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Discovery and Synthesis of a Potent Sulfonamide ET_B Selective Antagonist

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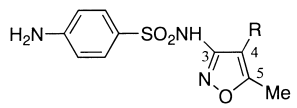
Abstract—The synthesis and structure–activity relationships of a series of sulfonamide endothelin antagonists are described. In the course of our modification studies, we discovered ET_B selective antagonists. The most potent compound **15f** displays IC_{50} values of 1.7 μM and 0.002 μM to ET_A and ET_B receptors, respectively.

Introduction

Endothelins (ET-1, ET-2, ET-3), 21-amino acid bicyclic peptides, are the most potent known vasoconstrictors.[1,2] Two distinct G-protein coupled receptors, ET_A and ET_B, were cloned and characterized with respect to their affinity for each endothelin.[3,4] The ET_A receptor is expressed on vascular smooth muscle cells and has high affinity for ET-1 and ET-2. The ET_B receptor is expressed on vascular endothelial and smooth muscle cells and has high affinity for all three endothelins. As these two subtypes of receptors are widely distributed in human tissues, the development of selective or non-selective endothelin antagonists is expected to be useful for the treatment of various diseases.[5] A number of groups have reported the discovery of non-peptide endothelin antagonists since 1994. Most of them were ET_A selective or non-selective antagonists,[6,7] and only recently have non-peptide ET_B selective antagonists been reported.[8]

In order to find low-molecular-weight non-peptide endothelin antagonists, we screened the Shionogi compound library for compounds capable of inhibiting specific [^{125}I]ET-1 binding. We identified sulfamethoxazole **5a**[9] and its iodide **5b** as ET_A selective antagonists (Fig. 1). We then started the modification of **5b** as a lead compound to enhance the binding affinity for the ET_A receptor. Our modification study led to the discovery of ET_B selective antagonists as well as non-selective and ET_A selective antagonists. In this paper, we wish to describe the discovery and synthesis of a potent ET_B selective antagonist.



	IC_{50} for ET_A
5a R=H (sulfamethoxazole)	333 μM
5b R=I	11 μM

Figure 1.

Chemistry

Sulfonamides **5a**–**m** of 4-substituted azoles (Table 1) were synthesized by condensation of the substituted benzenesulfonyl chlorides with the 4-substituted aminoisoxazoles **4A** (X = N, Y = O), **4B** (X = O, Y = N) or 4-substituted aminoisothiazoles **4C** (X = N, Y = S), and **4D** (X = S, Y = N) (Scheme 1). As most of the azoles **4** were not readily available, we developed a general synthetic method for preparing 4-substituted aminoazoles **4** starting from **1**.[10] Our method was based on directed *ortho*-metallation and was successfully used to prepare 4-substituted aminoisoxazoles **4A** bearing various substituents (R^2) at the 4-position. Substituted 4-lithioisoxazole was prepared from **1A**[10] using alkyllithium for the directed *ortho*-metallation, which was reacted with various electrophiles (iodomethane, iodoethane, benzyl bromide, methyl disulfide, disulfide **9**) to give **3A**. Deprotection of the amino group gave **4A**, from which sulfonamides

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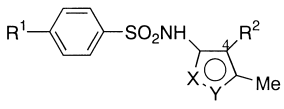
Table 1.



Compound	X	Y	R^1	R^2	IC_{50} (μM) ET_A	IC_{50} (μM) ET_B
5a	N	O	NH_2	H	333	ND
5b	N	O	NH_2	I	11	>1000
5c	N	O	NH_2	Cl	3.5	ND
5d	N	O	NH_2	Me	5.0	ND
5e	N	O	NH_2	Et	3.4	ND
5f	N	O	NH_2	$PhCH_2$	71	ND
5g	O	N	NH_2	Me	9.0	ND
5h	N	S	NH_2	Me	28	ND
5i	S	N	*t*-Bu	Me	>500	ND
5j	N	O	*t*-Bu	I	>100	30
5k	N	O	*t*-Bu	Me	28	ND
5l	N	O	*t*-Bu	MeS	>100	35
5m	N	O	*t*-Bu	3-MeO-PhS	0.53	0.30

ND, not determined.

5d–f, **5k–m** were prepared by treatment with 4-acetamido- and 4-*tert*-butyl-benzenesulfonyl chlorides. In contrast to the isoxazoles, we could not prepare isothiazole derivatives (**5h**, **5i**) efficiently by our method because a directed *ortho*-lithiation of **1C** or **1D**[11] competed with lithiation of the methyl group. Thus, 4-lithioisothiazoles were prepared via halogen–metal exchange reaction of bromide **2C** or iodide **2D** obtained by halogenation of the parent aminoisothiazole. The resulting lithium compounds were reacted with iodomethane to give methylated isothiazoles, which were then converted to sulfonamide **5h**, **5i**.

