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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1061–1064
Bioorganic & Medicinal Chemistry Letters

Tryptamine-based human β_3-adrenergic receptor agonists. Part 3: Improved oral bioavailability via modification of the sulfonamide moiety

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Received 26 October 2004; revised 10 December 2004; accepted 10 December 2004
Available online 18 January 2005

Abstract—The continued SAR investigation of tryptamine-based human β_3-adrenergic receptor (AR) agonists is reported. Prior efforts resulted in the identification of **2** as a potent β_3-AR agonist. Further modification of the left side arylsulfonamide portion in **2** provided compounds with good cell permeability, which have potent agonistic activity for β_3-AR. Cinnamylamine analog **16i** exhibited an excellent agonistic profile in vitro and good oral bioavailability in rats.

1. Introduction

In an effort to find novel therapy for the treatment of diseases such as non-insulin dependent diabetes mellitus (NIDDM), obesity, and frequent urination, we have recently disclosed a series of selective human β_3-adrenergic receptor (AR) agonists based on a tryptamine scaffold (Fig. 1).[1–3] It has been suggested that stimulation of β_3-AR results in various pharmacological effects, such as lipolysis and thermogenesis in adipocytes,[4] and relaxation of urinary bladder detrusor urination.[5] The structure–activity relationship (SAR) study of the tryptamine-based analogs revealed that the 7-position of the indole ring was important for both activity and selectivity. Although the optimization of β_3-agonistic activity within this series led us to several potent and selective β_3-agonists exemplified by **2**, these compounds had extremely low oral bioavailability due to the lack of cellular permeability.

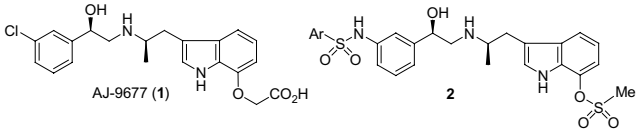

Figure 1. Structures of tryptamine-based human β_3-AR agonists.

In the course of our work on the tryptamine-based analogs, it became clear that Caco-2 cell monolayer permeability was greatly decreased due to the acidic property of incorporating a sulfonamide moiety into the left-hand side of the molecule. In fact, few compounds in this series lacking an acidic proton of the sulfonamide exhibited improved Caco-2 cell permeability (**3a** vs **3b** and **3c**, Table 1), although they showed moderate agonistic activity. These results prompted us to further optimize the left side of the molecule. We now wish to report further on the results of our investigation into the SAR of the tryptamine-based β_3-AR agonists in activity and Caco-2 cell permeability.

2. Chemistry

The benzenesulfonamide **3a** and *N*-methyl benzenesulfonamide **3b** were prepared as shown in Scheme 1. Thus,

Keywords: β_3-Adrenergic receptor; Agonist; Tryptamine.
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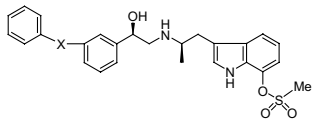
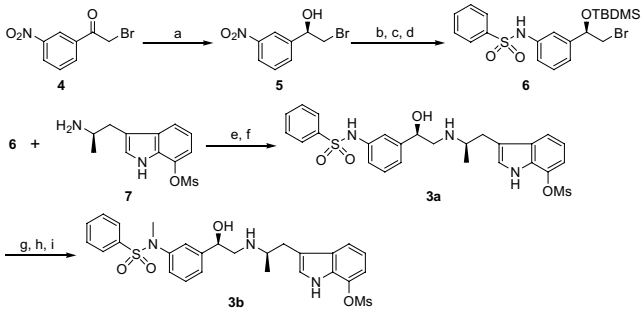
doi:10.1016/j.bmcl.2004.12.033

