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Tryptamine-based human β_3-adrenergic receptor agonists. Part 1: SAR studies of the 7-position of the indole ring

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Abstract—A series of tryptamine-based 2-thiophenesulfonamide derivatives were prepared and their agonistic activity for the β-adrenergic receptors (ARs) was evaluated. Compound **54**, containing 7-methanesulfonyloxy tryptamine, was found to be a highly potent β_3-AR agonist (EC_{50} = 0.21 nM, IA = 97%) with excellent selectivity for the β_3-AR over the β_1- and β_2-ARs (210- and 86-fold, respectively).

1. Introduction

The definitive subclassification of β-adrenergic receptors (β-ARs) into β_1- and β_2-ARs by Lands et al. in 1967[1] has contributed to the development of β_1- and β_2-AR agonists and/or antagonists, which have been useful in treating cardiovascular diseases and asthma.[2] After the inspired work of Lands' group, the existence of another β-AR was reported in the early 1980s.[3] Cloning of this novel β-AR (β_3-AR) by Emorine et al. revealed this receptor mediates metabolic effects such as lipolysis and thermogenesis in adipose tissues.[4] Fisher et al. demonstrated that chronic treatment of monkeys with L-755,507, a selective β_3-AR agonist, elicited lipolysis and increased energy expenditure.[5] Therefore, potent and selective β_3-AR agonists have the possibility to treat obesity and noninsulin dependent diabetes mellitus (NIDDM).[6]

In a previous paper, we demonstrated that a series of arylethanolamine derivatives with an indole ring were potent agonists of the human β_3-AR.[7] In this series, compound **1** (AJ-9677), having a carboxylmethoxy group at the 7-position of the indole ring, showed the best potency and selectivity for the human β_3-AR. In the course of our work on human β_3-AR agonists, it became clear that the indole ring unit is crucial in terms of preserving high potency. Subsequently, we have investigated the structure–activity relationship (SAR) of the tryptamine derivatives and found that replacement of the *meta*-chlorine atom with a 2-thiophenesulfonamide group, represented by compound **2**, further improved the selectivity for the human β_3-AR. To better understand the SAR of the tryptamine-based β_3-AR agonists, we investigated the effects of substituents at the 7-position of the indole ring on potency and selectivity while keeping the 2-thiophenesulfonamide moiety constant as the left-hand side. In this study, we report the synthesis and evaluation of a series of 2-thiophenesulfonamide derivatives with various substituents at the 7-position of the indole ring (Fig. 1).

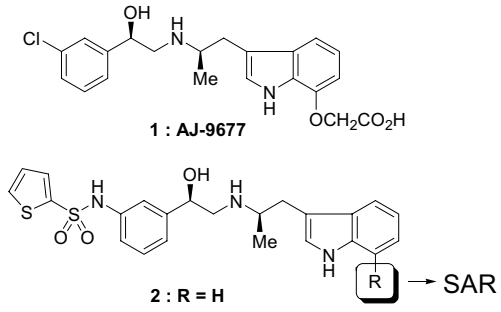

Figure 1.

Keywords: β_3-Adrenergic receptor; Agonist; Tryptamine.
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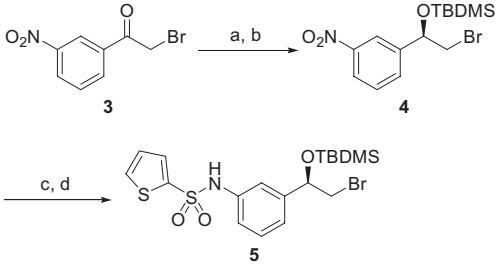
2. Chemistry

All compounds presented in this study were prepared by a convergent route in which various tryptamine derivatives were coupled to the sulfonamide **5**.[8] Synthesis of the sulfonamide **5** was carried out as depicted in Scheme 1. Asymmetric reduction of the commercially available 3-nitrophenacyl bromide **3** was accomplished by treatment with Corey's CBS-borane reagent[9] to afford the corresponding (*R*)-alcohol with high enantiomeric purity.[10] The secondary alcohol was then protected with a *tert*-butyldimethylsilyl (TBDMS) group to give **4**, and its nitro group was reduced with Fe and NH_4Cl. The resulting aniline was treated with 2-thiophenesulfonyl chloride to provide the desired sulfonamide **5**.