As a typical example of **15a–n**, the synthesis of **15f** is shown in Scheme 2. The isoxazole ring was constructed by 1,3-dipolar cycloaddition reaction of acetylene **7** and the nitrile oxide which was prepared from **10**.[12] Hydrolysis of the ester gave carboxylic acid **11** which was converted to the protected amine **12** by Curtius rearrangement. The 4-position of **12** was lithiated, and the resulting lithium compound was treated with disulfide **9**[13] to give **13**. Silyloxy compound **13** was converted to an acetate, and deprotection of its Boc group gave amine **14**. Sulfonylation of **14** with 4-*tert*-butylbenzenesulfonyl chloride, and subsequent hydrolysis of the acetate gave sulfonamide **15d**. Alcohol **15d** was converted to nitrile **15k**, and reduction of the nitrile by DIBAH gave aldehyde **15f**. Aldehyde **15f** was subsequently converted to ester **15h** and amide **15i** through carboxylic acid **15g** as well as to ketone **15j** and oxime **15l** (Scheme 3).

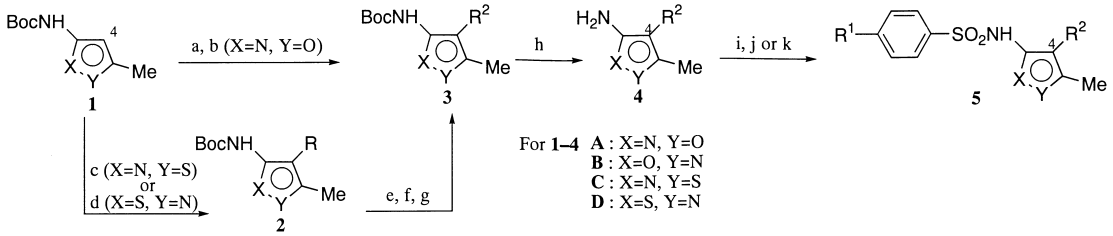
Results and Discussion

Structure–activity relationships were discussed using IC_{50} values obtained from radioreceptor binding studies. For ET_A receptor binding assay, we employed rat aortic smooth muscle A7r5 cells. For ET_B receptor binding assay, we employed COS-7 cells transfected with porcine ET_B receptor. IC_{50} data were recorded by measuring the displacement of [^{125}I]ET-1 binding from ET_A receptor or [^{125}I]ET-3 binding from ET_B receptor.

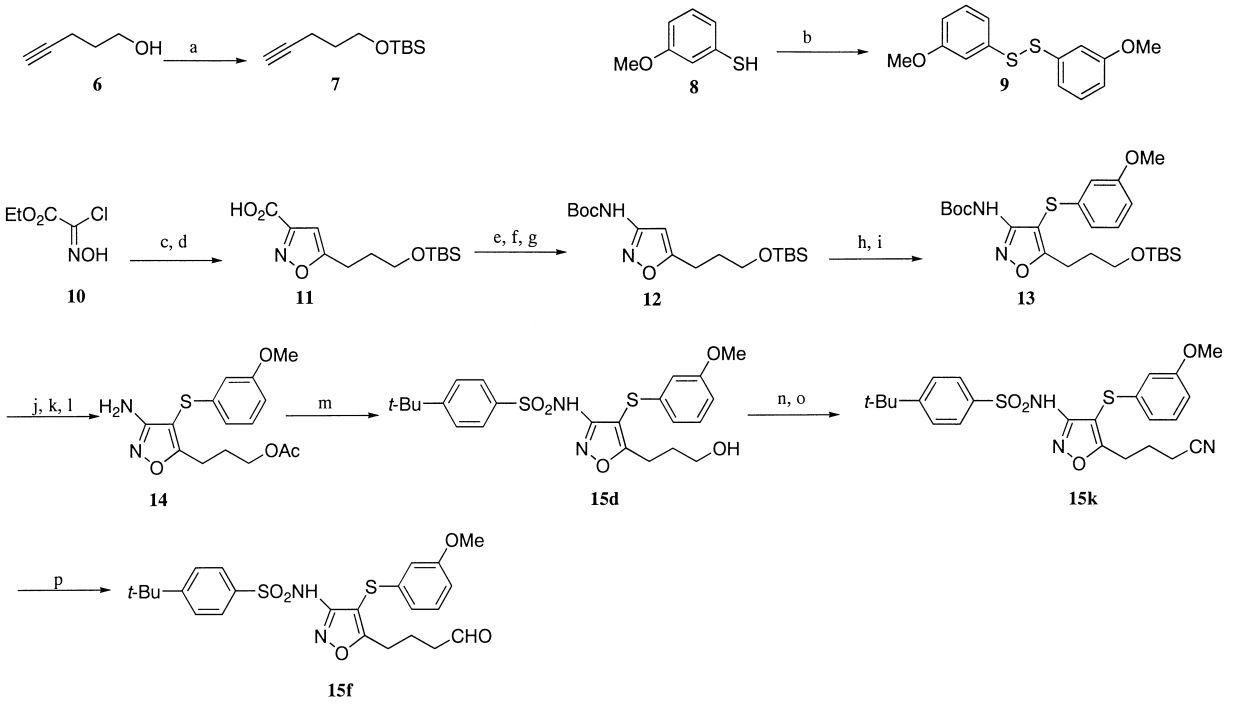
Table 1 shows the influences of substituent R^2 in the azole ring and substituent R^1 in the sulfonamide of compounds **5a–m**. We expected that introduction of a variety of groups for R^2 would lead to the enhancement of the binding affinity for the ET_A receptor because our lead compound **5b** had a higher binding affinity than the parent **5a**. We first investigated the influence of the R^2 substituent on the antagonistic activity. Halogen (**5b**, **5c**) or small alkyl groups (**5d**, **5e**) were needed for the activity, but their binding affinities were moderate. A large benzyl substituent decreased the activity (**5f**). Isomeric isoxazole ring (**5g**) or isothiazole analogues (**5h**, **5i**) had a lower binding affinity than the corresponding compound **5d**.