Table 1. Activity and cell permeability of tryptamine-based β_3-agonists

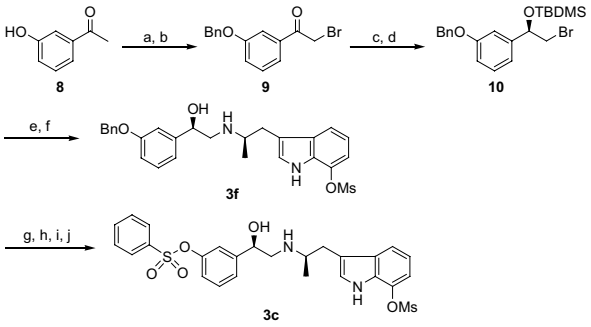


Compd	X	EC_{50}, nM[a] (IA, %)[b]			Permeability[c]
		β_3	β_1	β_2	$P_{app} \times 10^{-6}$ cm/s
3a	$-SO_2NH-$	0.54 (90)	13 (62)	19 (46)	nd (low)
3b	$-SO_2N(Me)-$	52 (38)	(3)[d]	(11)[d]	4.7 (high)
3c	$-SO_2O-$	32 (60)	(13)[d]	(4)[d]	7.0 (high)
3d	–CONH–	160 (96)	120 (96)	69 (82)	0.9 (moderate)
3e	$-SO_2CH_2-$	100 (54)	(4)[d]	(7)[d]	0.9 (moderate)
3f	$-CH_2O-$	15 (90)	(21)	46 (35)	6.7 (high)
3g	$-CH_2NH-$	11 (87)	(28)	16 (43)	9.0 (high)

[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) as a percentage of maximal stimulation with isoproterenol.
[c] Permeability of compounds was determined in Caco-2 cell monolayer permeability assay. The test compounds were classified for their absorption potential using high (>1.0), moderate (0.5–1.0) and low (<0.5) descriptions. nd = Not detected.
[d] EC_{50} was not determined. Values in parentheses represent percent activity at 1000 nM.



Scheme 1. Reagents: (a) (*R*)-2-methyl-CBS-oxazaborolidine, BH_3, THF; (b) TBDMSCl, imidazole, DMF; (c) Fe, NH_4Cl, EtOH, H_2O; (d) benzenesulfonyl chloride, pyridine, CH_2Cl_2; (e) *i*-Pr_2NEt, KI, MeCN; (f) 4 N HCl–AcOEt, EtOH; (g) $(Boc)_2O$, $CHCl_3$; (h) MeI, K_2CO_3, DMF; (i) 4 N HCl–AcOEt.

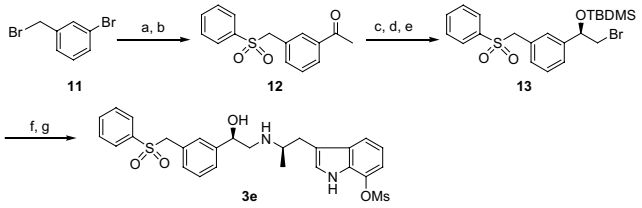


Scheme 2. Reagents: (a) benzyl bromide, K_2CO_3, DMF; (b) phenyltrimethylammonium tribromide, THF; (c) (*R*)-2-methyl-CBS-oxazaborolidine, BH_3, THF; (d) TBDMSCl, imidazole, DMF; (e) **7**, *i*-Pr_2NEt, KI, MeCN; (f) 4 N HCl–AcOEt, EtOH; (g) $(Boc)_2O$, $CHCl_3$; (h) H_2, 20% $Pd(OH)_2$, EtOH; (i) benzenesulfonyl chloride, Et_3N, CH_2Cl_2; (j) 4 N HCl–AcOEt.

