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Modifications and Structure–Activity Relationships at the 2-Position of 4-Sulfonamidopyrimidine Derivatives as Potent Endothelin Antagonists

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Abstract—To improve water solubility and to study structure–activity relationships, we modified the structure of the pyrimidine nucleus of each of a series of potent ET_A antagonists, **3a** and **4a**, at the 2-position. In a previous study, each of these antagonists showed an extremely high affinity for the ET_A receptor in porcine aortic membrane (IC_{50} **3a**; < 0.001 nM, **4a**; 0.0039 nM). Two modification methods, one being the addition of organolithium followed by DDQ oxidation and the other being the nucleophilic substitution of 2-(methylsulfonyl)pyrimidine, were applied individually to synthesize 2-substituted-4-sulfonamidopyrimidine derivatives. The introduction of aryl, heteroaryl, alkyl, amino, alkoxy, or alkylthio groups into the 2-position varied the affinity. Derivatives with hydrophilic groups at the 2-position showed higher water solubility but tended to reduce the affinity for the ET_A receptor.

Endothelin (ET)-1 is a 21-amino acid peptide first isolated from cultured porcine vascular endothelial cells in 1988. It belongs to a family of three isopeptides ET-1, ET-2, and ET-3 each of which has a distinct specificity for receptor subtypes ET_A (binding affinity: ET-1 = ET-2 > ET-3) and ET_B (binding affinity: ET-1 = ET-2 = ET-3).[1–3] ETs have been implicated as pathogenic factors for a variety of disease states, including essential hypertension, congestive heart failure, pulmonary hypertension, subarachnoid hemorrhage, cerebral ischemia, vasospasm, cyclosporin-induced renal failure, atherosclerosis, and asthma.[4–7] The ET antagonists have been suggested to offer potential utility in the treatment of these disorders.[8–10]

The first orally active non-peptidic sulfonamide derivatives, Ro.46-2005 (**1**)[11] and bosentan (**2**),[12] were $ET_{A/B}$ mixed type antagonists disclosed in 1993 (Fig. 1). The selective ET_A antagonist is expected to be a useful therapeutic agent for these cardiovascular diseases because the effect of ET-1 on the cardiovascular system is mediated predominantly by the ET_A receptor.[3] We have already developed a series of potent and ET_A-selective antagonists, **3a** and the related compounds **4a** and **5** (shown in Fig. 1), and we have reported the structure–activity relationships (SAR) at the 4- and 5-positions of the pyrimidine nucleus.[13] The IC_{50}s of **3a**, **4a**, and **5** for the ET_A receptor in porcine aortic membrane were < 0.001, 0.0039, and 0.051 nM, respectively. At the 4-position, the benzenesulfonamide moiety with a bulky hydrophobic group at *para*-position showed the high affinity. With respect to the 5-position, the phenyl or phenoxy group with small substituents allowed the high affinity to be maintained. However, these derivatives showed low water solubility (**3a**, 0.0016 mg/mL at pH 7.5). Therefore, we intended to improve the water solubility by introducing hydrophilic groups into the 2-position of the pyrimidine nucleus, which had not been modified. Here we describe the efficient modifications and SAR at the respective 2-positions of the compounds **3a** and **4a**.

The general method of synthesizing the substituted pyrimidines is shown in Scheme 1 (Method A).[14–17] First, we chlorinated 2,5-disubstituted-4,6-dihydroxypyrimidine **8**, prepared from the substituted malonate **6** and the amidine derivative **7**. This was followed by substitution at the 4- and 6-positions to afford the 2,4,5,6-tetrasubstituted pyrimidines **3b–c** and **4b–d** (see Table 1). With this method, the modification at the 2-position

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would not be efficient since it requires a variety of starting material **7**. Therefore, we developed the following two routes of synthesis for convenient modification at the 2-position of the pyrimidine nucleus.