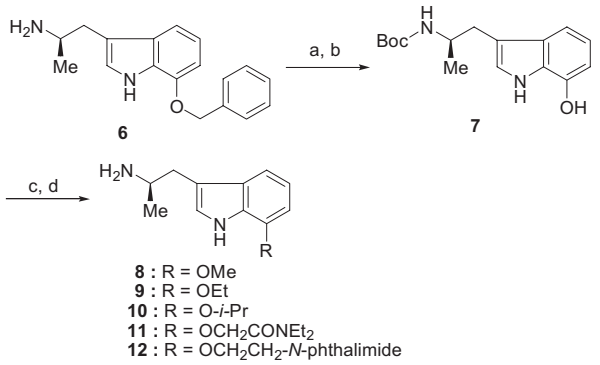
We then concentrated on the synthesis of the tryptamine right-hand moiety (Schemes 2 and 3). The 7-alkoxytryptamine derivatives **8**–**12** were synthesized as shown in Scheme 2. The amino group in **6**[11] was first protected with a Boc group, and then the benzyl group was removed by hydrogenation to give 7-hydroxyindole **7**. The hydroxyindole **7** was treated with the appropriate alkyl halides in the presence of potassium carbonate, followed by treatment with 4 N HCl in EtOAc to afford **8**–**11**. Alternatively, Mitsunobu alkylation of **7** with *N*-(2-hydroxyethyl)phthalimide and subsequent deprotection of the Boc group provided the desired compound **12**.

The 7-sulfonate derivatives **22**–**29** were also prepared from **7** as shown in Scheme 3. The hydroxyindole **7** was treated with commercially available sulfonyl chlorides to afford sulfonates **13**–**20**. Removal of the Boc group in **13**–**19** provided the desired compounds **22**–**28**. Ethyl ester **29** was obtained by esterification of **20** and subsequent deprotection of the Boc group.

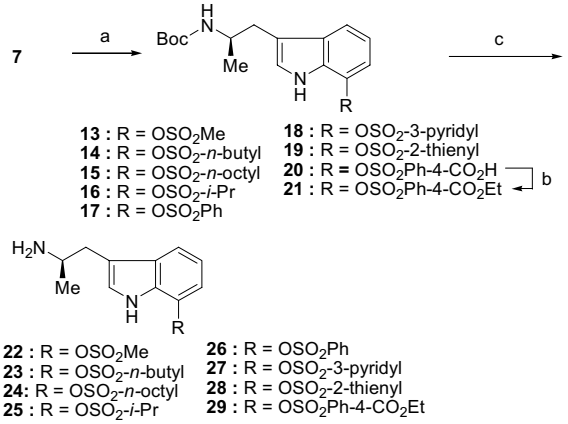
The tryptamine derivatives obtained above (**6**, **8**–**12**, **22**–**29**), and (*R*)-α-methyltryptamine **30**[12] were treated with the sulfonamide **5** in the presence of *i*-Pr_2NEt and KI to give **31**–**45**. Removal of the TBDMS group provided the desired **2**, **46**–**50**, and **54**–**61**. Alkaline hydrolysis of **50** gave the desired carboxylic acid **51**. The phthalimide **52** was treated with hydrazine hydrate in MeOH to supply the desired amine **53**. Carboxylic acid **62** was obtained by acidic hydrolysis of the ethyl ester **61** (Scheme 4).



Scheme 1. Reagents and conditions: (a) (*R*)-2-methyl-CBS-oxazaborolidine, BH_3, THF, 80%; (b) TBDMSCl, imidazole, DMF, 96%; (c) Fe, NH_4Cl, EtOH, H_2O, 99%; (d) 2-thiophenesulfonyl chloride, pyridine, CH_2Cl_2, 99%.