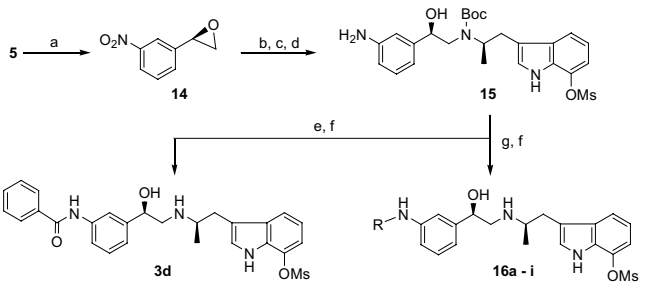
commercially available *m*-nitrophenacyl bromide **4** was reduced stereoselectively with borane–THF complex in the presence of (*R*)-2-methyl-CBS-oxazaborolidine providing chiral alcohol **5**.[2] Protection of the chiral alcohol **5** with TBDMS, followed by reduction of the nitro group with Fe, gave the corresponding aniline, which was treated with benzenesulfonyl chloride to afford **6**. Coupling of the resulting bromide **6** with (*R*)-α-methyl-7-methanesulfonyloxytryptamine **7**[2] in the presence of diisopropylethylamine and KI, followed by deprotection of TBDMS afforded the benzenesulfonamide **3a**, which was then converted to the *N*-methyl benzenesulfonamide **3b** through a three-step procedure involving protection of the secondary amine, N-methylation, and deprotection.

The benzenesulfonate **3c** was synthesized similarly to the preparation of **3a**, except reversing the order of the benzenesulfonyl group introduction (Scheme 2). Protection of the phenol **8** with the benzyl group, followed by bromination of the acetyl group using phenyltrimethylammonium tribromide gave **9**. Stereoselective reduction of **9** and protection of the resulting alcohol afforded **10**. The bromide **10** was then allowed to react with tryptamine **7** and deprotected to give the benzyloxy analog **3f**. Protection of the secondary amine with Boc and the benzyl group was removed by hydrogenolysis, exposing the phenolic alcohol, which was functionalized through esterification with benzenesulfonyl chloride. Deprotection of the Boc group afforded the desired benzenesulfonate **3c**.

The benzenesulfonylmethyl analog **3e** was prepared according to Scheme 3. The key step was the palladium-catalyzed conversion of an aryl bromide to the corresponding acetophenone **12**. The reaction of 3-bromobenzyl bromide **11** with benzenesulfinic acid sodium salt gave the benzenesulfonylmethylphenyl bromide,[6] which was used to palladium-catalyzed α-arylation of butyl vinyl ether.[7] The resulting acetophenone **12** was brominated, reduced stereoselectively, protected as de-



Scheme 3. Reagents: (a) benzenesulfinic acid sodium salt, DMF; (b) butyl vinyl ether, K_2CO_3, $Pd(OAc)_2$, 1,3-bis(diphenylphosphino)propane, DMF, H_2O; (c) phenyltrimethylammonium tribromide, THF; (d) (*R*)-2-methyl-CBS-oxazaborolidine, BH_3, THF; (e) TBDMSCl, imidazole, DMF; (f) **7**, *i*-Pr_2NEt, KI, MeCN; (g) 4 N HCl–AcOEt, EtOH.

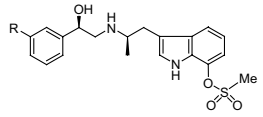


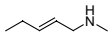
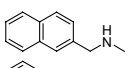
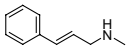
Scheme 4. Reagents: (a) 2 N NaOH, THF; (b) **7**, DMF; (c) $(Boc)_2O$, $CHCl_3$; (d) Fe, NH_4Cl, EtOH, H_2O; (e) PhCOCl, Et_3N, $CHCl_3$; (f) 4 N HCl–AcOEt; (g) R–Br, K_2CO_3 or Et_3N, DMF.

scribed in Scheme 2, and provide bromide **13**. Alkylation of tryptamine **7** with bromide **13** and the subsequent deprotection of Boc afforded the benzenesulfonylmethyl analog **3e**.