We next replaced the hydrophilic amino group R^1 with the hydrophobic *tert*-butyl group, and investigated the effect of R^2. Although compound **5j–l** showed no or decreased binding affinity for the ET_A receptor, compounds **5j** and **5l** were found to be ET_B selective antagonists with moderate affinity. This indicated that introduction of a bulky hydrophobic substituent R^1 is effective for ET_B affinity. Therefore, in order to increase the binding affinity for both subtypes, we modified the 4-position and introduced the 3-methoxy phenylthio group by an analogy to the known non-selective endothelin antagonist.[7a] By this modification, we obtained the non-selective antagonist **5m** with improved IC_{50} values in the sub-micromolar range for both subtypes.

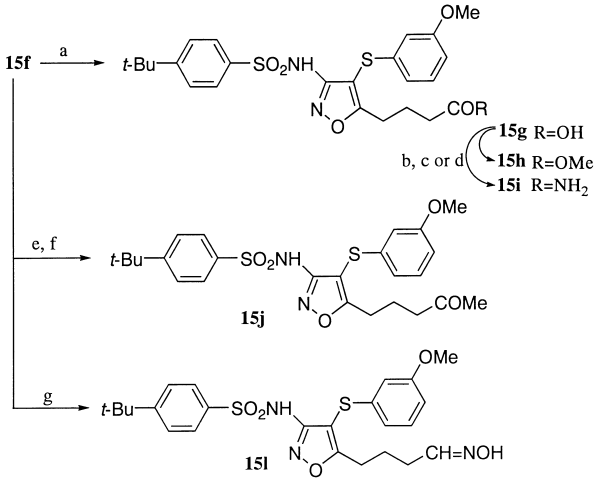
Next, we selected **5m** as the second lead compound for a non-selective endothelin antagonist, and modified the 5-position of the isoxazole ring (Table 2). One carbon homologation of **5m** decreased the affinity for both subtypes (**15a**). Therefore, we decided to introduce a functional group with the expectation of specific interaction



Scheme 1. (a) *n*-BuLi, THF, −78 °C to rt; (b) electrophile, THF, −78 °C; (c) Br_2, AcONa, AcOH, rt; (d) ICl, AcOH, rt; (e) NaH, THF, rt; (f) *t*-BuLi, THF, −78 °C; (g) MeI, THF, −78 °C; (h) TFA, rt; (i) 4-AcNHPhSO_2Cl, pyridine, rt; (j) aq NaOH, MeOH, reflux; (k) 4-*t*-BuPhSO_2Cl, pyridine, rt.