Scheme 2 summarizes the method to introduce the aryl group into the 2-position of the pyrimidine nucleus (Method B). Nucleophilic addition of an aryl lithium to **11**, followed by DDQ oxidation, provided **12**.[18] Substitution by 4-*tert*-butylbenzenesulfonamide and ethylene glycol at the 4- and 6-positions, followed by the introduction of 5-bromopyrimidine into the terminal hydroxyl group, afforded the targeted 2-aryl derivatives **3d–f**. By this method, however, heteroatoms, such as amine, alcoholate, and thiolate, are introduced not into the 2-position but into the 4-position of **11**.

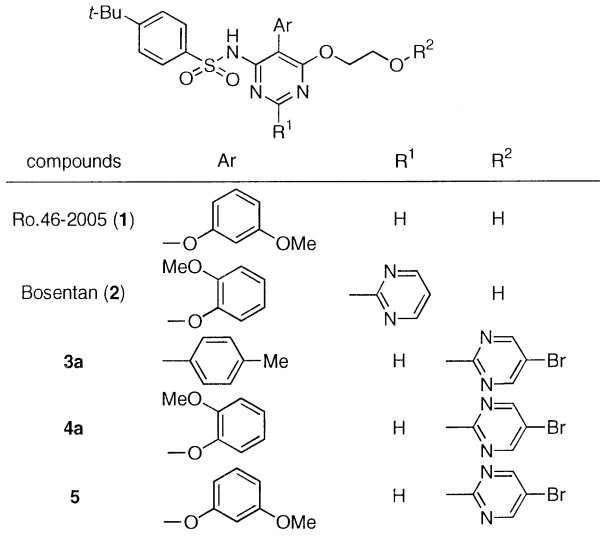
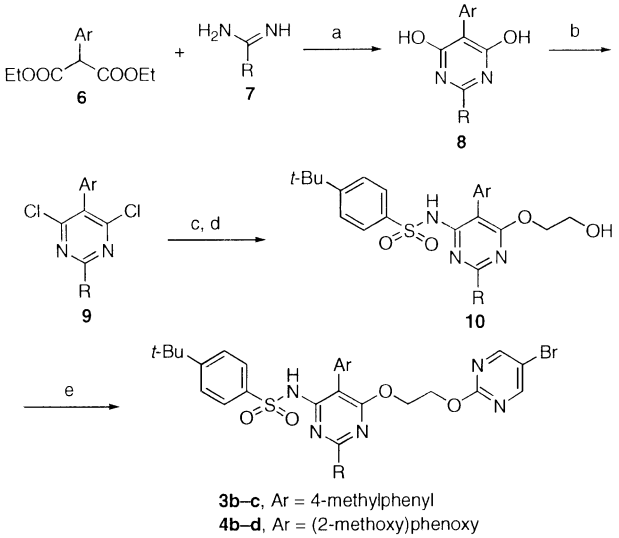
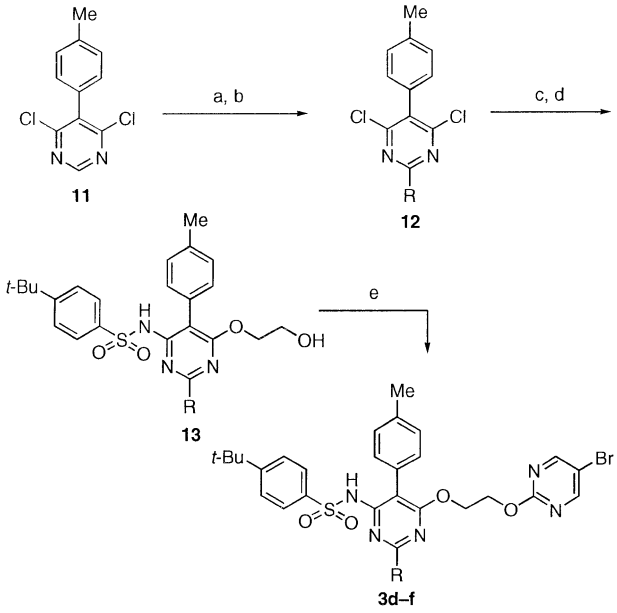


Figure 1. Structure of Ro.46-2005, bosentan and our *N*-(6-(2-((5-bromo-2-pyrimidinyl)oxy)ethoxy)-4-pyrimidinyl)sulfonamide derivatives.