The general procedure for the preparation of 3-aniline derivatives with various R groups, through the alkylation of the key aniline intermediate **15**, is listed in Table 2 and shown in Scheme 4,. Therefore, the chiral alcohol **5** was treated with alkaline to form epoxide **14**. The reaction of the resulting epoxide **14** with tryptamine **7** afforded a secondary amine, which was then protected with Boc, and subsequently reduced the nitro group to give the aniline **15**. The functionalization of the amino group was accomplished by alkylation with the appropriate allyl or aralkyl bromide. Finally, deprotection of the Boc group furnished the desired anilines **16a**–**i**. Alternatively, the reaction of the aniline **15** with benzoyl chloride, followed by deprotection of the Boc group afforded the amide **3d**.

Table 2. Activity of tryptamine-based β_3-agonists[a]



Compd	R	EC_{50}, nM (IA, %)		
		β_3	β_1	β_2
16a		3.2 (110)	(49)[d]	2.2 (78)
16b		1.2 (102)	170 (30)	10 (64)
16c		9.0 (101)	(38)[d]	11 (49)
16d		36 (79)	160 (51)	36 (59)
16e		17 (114)	(27)[d]	1.5 (60)
16f		10 (102)	45 (52)	9.6 (67)
16g		6.5 (90)	(45)[d]	18 (61)
16h		2.9 (107)	44 (58)	7.6 (75)
16i		1.0 (97)	(36)[d]	13 (81)

[a] See footnotes in Table 1.
[d] EC_{50} was not determined. Values in parentheses represent percent activity at 1000 nM.

All final compounds synthesized were characterized using NMR, mass spectrometry, and HPLC.

3. Biological evaluation

All compounds were tested in vitro for their ability to stimulate cAMP accumulation in CHO cells expressing the cloned human β_1-, β_2-, and β_3-ARs.[8] Compounds with high potency were evaluated for their ability to permeate Caco-2 cell monolayers.[9]

To identify compounds with improved Caco-2 cell permeability, we initially prepared tryptamine analogs with various linkages instead of the sulfonamide linkage as shown in Table 1. The replacement of the sulfonyl group with carbonyl (amide **3d**) resulted in a substantially diminished potency for β_3-AR. The geometry of the planar amide in this position is not well-tolerated by β-ARs. A dramatic decrease in potency for all β-ARs was observed when the sulfonamide nitrogen atom was exchanged for a methylene group (sulfone **3e**), indicating that the heteroatoms play a role in receptor binding. The importance of this heteroatom was further confirmed with the improved potency of the benzyloxy (**3f**) and benzylamine (**3g**) analogs. Interestingly, the benzyloxy analog **3f** exhibited good agonistic activity for β_3-AR with improved Caco-2 cell permeability, whereas the sulfonate analog **3c** was a weak agonist and was only partially agonistic for β_3-AR. The benzylamine analog **3g** also exhibited good potency for β_3-AR with excellent subtype selectivity. It was noteworthy that benzylamine **3g** showed considerably improved Caco-2 cell monolayer permeability.

Since benzylamine analog **3g** exhibited a good agonistic profile with high cellular permeability properties, we used the aniline portion of **3g** as a template for a second SAR study wherein substituents on amino groups were examined (Table 2). We designed some allyl derivatives to reduce the molecular weight in anticipation of further improvement of cellular permeability. The replacement of the benzene ring in **3g** with a simple olefin dramatically improved agonistic activity for β_3-AR, but resulted in an increase in potency against β_2-AR (**16a**–**c** vs **3g**). Crotyl analog **16b** exhibited maximum potency in this series (EC_{50} = 1.2 nM, IA = 102%). Geminal substitution of the double bond in **16a** was unfavorable to

Table 3. Binding affinity and cell permeability of tryptamine-based β_3-agonists

Compd	Binding K_i, nM[a]			Permeability[b]
	β_3	β_1	β_2	$P_{app} \times 10^{-6}$ cm/s
3a	3.3	44	23	nd (low)
16b	33	150	18	7.3 (high)
16i	15	48	25	2.1 (high)

[a] Binding potency is reported as K_i, the binding inhibition constant, determined by inhibition of ^{125}I-iodocyanopindolol binding.
[b] See footnote c in Table 1.