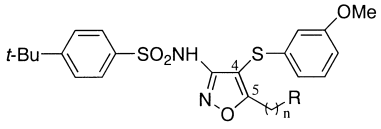
Scheme 2. (a) *t*-BuSiMe$_2$Cl, imidazole, CH$_2$Cl$_2$, rt, 97%; (b) DMSO, 85 °C, 80%; (c) Et$_3$N, **7**, rt, 76%; (d) aq NaOH, MeOH, rt, 81%; (e) SOCl$_2$, pyridine, CH$_2$Cl$_2$, 0 °C; (f) NaN$_3$, acetone–H$_2$O, 0 °C; (g) *t*-BuOH, toluene, reflux, 41% (three steps); (h) *n*-BuLi, THF, −78 °C to rt; (i) **9**, THF, −78 °C, 67% (two steps); (j) *n*-Bu$_4$NF, THF, rt; (k) Ac$_2$O, DMAP, pyridine, CH$_2$Cl$_2$, rt; (l) TFA, anisole, rt, 86% (three steps); (m) 4-*t*-BuPhSO$_2$Cl, DMAP, pyridine, 50 °C, 64%; (n) aq NaOH, MeOH–THF, rt, 92%; (o) MsCl, pyridine, 0 °C; (p) aq NaOH, MeOH–THF, rt; (q) NaCN, DMF, 80 °C; (q) DIBAH, toluene, −78 °C to rt, 75% (four steps).



Scheme 3. (a) PDC, DMF, rt, 79%; (b) SOCl$_2$, pyridine, Et$_2$O, 0 °C; (c) MeOH, 0 °C, 37% (two steps); (d) 28% aq NH$_3$, 0 °C, 24%; (e) MeLi, THF, −78 °C to 0 °C; (f) PDC, DMF, rt, 29% (two steps); (g) NH$_2$OH HCl, EtOH–pyridine, rt, 100%.

between the functional group and the receptor. We first introduced a hydroxyalkyl group ((CH$_2$)$_n$OH) which had both hydrogen bond donating and accepting properties and optimized the length of the alkyl chain (n = 1–4, **15b–e**). Of these compounds, **15d** (n = 3) showed the best affinity for both subtypes. Next, we introduced other functional groups, keeping the length of the alkyl chain unchanged (n = 3, **15f–l**). Interestingly, aldehyde **15f** had highly improved affinity and selectivity for the ET$_B$ receptor. The IC$_{50}$ values of **15f** were 1.7 μM and 0.002 μM for ET$_A$ and ET$_B$ receptors, respectively, and

Table 2.



Compound	n	R	IC$_{50}$ (μM) ET$_A$	IC$_{50}$ (μM) ET$_B$	ET$_B$ selectivity (IC$_{50}$ ET$_A$/IC$_{50}$ ET$_B$)
5m	1	H	0.53	0.30	1.8
15a	2	H	0.80	2.8	0.3
15b	1	OH	6.2	0.56	11
15c	2	OH	0.77	0.43	1.8
15d	3	OH	0.32	0.14	2.3
15e	4	OH	0.63	0.24	2.6
15f	3	CHO	1.7	0.002	850
15g	3	CO$_2$H	4.3	0.50	8.6
15h	3	CO$_2$Me	0.40	0.31	1.3
15i	3	CONH$_2$	6.3	0.24	26
15j	3	COCH$_3$	1.3	0.40	3.3
15k	3	CN	1.3	0.52	2.5
15l	3	CH=NOH	1.5	0.085	18
15m	2	CHO	0.38	0.50	0.8
15n	4	CHO	1.4	0.020	70

the ET$_B$ selectivity (IC$_{50}$ ET$_A$/IC$_{50}$ ET$_B$) was 850. This compound was found to be one of the most potent ET$_B$ selective antagonists reported so far.[8] Amide **15i** and oxime **15l** were also ET$_B$ selective antagonists, although they had a lower activity than **15f**. Other derivatives, such as carboxylic acid **15g**, ester **15h**, ketone **15j**, and nitrile **15k** had decreased binding affinity for both subtypes compared with **15d**, and their ET$_B$ selectivities were low. Aldehyde analogues with shorter (n = 2, **15m**)

or longer ($n=4$, **15n**) alkyl spacers had lower activity and selectivity for the ET_B receptor than **15f**. These findings demonstrate that the substituent in the 5-position of the isoxazole ring plays a crucial role in the ET_B receptor binding. Adequate spatial arrangement of the specific functional group is needed for the high affinity binding to the ET_B receptor. Our results indicate that compound **15f** displays specific interaction between its aldehyde group and the ET_B receptor binding site.

In conclusion, we have discovered a potent ET_B selective antagonist **15f**. Unfortunately, **15f** had insufficient oral bioavailability. However, it should be useful for understanding the role of the ET_B receptor in pathological conditions. Further modification of **15f** is in progress and will be reported elsewhere.

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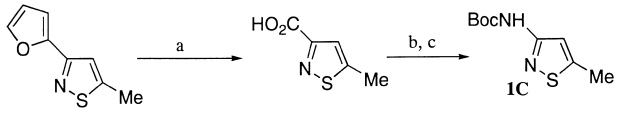

Scheme 4. (a) $KMnO_4$, benzene–acetone, rt, 67%; (b) $ClCO_2Et$, Et_3N then NaN_3, H_2O–acetone; (c) *t*-BuOH, toluene, rt, 70%.

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