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Novel and Potent Human and Rat β_3-Adrenergic Receptor Agonists Containing Substituted 3-Indolylalkylamines

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Abstract—A novel series of 2-(3-indolyl)alkylamino-1-(3-chlorophenyl)ethanols was prepared and evaluated for in vitro ability to stimulate cAMP production in Chinese hamster ovary cells expressing cloned human β_3-AR. The optically active **30a** was found to be the most potent and selective human β_3-AR agonist in this series with an EC_{50} value of 0.062 nM. In addition, **30a** selectivity for human β_3-AR was 210-fold and 103-fold that for human β_2-AR and β_1-AR, respectively. Furthermore, **30a** showed potent agonistic activity at rat β_3-AR.

Introduction

β-Adrenergic receptors (β-ARs) have been subclassified as β_1- and β_2-ARs since 1967.[1] A third β-AR, initially referred to as 'atypical'[2] and later called β_3-AR[3,4] has been found in a number of species,[5–7] including man in the early 1980s.[4] The β_3-AR is located on the cell surface of both white and brown adipocytes and its stimulation promotes both lipolysis and energy expenditure.[8]

Since the discovery of β_3-AR, a number of laboratories have been engaged in developing potent and selective β_3-AR agonists for the treatment of obesity and non-insulin dependent (Type-II) diabetes.[9] Early β_3-AR agonists (the 'first generation' of potent and selective rat β_3-AR agonists) such as, BRL 37344,[10] CL 316243,[11] and SR 58611A,[12] having a 3-chlorophenyl moiety in the left-hand side and a carboxylic acid or an ester functionality in the right-hand side as shown in Figure 1, were reported to be effective anti-obesity and anti-diabetic agents in rodents.[13]

However, human clinical trials with these drugs for the treatment of metabolic disorders have been disappointing due to a lack of selectivity and/or potency or poor pharmacokinetics.[14] Because of the structural differences between human and rat β_3-ARs, activity at the rat β_3-AR could not effectively predict that at the human β_3-AR.[15] Thus, a new generation of human β_3-AR agonists with minimal side effects associated with activation of human β_1- and β_2-ARs has long been needed.

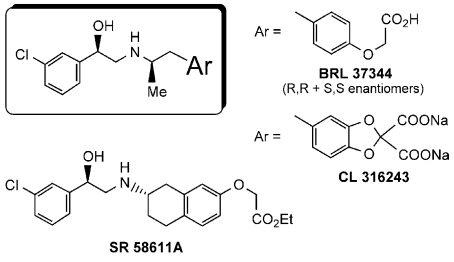

Figure 1.

At the beginning of 1990 and on the basis of results obtained from random screening for rat β_3-AR agonists, we found that a novel 2-[2-(3-indolyl)ethylamino]-1-(3-chlorophenyl)ethanol (**7**) having the 3-chlorophenyl moiety structure known to be required for β_3-AR agonistic activity, potently inhibited rat spontaneous colonic contraction (β_3-AR; $EC_{50} = 22.9 \pm 3.1$ nM) and only slightly relaxed both rat uterus (β_2-AR; $EC_{50} = 577.3 \pm 149.4$ nM) and guinea-pig trachea (β_1-AR; $EC_{50} = > 10{,}000$ nM). In order to improve the selectivity of lead compound **7**, we focused on the introduction of various substituents into the indole nucleus and the

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side-chain at the 3-position of the indole ring, and performed optical resolution of selected compounds. Additionally, to develop potent and selective human β_3-AR agonists, we examined adenylyl cyclase activity using Chinese hamster ovary (CHO) cell lines expressing human β_1-, β_2-, and β_3-ARs.

Structure–activity relationship (SAR) studies of various 2-(3-indolyl)alkylamino-1-(3-chlorophenyl)ethanols led to the discovery of the optically active **30a**, which is a potent human and rat β_3-AR agonist with low activity for human β_1- and β_2-ARs.

