of the (2-pyridyl)carbamoyl substructure incorporated in **2d** which gives rise to 250-fold improvement of binding affinity for ET_A receptors over the non-functionalized intermediate **1b**. The exact positioning of the nitrogen in this substructure was found to be crucial for high affinity binding as demonstrated by the largely reduced ET_A receptor affinities of the (3- and 4-pyridyl)-carbamoyl derivatives **2e,f** (185- and 300-fold, compared to **2d**). The (2-pyridyl)ureido analogue **5**, Scheme 1, showed reduced affinity for ET_A receptors in comparison to **2d** (13-fold), as well as the carboxylic ester derivatives **2a-c**. This indicates a particular role of the (2-pyridyl)carbamoyl group in providing additional binding contacts to the ET_A receptor. The functional antagonistic activity of **2d**, measured on rat aortic rings, was found to be only slightly improved over Bosentan (pA_2: 7.5 for **2d** vs. pA_2: 7.3 for **1a**). Subsequently, different substituents were introduced to the 2-position of the central pyrimidine ring (R_1), to investigate their effects on functional potency and to obtain antagonists with high affinity also for ET_B receptors.

Table 2. Selected compounds with different substituents at R_1

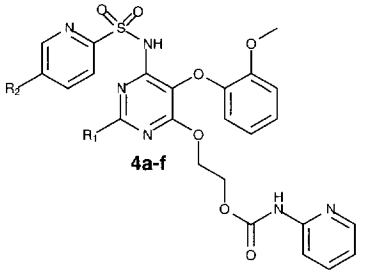


3a-g

Compd.	R_1	IC_{50} (μM) ET_A (Sf9)	ET_B	pA_2 (ET_A)
3a	propyl	0.009	0.063	7.9
3b	i-propyl	0.007	0.16	7.8
3c	t-butyl	0.014	0.144	7.6
3d	cyclopropyl	0.007	0.06	7.7
3e	2-thienyl	0.03	0.2	8.0
3f	**2-pyrimidyl**	**0.006**	**0.043**	9.0
3g	**4-morpholino**	**0.017**	**0.019**	8.0

Implementation of heterocyclic substituents instead of alkyl groups led to further improved compounds, particularly with respect to functional activities, Table 2. This might be due to increased lipophilicity of these derivatives giving rise to higher tissue penetration. Compound **3f** - incorporating the 2-pyrimidyl substituent of Bosentan - showed the highest activity in this series, whereas the morpholino derivative **3g** gave the most balanced receptor subtype selectivity profile, combined with high functional potency. Further modifications of the arylsulfonamide group, to modulate its lipophilicity and acidity via nitrogen insertion and substitution at R_2, led to the identification of Ro 48-5695 (**4d**), Table 3.

Table 3 Selected compounds incorporating an alkylpyridylsulfonamide group

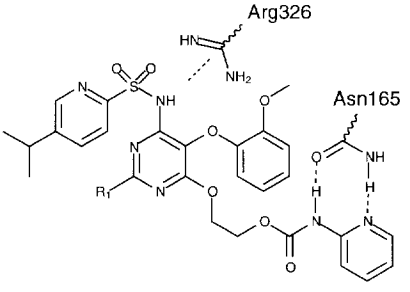


Compd.	R_1	R_2	IC_{50} (μM) ET_A (Sf9)	ET_B	pA_2 (ET_A)
4a	2-pyrimidyl	CH_3	0.004	0.17	9
4b	2-pyrimidyl	$CH(CH_3)_2$	0.004	0.049	10
4c	4-morpholino	CH_3	0.0002	0.025	9.7
4d	**4-morpholino**	**$CH(CH_3)_2$**	**0.0003**	**0.005**	**9.3**
4e	4-thiomorpholino	$CH(CH_3)_2$	0.001	0.014	8.8
4f	4-piperidino	$CH(CH_3)_2$	0.003	0.025	8.3

Introduction of the isopropylpyridylsulfonamide group to position 4 of the central pyrimidine gave **4b** and **4d** with further improved binding affinities and functional antagonistic activities for ET_A receptors. The derivatives **4a** and **4c**, which incorporate the methylpyridylsulfonamide group, have less balanced receptor subtype selectivity profiles, with reduced affinities for ET_B receptors in comparison to the previous compounds. **4d** showed overall the highest *in vitro* potency on both receptor subtypes. Attempts to further improve this compound by replacing the morpholino substituent (R_1) with other heterocycles (thiomorpholino, piperidino: **4e** and **4f**) did not yield compounds with improved activity.

Model of receptor-antagonist interaction

The study of Bosentan-binding determinants on the ET_A receptor has led to a 3-D model of receptor-antagonist interaction.[9] In accordance with this model important features of subnanomolar binding of **4d** to the ET_A receptor might comprise: (i) salt bridge formation between the acidic pyridylsulfonamide group (**4d**; pKa: 6.3) and Arg 326, and, (ii) formation of a tight hydrogen-bonding network between the (2-pyridyl)carbamoyl moiety and Asn165, as depicted below.



Biological activity profiles

The *in vitro* activity profile of **4d** compared to that of Bosentan and ET-1, is summarized in Table 4. Binding affinity of **4d** for ET_A receptors expressed in CHO cells is about 2-fold lower in comparison to the value obtained with receptor preparations from Sf9 cells. This might be attributed to differences in postranslational receptor glycosylation. **4d** is almost equipotent to ET-1 with respect to affinity for ET_A receptors and 25-fold less potent with respect to ET_B receptors. Compared to Bosentan, ET_A receptor affinities are improved by a factor of 266 and 11, for the Sf9 and CHO cell assays, respectively. Functional potency for ET_A receptors

measured as pA_2 values on rat aortic rings is 100-fold improved over Bosentan, with a similar improvement for ET_B receptors.

Table 4. In vitro biological profiles

	IC_{50} (nM)					pA_2	
	Binding			Aequorin Ca		Rat aorta	Rat trachea
	ET_A (Sf9)	ET_A (CHO)	ET_B	ET_A	ET_B	ET_A	ET_B
Bosentan (**1a**)	80	8	150	-	-	7.3	5.8
Ro 48-5695 (**4d**)	0.3	0.7	5	0.9	6	9.3	7.6
ET-1	0.2	0.3	0.2	-	-	-	-

4d inhibits intracellular calcium mobilization induced by ET-1 in ET_A and ET_B receptor transfected HEK-293 cells with potencies (IC_{50} values) in the low nanomolar range (Table 4).

The compound, applied as its sodium salt (hydrosolubility: 2mg/l), proved to be orally active in dogs and rats (40-60% bioavailability) and showed good *in vivo* activity in different preclinical animal models. The results will be reported elsewhere.

Conclusion

Chemical optimization in a class of functionalized arylsulfonamido pyrimidines led to the identification of Ro 48-5695 (**4d**), which is derived from Bosentan by pyridylcarbamoyl group implementation and introduction of an isopropylpyridylsulfonamide substituent as key characteristics. With respect to affinity for ET_A receptors, the compound is almost equipotent to ET-1. It shows a balanced affinity profile for both receptor subtypes and high functional antagonistic potencies *in vitro*. Ro 48-5695 is orally bioavailable and has been selected for in-depth pharmacological characterization.

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