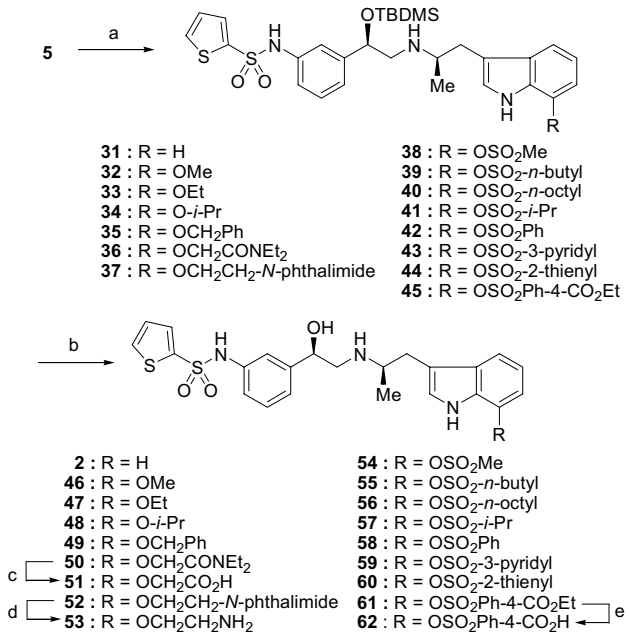
Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, $CHCl_3$, 97%; (b) 5% Pd–C, HCO_2NH_4, MeOH, 99%; (c) appropriate alkyl iodides (or alkyl chlorides, KI), K_2CO_3, acetone, 71–99% or *N*-(2-hydroxyethyl)phthalimide, Ph_3P, DEAD, THF, 29%; (d) 4 N HCl–EtOAc, 88–99%.



Scheme 3. Reagents and conditions: (a) appropriate sulfonyl chlorides, TEA, CH_2Cl_2, 53–99%; (b) EtOH, WSC, DMAP, CH_2Cl_2, 98%; (c) 4 N HCl–EtOAc, 67–99%.

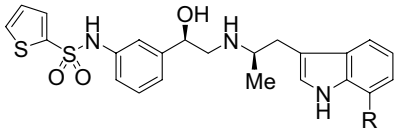
3. Results and discussion

First, a variety of tryptamine derivatives with different alkoxy substituents at the 7-position of the indole ring were prepared, and their agonistic activity for the β_{1-3}-ARs was evaluated. Their potency was determined by measuring their ability to stimulate increases in cAMP in CHO cells expressing cloned human β-ARs.[13] In a previous report,[7] we used CHO cells expressing a high level of β_3-AR to measure compound activity, that is, the receptor densities were 150,000 receptors/cell (β_3-AR), 12,000 receptors/cell (β_1-AR), and 30,000 receptors/cell (β_2-AR). In this study, to better evaluate subtype selectivity, we used CHO cells expressing a low density of β_3-AR (13,000 receptors/cell) and high densities of β_1- and β_2-ARs (320,000 and 600,000 receptors/cell, respectively). As shown in Table 1, all 7-alkoxy derivatives **46**–**53** showed good agonistic activity for theβ_3-AR. In particular, the methoxy derivative **46** exhibited potent agonistic activity for the β_3-AR



Scheme 4. Reagents and conditions: (a) appropriate tryptamines [**6**, **8**–**12**, **22**–**29** (*R*)-α-methyl-tryptamine (**30**)], *i*-Pr_2NEt, KI, MeCN, 31–69%; (b) 10% HCl–EtOH, 76–99%; (c) NaOH, EtOH–H_2O, 71%; (d) NH_2NH_2–H_2O, MeOH, 50%; (e) 2 N HClaq–EtOH = 2:1, 42%.