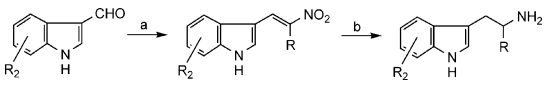
In this paper, we describe the synthesis and SARs of 3-indolylethanolamine derivatives while keeping the 3-chlorophenyl moiety constant as an aryl group.

Chemistry

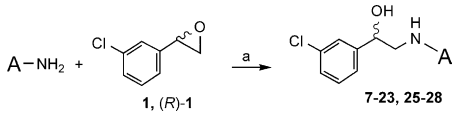
The requisite intermediate tryptamine derivatives were prepared using procedures previously described in the literatures.[16] In general, the 3-formylindoles obtained by Vilsmeier reaction ($POCl_3$, DMF) of the substituted indoles were treated with nitroalkane in AcOH to produce nitroolefins in good yield. Conjugated nitroolefins were directly reduced by $LiAlH_4$ to give saturated primary amines although the yield was moderate to low (Scheme 1).

Most of the compounds (**7**–**28** except **24**) listed in Tables 1, 3, and 4 were prepared by coupling reaction of the racemic 3-chlorostyrene oxide **1** or its optical isomer[17] (*R*)-**1** with various tryptamine derivatives in MeOH (Scheme 2).

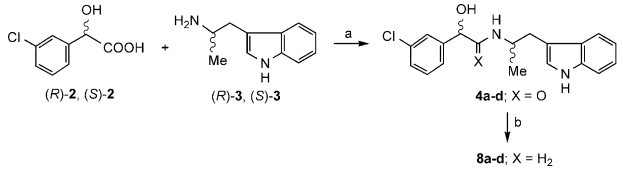
The optically active **8a**–**d** listed in Table 2 were synthesized by treatment of the optically active 3-chloromandelic acids[18] (*R*)-**2** and (*S*)-**2** with the optically active α-methyltryptamines[19] (*R*)-**3** and (*S*)-**3** using BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate) as a coupling reagent followed by reduction of the amide group of **4a**–**d** with borane (Scheme 3).



Scheme 1. (a) $MeNO_2$ or $EtNO_2$, AcOH; (b) $LiAlH_4$, THF.

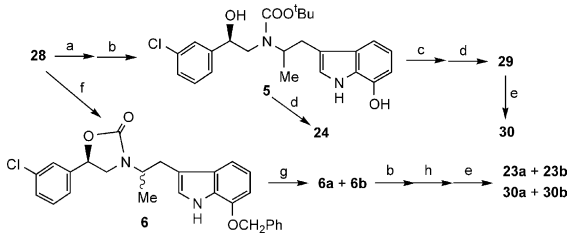


Scheme 2. (a) MeOH.



Scheme 3. (a) Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), DMF; (b) BH_3·THF.

The 2-[3-(7-*O*-substituted 3-indolyl)-2-propylamino]-1-(3-chlorophenyl)ethanols **24** and **30** as a mixture of diastereomers and the selected optical isomers **23a**,**b** and **30a**,**b** listed in Tables 4 and 5 were synthesized as shown in Scheme 4.

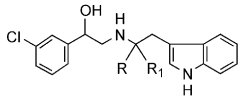


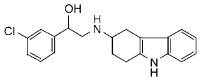
Scheme 4. (a) Boc_2O; (b) Pd/C, H_2, chlorobenzene; (c) $ClCH_2CO_2Me$, K_2CO_3, KI; (d) aqueous HCl; (e) aqueous NaOH; (f) *N*,*N*′-carbonyldiimidazole; (g) separation by silica gel column chromatography; (h) $ClCH_2CO_2Me$ or MeI.

Protection of the secondary amine functionality of the 7-benzyloxytryptamine **28** with Boc group followed by catalytic hydrogenation in the presence of chlorobenzene to avoid removal of the 3-chlorine atom in the benzene ring produced the 7-hydroxytryptamine **5**. Reaction of **5** with methyl chloroacetate, followed by removal of the Boc protecting group under acidic conditions furnished the 7-methoxycarbonylmethoxytryptamine **29**. Subsequent alkaline hydrolysis of **29** and acid hydrolysis of **5** gave **30** and **24**, respectively.