β_3-agonistic activity (**16d** and **16e**). Interestingly, **16e** showed strong activity against β_2-AR (EC_{50} = 1.5 nM), while the potency against β_1-AR was extremely weak. Acetylene substituents tended to exert little influence on the potency for β_3-AR (**16f** and **16g** vs **3g**).

A very interesting observation was made upon the expansion of the benzene ring to the naphthalene ring (Table 2). The β_3-agonistic potency of **16h** increased 4-fold compared to that of **3g**. As with the olefin compounds, geminal substitution of the double bond was expected to decrease the potency for β_3-AR, we therefore, designed cinnamylamine analog **16i**. As expected, cinnamylamine analog **16i** showed strong activity for β_3-AR (EC_{50} = 1.0 nM, IA = 97%). This compound also showed excellent selectivity against β_1-AR and modest activity against β_2-AR.

To evaluate the possibility of this series for drug candidates, the selected compounds were subjected to receptor binding assays. As shown in Table 3, the binding potency of crotyl analog **16b** decreased by one order of magnitude compared to that of **3a** (K_i = 33 and 3.3 nM, respectively). Furthermore, **16b** exhibited reversal selectivity in binding regarding β_3 versus β_2-AR compared with the agonistic activity. Cinnamylamine analog **16i** also showed a decreased binding constant (K_i = 15 nM), but possessed moderate selectivity against β_1 and $_2$-AR. Since the cinnamylamine analog **16i** showed high Caco-2 cellular permeability (P_{app} = 2.1 $P_{app} \times 10^{-6}$ cm/s), the pharmacokinetic property was determined in rats (5 mg/kg po, 0.5 mg/kg iv).[10] As expected from the Caco-2 experiments, a large improvement was observed with the cinnamylamine analog **16i**; its oral bioavailability in the rat was 21%. In addition, the half-life of **16i** was 4.2 h. These data indicate that the sulfonamide moiety is detrimental to oral absorption.

In conclusion, we have discovered aniline analogs as potent and selective β_3-agonists with excellent Caco-2 cell monolayer permeability. The cinnamylamine analog **16i** exhibited good permeability and showed improved oral bioavailability in rats. Further studies are being conducted to optimize the in vitro profile of this series and will be reported in due course.

Acknowledgements

We would like to thank San Francisco Edit (www.sfedit.net) for their assistance in editing this manuscript.

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8. In a previous report,[1] 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). To better evaluate the subtype selectivity, we used CHO cells expressing low densities of β_3-AR (13,000 receptors/cell), and high densities of β_1- and β_2-ARs in this report (320,000 and 600,000 receptors/cell, respectively). The CHO cells expressing either human β_1-, β_2-, or β_3-AR were prepared as described in Kato, S.; Harada, H.; Taoka, I.; Kawashima, H. PCT Patent Application, WO 2000044721, 2000; Kato, S.; Harada, H.; Hirokawa, Y.; Yoshida, N.; Kawashima, H. PCT Patent Application, WO 9616938, 1996.
9. The apparent permeability coefficients (Papp) were determined in the apical-to-basolateral (A-to-B) direction across the cell's monolayers cultured on polycarbonate membrane filters.
10. Compound **16i** was dosed orally at 5 mg/kg in 1% hydroxypropyl cellulose aqueous solution and intravenously at 0.5 mg/kg in a vehicle consisting of 10% DMSO, 10% EtOH, 20% polyethylene glycol and saline. After dosing, blood samples were collected at 5 and 15 min (iv only), and 0.5, 1, 2, 4, 6, 8, 24 h (iv and po) to determine plasma drug concentrations by LC/MS/MS.