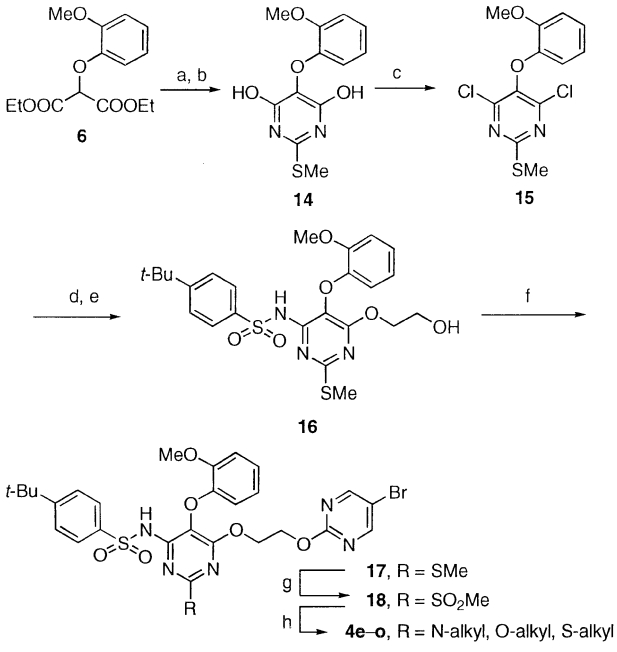


Scheme 1. General method of synthesizing the substituted pyrimidine (Method A): (a) NaOMe, MeOH, room temperature; (b) $POCl_3$, reflux; (c) 4-*tert*-butylbenzenesulfonamide, K_2CO_3, DMSO, 80 °C; (d) ethylene glycol, NaH, 100 °C; (e) 5-bromo-2-chloropyrimidine, NaH, THF–DMAc, room temperature.

By method C shown in Scheme 3, a variety of heteroatoms were introduced at the 2-position at the last synthetic step. 2-Methylthio-4,6-dihydroxypyrimidine derivative **14**, prepared from **6** and thiourea, was converted to the 4,6-dichloro-2-methylthiopyrimidine derivative **15**, which led to the compound **17**. After



Scheme 2. Nucleophilic addition at the 2-position and following oxidation of pyrimidine (Method B): (a) RLi, THF, −60 °C; (b) DDQ, aqueous AcOH, 0 °C; (c) 4-*tert*-butylbenzenesulfonamide, K_2CO_3, DMSO, 80 °C; (d) ethylene glycol, NaH, 100 °C; (e) 5-bromo-2-chloropyrimidine, NaH, THF–DMAc, room temperature.



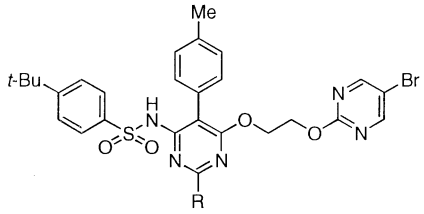
Scheme 3. Nucleophilic substitution into (2-methylsulfonyl)pyrimidine with a variety of heteroatoms (Method C). (a) thiourea, NaOMe, MeOH, room temperature; (b) MeI, K_2CO_3, H_2O, room temperature; (c) $POCl_3$, $PhNMe_2$, reflux; (d) 4-*tert*-butylbenzenesulfonamide, K_2CO_3, DMSO, 80 °C; (e) ethylene glycol, NaH, 100 °C; (f) 5-bromo-2-chloropyrimidine, NaH, THF–DMAc, room temperature; (g) mCPBA, $CHCl_3$, room temperature; (h) amine, alcoholate, or thiolate, room temperature −140 °C.

oxidative conversion of the methylthio group of **17** to methylsulfone as an activated leaving group (**18**), a variety of heteroatoms, such as *N*-alkyl, *O*-alkyl, and *S*-alkyl groups, were introduced into the 2-position by nucleophilic substitutions (**4e–o**).