Table 1. Agonistic activity of substituted tryptamine derivatives for human β-ARs (**1**)



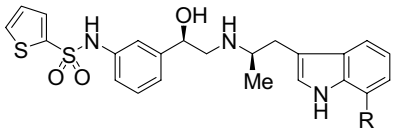
Compd	R	EC_{50}, nM[a] (IA, %)[b]		
		β_3	β_1	β_2
2	H	0.88 (96)	66 (50)	21 (50)
46	OMe	0.55 (101)	29 (36)	6.6 (67)
47	OEt	1.0 (98)	nd[c] (26)[d]	7.7 (67)
48	O–*i*-Pr	2.0 (73)	nd[c] (30)[d]	12 (77)
49	OCH_2Ph	0.76 (87)	54 (33)	6.6 (75)
50	OCH_2CONEt_2	1.3 (90)	19 (42)	6.8 (69)
51	OCH_2CO_2 H	1.7 (103)	180 (39)	19 (51)
53	$OCH_2CH_2NH_2$	4.3 (75)	29 (34)	nd[c] (23)[d]

[a] Agonistic activity was assessed by measuring cAMP accumulation in CHO cells expressing human β-ARs.
[b] Values in parentheses represent the intrinsic activity (IA) given as percentage of maximal stimulation with isoproterenol.
[c] nd = not determined.
[d] % Activity at 1000 nM.

(EC_{50} = 0.55 nM, IA = 101%) with good selectivity over the β_1-AR (53-fold). However, the selectivity of **46** over the β_2-AR was lower than that of the parent compound **2** (12- and 24-fold, respectively). Although the agonistic activity of the isopropoxy derivative **48** for the β_3-AR was decreased, compound **49**, which also has a large benzyloxy group, showed potent agonistic activity for the β_3-AR (EC_{50} = 0.76 nM, IA = 87%). Nevertheless, the selectivity of **49** over the β_2-AR was further decreased (8.7-fold). Interestingly, introduction of a carboxymethoxy group (**51**) dramatically decreased the agonistic activity against the β_1-AR (EC_{50} = 180 nM, IA = 39%), maintaining potent agonistic activity for the β_3-AR (EC_{50} = 1.7 nM, IA = 103%). However, masking the negative charge of the carboxylate (**50**) resulted in the loss of selectivity against the β_1-AR. As can be seen from Table 1, most of the 7-alkoxy derivatives synthesized in this study exhibited relatively strong agonistic activity against the β_2-AR, and therefore, their subtype selectivity for the β_3-AR was insufficient for further development.

Researchers at Merck have extensively reported a series of potent β_3-AR agonists containing a sulfonamide group on the right-hand side.[14] Alternatively, Sum et al. reported that novel cyclic amine sulfonamides, such as piperidine sulfonaminde, were also potent β_3-AR agonists.[15] On the basis of these findings, we became interested in the synthesis of analogues containing a sulfonate group at the 7-position of the indole ring. As shown in Table 2, incorporation of a sulfonate moiety at the 7-position of the indole ring increased its agonistic activity for the β_3-AR except for **61** and **62**. It is noteworthy that the methanesulfonate **54** exhibited highly potent agonistic activity for the β_3-AR (EC_{50} = 0.21 nM, IA = 97%). In addition, **54** showed remarkable selectivity over the β_1- and β_2-ARs (210- and 86-fold, respectively). Replacement of the methyl group in **54** with other aliphatic or aromatic groups (**55**–**60**) decreased the selectivity for the β_3-AR over the β_2-AR, while good agonistic activity for the β_3-AR was maintained. In particular, 7-aryl sulfonate derivatives (**58**–**60**) showed strong agonistic activity against the β_2-AR (EC_{50} = 3.1, 1.3, and 1.2 nM, respectively) with high intrinsic activity (IA = 91%, 90%, and 86%, respectively). However, introduction of a carboxyl group at the *para*-position of the phenyl ring (**62**) greatly