The optically active **23a**,**b** and **30a**,**b** having 7-methoxy- and 7-carboxylmethoxy groups, respectively (Table 5), were synthesized as follows. Protection of the aminoethanol moiety of **28** with a carbonyl group, followed by silica gel column chromatography separation of the resulting diastereomer **6** gave the optical isomers **6a**,**b** having *R*- and *S*-configuration in the α-methyl group, respectively. A similar method to that described for the

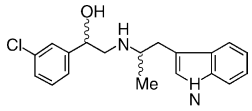
Table 1. Human β_3-AR agonistic activity of 2-(3-indolyl)alkylamino-1-(3-chlorophenyl)ethanols **7**–**11**[a]



Compd	R	R_1	Human β_3-AR	
			EC_{50} (nM) (IA%)[b]	cAMP accumulation (% at 10^{-7} M)[c]
7[d]	H	H	69 (99)	
8[e]	Me	H	12 (114)	
9[e]	Et	H		20
10[d]	Me	Me	70 (118)	
11[e]				0

[a]β_3-AR agonistic activity was assessed by measuring cAMP accumulation in CHO cells expressing human β_3-AR (150,000 receptors/cell).
[b]The maximal amount of cAMP obtained by (−)-isoproterenol and the amount of cAMP in the absence of agonists were defined as 100 and 0%, respectively, and the relative maximal response of each compound is expressed as intrinsic activity (IA). EC_{50} value is a concentration of the test compound to be required to achieve 50% of cAMP accumulation.
[c]Activity relative to (−)-isoproterenol.
[d]Racemic mixture.
[e]Mixture of four diastereomers.

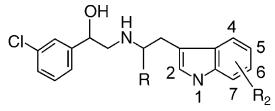
Table 2. β-AR agonistic activity of compound **8** and its individual diastereomers at cloned human β_1-, β_2-, and β_3-ARs and at the cloned rat β_3-AR[a]



Compd	Configuration of hydroxy center	Configuration of methyl center	EC_{50} (nM) (IA%)[b]			
			Human β_3-AR	Human β_2-AR	Human β_1-AR	Rat β_3-AR
8	Mixture	Mixture	12 (114)	23 (46)	NT[d]	0.97 (107)
8a	*R*	*R*	5.4 (110)	25 (50)	1.9 (65)	0.36 (98)
8b	*R*	*S*	240 (97)	—[c]	9.4 (50)	13 (96)
8c	*S*	*R*	220 (119)	330 (23)	47 (70)	11 (108)
8d	*S*	*S*	3300 (62)	—[c]	140 (47)	33 (108)

[a]β-ARs agonistic activity were assessed by measuring cAMP accumulation in CHO cells expressing various β-ARs. Expression levels[21] of β-ARs were 150,000 receptors/cell, 30,000 receptors/cell, 12,000 receptors/cell and 880,000 receptors/cell for human β_3-, β_2-, β_1-, and rat β_3-ARs.
[b]See footnote b in Table 1.
[c]—, could not be calculated because of low IA.
[d]NT, not tested.

Table 3. Human β_3-AR agonistic activity of substituted indole derivatives **12**–**22**[a]



Compd	R	R_2	Human β_3-AR	
			EC_{50} (nM) (IA%)[b]	cAMP accumulation (% at 10^{-7} M)[c]
12[d]	H	1-Me		6
13[d]	H	2-Me		5
14[d]	H	4-Me		20
15[e]	Me	4-Me	96 (165)	
16[d]	H	5-Me		7
17[e]	Me	6-Me	12 (95)	
18[d]	H	7-Me		0
19[e]	Me	6-MeO	22 (102)	
20[e]	Me	7-MeO	1.7 (113)	
21[e]	Me	6-Cl	28 (131)	
22[e]	Me	6-Br	38 (98)	

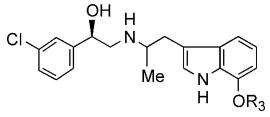
[a]See footnote a in Table 1.
[b]See footnote b in Table 1.
[c]See footnote c in Table 1.
[d]See footnote d in Table 1.
[e]See footnote e in Table 1.