Tables 1 and 2 show the SAR of the modified compounds at the 2-position. With respect to the 5-(4-methyl)phenyl derivatives (**3**), the compound with 2-pyridyl (**3d**) group showed a relatively high affinity for the ET_A receptor ($IC_{50} = 0.043$ nM). However, other groups at this position, such as the 2-thienyl (**3e**), phenyl (**3f**), and alkyl groups (**3b** and **c**), reduced the affinity drastically. With respect to the 5-(2-methoxy)phenoxy derivatives (**4**), the 2-hydroxyethylamino (**4g**) and 4-hydroxypiperidino (**4n**) derivatives maintained the high affinity for the ET_A receptor and showed high ET_A-selectivity (IC_{50}s for ET_B receptors in rat cerebellum membrane were larger than 1 nM). Minor changes to the substituents in **4g** and **4n**, such as replacement of the NH or OH group with another (**4i**, **j**, or **o**) and chain elongation (**4h**), decreased the affinity for the ET_A receptor. Furthermore, carboxylic acid (**4m**) and other smaller substituents, such as Me, CF_3, OMe, and NH_2 (**4b**, **d**, **e**, and **f**), also reduced the affinity. These results suggest that the ET_A receptor could have specific interactions with the 2-hydroxyethylamino and 4-hydroxypiperidino groups at the 2-position of the pyrimidine nucleus. In addition, the introduction of these groups into the 2-position improved the water solubility. The water solubility of **4a**, **4g**, and **4n** at pH 7.4 were 0.16, 7.32, and 2.57 μg/mL, respectively.

In summary, to study SAR for ET_A receptor antagonist activity, modifications at the 2-position of the sulfonamidopyrimidine derivatives **3a** and **4a** with a variety of substituents were carried out. The 2-hydroxyethylamino (**4g**) and 4-hydroxypiperidino (**4n**) derivatives each showed an affinity nearly equal to or a little higher than that for the ET_A receptor compared with unsubstituted ones (**3a** and **4a**).[13] The potency of each of these compounds was about 1000 times higher than that of bosentan. Further investigation is now under way.

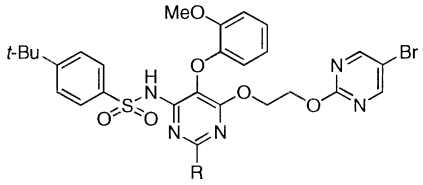
Table 1. SAR of the 2-substituted derivatives **3**



Compd	R	IC_{50} (nM)[a]	Method	Mp (°C)
3a	H	< 0.001		
3b	*i*-Pr	1.1	A	166–168
3c	*n*-Pr	1.6	A	184–186
3d	2-Pyridyl	0.043	B	169–171.5
3e	2-Thienyl	3.9	B	209–210
3f	Phenyl	> 10	B	173.5–174.5

[a]Inhibition of [^{125}I]ET-1 binding in vitro to ET_A receptors in porcine aortic membrane. Values are from a single experiment.