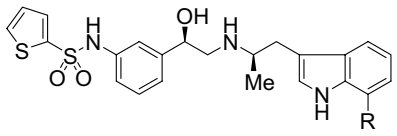
Table 2. Agonistic activity of substituted tryptamine derivatives for human β-ARs (**2**)



Compd	R	EC_{50}, nM[a] (IA, %)[b]		
		β_3	β_1	β_2
54	OSO_2Me	0.21 (97)	44 (36)	18 (50)
55	OSO_2–*n*-butyl	0.59 (86)	26 (48)	7.3 (77)
56	OSO_2–*n*-octyl	0.28 (80)	20 (49)	5.6 (62)
57	OSO_2–*i*-Pr	0.51 (93)	40 (48)	6.2 (84)
58	OSO_2Ph	0.87 (92)	72 (40)	3.1 (91)
59	OSO_2–3-pyridyl	0.26 (83)	22 (47)	1.3 (90)
60	OSO_2–2-thienyl	0.64 (100)	49 (47)	1.2 (86)
61	OSO_2Ph–4-CO_2Et	1.2 (96)	58 (59)	7.2 (48)
62	OSO_2Ph–4-CO_2H	1.1 (88)	>85 (51)[c]	60 (70)

[a] Agonistic activity was assessed by measuring cAMP accumulation in CHO cells expressing human β-ARs.
[b] Values in parentheses represent the intrinsic activity (IA) given as percentage of maximal stimulation with isoproterenol.
[c] % Activity at 1000 nM.

Table 3. Binding affinity of compounds **2**, **46**, **51**, and **54** for human β-ARs and selectivity versus β_1- and β_2-ARs



Compd	R	Binding K_i (nM)[a]			Selectivity[b]	
		β_3	β_1	β_2	Versus β_1	Versus β_2
2	H	30	110	51	3.6	1.7
46	OMe	14	43	25	3.2	1.9
51	OCH_2CO_2H	17	480	160	29	9.5
54	OSO_2Me	4.0	66	48	17	12

[a] Binding affinity is reported as K_i, the binding inhibition constant, determined by inhibition of ^{125}I-iodocyanopindolol binding.

[b] Binding selectivity is defined as the ratio of β_3 (K_i) to β_1 (K_i) or β_2 (K_i).

decreased the agonistic activity for the β_2-AR (EC_{50} = 60 nM).

The selected compounds **2**, **46**, **51**, and **54** were then subjected to binding assays for the human β_{1-3}-ARs. As shown in Table 3, the methanesulfonate **54** exhibited a high affinity for the β_3-AR with a binding constant (K_i) of 4.0 nM. Binding selectivity of **54** for the β_3-AR over the β_1- and β_2-ARs was 17- and 12-fold, respectively. Although the K_i value of **51** for the β_3-AR (K_i = 17 nM) was lower than that of **54**, **51** showed excellent selectivity over the β_1-AR (29-fold). The methoxy derivative **46** also showed moderate affinity for the β_3-AR (K_i = 14 nM), but the selectivity over the β_1- and β_2-ARs was low (<4-fold). It is interesting to note that the binding affinity of the methanesulfonate **54** is superior to those of the carboxylic acid derivative **51** and the methoxy derivative **50**. In addition, **54** has excellent subtype selectivity comparable to **47**. These data indicate that the sulfonate moiety contributes not only to potency but also to selectivity over the β_1- and β_2-ARs.

In summary, we synthesized the tryptamine-based arylsulfonamide derivatives with various alkyloxy or sulfonate groups at the 7-position of the indole ring, and evaluated their agonistic activity for the β_{1-3}-ARs. We found that the methanesulfonate **54** was a highly potent β_3-AR agonist (EC_{50} = 0.21 nM, IA = 97%) with excellent subtype selectivity over the β_1- and β_2-ARs (210- and 86-fold, respectively). In a binding assay, **54** exhibited a strong affinity for the β_3-AR (K_i = 4.0 nM), and good selectivity over the β_1- and β_2-ARs (17- and 12-fold, respectively). Further studies on **54** and related compounds will be reported in due course.

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