preparation of **30** from **28** was applied to the preparation of the desired optical isomers **23a**,**b** and **30a**,**b**. The absolute configuration of **6b** (*S*-configuration) and **30a** (*R*-configuration) was confirmed by X-ray crystallography, and the ORTEP diagram of **30a** is shown in Figure 2.[20]

Results and Discussion

Activation of β-ARs by the various compounds prepared in this study was assessed by measuring cAMP accumulation in CHO cells expressing cloned human β_1-, β_2-, and β_3-ARs and rat β_3-AR. As shown in Table 1, the lead compound **7** exhibited a relatively modest agonistic activity at the human β_3-AR ($EC_{50} = 69$ nM).

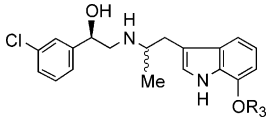
Table 4. Human β_3-AR agonistic activity of 7-*O*-substituted indole derivatives **23**–**30**[a]



Compd[c]	R_3	Human β_3-AR
		EC_{50} (nM) (IA%)[b]
23	Me	0.67 (114)
24	H	1.7 (128)
25	Et	0.96 (96)
26	Pr	14 (103)
27	*iso*-Pr	2.8 (87)
28	CH_2Ph	11 (114)
29	CH_2CO_2Me	0.92 (102)
30	CH_2CO_2H	0.11 (124)

[a]See footnote a in Table 1.
[b]See footnote b in Table 1.
[c]Mixture of *R,R* and *R,S* diastereomers.

Table 5. β-AR agonistic activity of optically active 7-*O*-substituted indole derivatives **23a**,**b**, **30a**,**b**, and reference compounds at cloned human β_1-, β_2-, and β_3-ARs and at the cloned rat β_3-AR[a]



Compd	Configuration of methyl center	R_3	EC_{50} (nM) (IA%)[b]			
			Human β_3-AR	Human β_2-AR	Human β_1-AR	Rat β_3-AR
23a	*R*	Me	0.36 (89)	5.2 (46)	0.13 (118)	0.15 (147)
23b	*S*	Me	120 (51)	—[c]	130 (83)	6.5 (121)
30a (AJ-9677)	*R*	CH_2CO_2H	0.062 (116)	13 (26)	6.4 (26)	0.016 (110)
30b	*S*	CH_2CO_2H	10 (84)	—[c]	14 (107)	1.2 (106)
BRL 37344			21 (95)	290 (31)	1700 (17)	0.095 (109)

[a]See footnote a in Table 2.
[b]See footnote b in Table 2.
[c]See footnote c in Table 2.

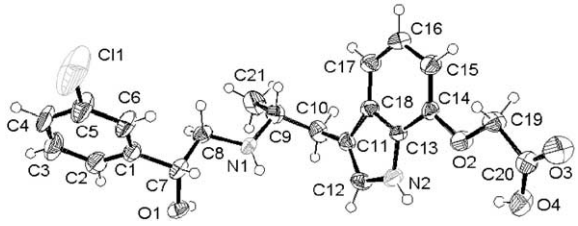


Figure 2. The ORTEP drawing of **30a** with thermal ellipsoids at 50% probabilities.

Introduction of a methyl group (yielding **8**) into the tryptamine side-chain of **7** resulted in good improvement in β_3-AR agonistic activity ($EC_{50} = 12$ nM, intrinsic activity (IA) value of 114%). However, introduction of an ethyl group (yielding **9**) resulted in low activity at the human β_3-AR. The α,α-dimethyltryptamine **10** had an activity nearly equipotent to that of the parent compound (**7**), and the tetrahydrocarbazole **11** showed significantly poor activity.