Table 2. SAR of the 2-substituted derivatives **4**



Compd	R	IC_{50} (nM)[a]	Method	Yield (%) from **18**	Mp (°C)
4a	H	0.0039[b]	A		–
4b	Me	2.7	A		148–149.5
4c	*n*-Pr	0.026	A		143–145
4d	CF_3	> 10	A		Amorphous
4e	OMe	> 10	C	42	78.5–80
4f	NH_2	> 10	C[c]	99[e]	197–198.5
4g	$NH(CH_2)_2OH$	0.0053[b]	C	12	Amorphous
4h	$NH(CH_2)_3OH$	> 10	C	14	Amorphous
4i	$NMe(CH_2)_2OH$	> 10	C	26	137–140
4j	$NH(CH_2)_2NMe$	> 10	C	51	Amorphous
4k	$S(CH_2)_2OH$	0.15	C	84	Amorphous
4l	$O(CH_2)_2OH$	0.20	C	91	Amorphous
4m	$S(CH_2)_2COOH$	10	C[d]	47[f]	Amorphous
4n	4-hydroxy-piperidino	0.0025[b]	C	16	Amorphous
4o	4-methyl-piperazin-1-yl	0.45	C	47	Amorphous
Bosentan		7.5			

[a]Inhibition of [^{125}I]ET-1 binding in vitro to ET_A receptors in porcine aortic membrane. Values are from a single experiment.
[b]IC_{50} for ET_B receptors in rat cerebellum membrane were larger than 1 nM. Values are from single experiment.
[c]Prepared by reduction of $R = N_3$ derivative.
[d]Prepared by hydrolysis of $R = S(CH_2)_2COOMe$ derivative.
[e]Yield of the $R = N_3$ derivative.
[f]Yield of the $R = S(CH_2)_2COOMe$ derivative.

Experimental

Compound 12. 1.66 M *n*-Butyllithium in hexane solution (11.4 mL) was added to a solution of thiophene (1.69 g) in dry THF (20 mL) dropwise at 0 °C. After 30 min, a solution of **11** (4.00 g) in dry THF (20 mL) was added to the mixture at −60 °C, and the whole was allowed to stir at 0 °C for 1.5 h. 85% aqueous AcOH then DDQ (5.70 g) were added. After 1 h, the reaction mixture was diluted with 10% aqueous citric acid and extracted with EtOAc. The extract was washed with saturated aqueous $NaHCO_3$, H_2O, and brine, dried over anhydrous Na_2SO_4, and concentrated. Purification by silica gel column chromatography followed by crystallization from hexane afforded **12** (2.64 g, 66%): mp 119.5–120 °C.

Compounds **13** and **3d–f** were synthesized as described in ref 13.

Compound 14. To a solution of diethyl (2-methoxy)-phenoxymalonate (10.00 g) and thiourea (4.04 g) in MeOH (100 mL) was added 28% sodium methoxide in methanol solution (17.07 g) dropwise over 30 min at 0 °C, and the mixture was stirred at room temperature for 16 h. MeOH was evaporated, and the residue was dissolved in H_2O (200 mL). MeI (3.30 mL) was added, and the mixture was stirred at room temperature for 3 h and acidified with 10% aqueous HCl. The resulting precipitate was collected, washed with H_2O, and air-dried to afford **14** (8.90 g, 90%): mp 206–210 °C.

Compounds **15**, **16**, and **17** were synthesized as described in ref 13.

Compound 18. To a suspension of **17** (16.24 g) in $CHCl_3$ (160 mL) was added 85.4% *m*-chloroperbenzoic acid (10.67 g) at 0 °C, and the mixture was allowed to stir at room temperature for 3 h, washed with saturated aqeous $NaHCO_3$, H_2O, and brine, dried over anhydrous Na_2SO_4, and concentrated. Purification by silica gel column chromatography afforded **18** as foam (13.45 g, 79%).

Compound 4n. A mixture of **18** (350 mg) and 4-hydroxy-piperidine (250 mg) in DMSO (3 mL) was stirred at 120 °C for 24 h. After cooling, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with EtOAc. The extract was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and concentrated. Purification by preparative TLC followed by trituration with *i*-Pr_2O afforded **4n** as amorphous powder (58 mg, 16%).

ET receptor binding assay. These binding experiments were carried out according to the reported method by Ihara et al.[19]

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