From the study on the β-ARs agonistic activity of the four optical isomers of BRL 37344, the (*R,R*)-configuration

had proved to be important for enhancing rat β_3-AR agonistic activity.[22] Thus, the four optical isomers **8a–d** of the selected compound **8** were prepared and their agonistic activity at the human β_1-, β_2-, and β_3-ARs and rat β_3-AR was evaluated. As expected, the optical isomer **8a**, having *R*-configuration in both the hydroxy and α-methyl centers, exhibited a potent agonistic activity at the human and rat β_3-ARs compared with other stereoisomers (**8b–d**). Although **8a** had an agonistic activity at human and rat β_3-ARs 2–3 times more potent than that of the original compound **8**, it was completely non-selective. Other enantiomers (**8b–d**) showed low activity at all β-ARs (Table 2).

Next, we examined the agonistic activity of various substituted tryptamine derivatives. Introduction of a methyl group at 1-, 2-, 4-, 5-, and 7-positions of the indole ring of **7** and **8** resulted in low agonistic activity at the human β_3-AR (compounds **12–16** and **18**, Table 3). The 6-methyl group was well tolerated and **17** displayed comparable agonistic activity to that of its parent **8**. The 6-methoxytryptamine **19** and the tryptamine derivatives **21** and **22** with chlorine and bromine at the 6-position, respectively, were weak human β_3-AR agonists compared with **8**. Fortunately, the 7-methoxyindole counterpart **20** showed much more potent agonistic activity than **8**. From the above SAR studies, the 7-methoxytryptamine **20** was found to exhibit the most preferred agonistic activity at human β_3-AR.

Because the (*R*)-hydroxy isomers of the hydroxy center were more potent than the corresponding (*S*)-hydroxy derivatives, 7-*O*-substituted indole analogues with an (*R*)-hydroxy group were prepared and tested for their agonistic activity at the human β_3-AR. As shown in Table 4, the 7-methoxyindole derivative **23** showed an activity ca. 2.5-fold more potent than that of **20**. Removal of the methyl group on the methoxy substitution gave derivative **24**, which exhibited a decreased agonistic activity at the human β_3-AR. When the methoxy group of **23** was replaced with an ethoxy and methoxycarbonylmethoxy groups, the resultants **25** and **29** exhibited approximately equal agonistic activity at human β_3-AR. A significant loss in activity was observed with the 7-propoxy (**26**), 7-isopropoxy (**27**), and 7-benzyloxy (**28**) derivatives. Replacement of the methoxy group of **23** by a carboxylmethoxy group (yielding **30**) led to a 6-fold improvement in human β_3-AR agonistic activity.

Finally, the 7-methoxy and 7-carboxylmethoxyindole derivatives (**23** and **30**, respectively) with potent agonistic activity at human β_3-AR were selected for diastereomers separation and agonistic activity examination at human β_1-, β_2-, and β_3-ARs and rat β_3-AR. As shown in Table 5, the optical isomers **23a** and **30a** with an (*R*)-α-methyl group were more potent than the corresponding diastereomers **23b** and **30b**. However, **23a** exhibited poor selectivity for human β_3-AR as it potently stimulated both human β_1- and β_2-ARs. On the other hand, the selectivity of **30a** for human β_3-AR was high. The optically active **30a** showed a potent agonistic activity at human and rat β_3-ARs with selectivity for human β_3-AR over 100-fold that for the β_1-AR and 200-fold that for the β_2-AR. Introduction of a carboxylmethoxy group into the indole ring of **30a** led to a much more potent agonistic activity at the human β_3-AR and ca. 6-fold increase in activity at the rat β_3-AR as compared to BRL 37344. The presence of the 7-carboxylmethoxy group and the *R*-configuration for the α-methyl group were therefore found to be necessary for potent agonistic activity and selectivity.

In conclusion, the synthesis and SAR studies of substituted tryptamine derivatives based on human β_3-AR agonistic activity have been discussed. Introduction of a carboxylmethoxy group at the 7-position of the indole ring resulted in the identification of a potent human ($EC_{50} = 0.062$ nM) and rat ($EC_{50} = 0.016$ nM) β_3-ARs full agonist **30a** (AJ-9677). Additionally, this compound (**30a**) showed good selectivity for human β_3-AR as compared to that for human β_1- and β_2-ARs.

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