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Discovery of a novel, potent and selective human β_3-adrenergic receptor agonist

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Abstract—The discovery of a novel, potent and selective β_3-adrenergic receptor (AR) agonist is described. SAR studies demonstrated the structural requirements for activity and selectivity. Compound **1c**, which showed good β_3-AR activity and selectivity, was identified and pharmacokinetics were investigated.

1. Introduction

The β_3-adrenergic receptor (AR), which is present on the surface of adipocytes, plays a significant role in mediating lipolysis in white adipocyte tissue and thermogenesis in brown adipocyte tissue.[1,2] It has been reported that stimulation of β_3-AR induces a variety of pharmacological effects such as increase of fat oxidation, enhancement of energy expenditure and improvement in insulin-mediated glucose uptake in rodent models and thus β_3-AR agonists have been developed as therapeutic candidates for obesity and type II diabetes.[3] Recent studies indicated that in addition to adipocytes, the β_3-AR is also distributed in gall bladder, gastrointestinal tract and prostate,[4] therefore new therapeutic applications of β_3-AR agonists in treatment of gastrointestinal and urinary disease have been studied.[5–7] Early β_3 agonists, which had been developed using rodent models showed insufficient effects in clinical trials due to weak agonistic activity for the human β_3 receptor in spite of high potency for rodent receptors.[8] Thus, potent β_3-AR agonists against the human receptor are required. Furthermore, β_3-AR selectivity over β_1-AR and β_2-AR is also important, because stimulation of β_1-AR and β_2-AR may induce severe side effects such as enhancement of heart rate and tracheal relaxation, respectively. In our laboratory and in others, the search for novel, potent human β_3-AR agonists has been ongoing for a number of years.[9]

2. Design

Our design concept is outlined in Figure 1. We planned to introduce a hydroxymethyl group into the core

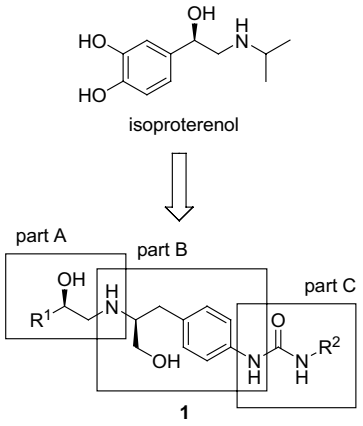

Figure 1. Design of β_3 agonists.

Keyword: β_3-Adrenergic receptor agonist.
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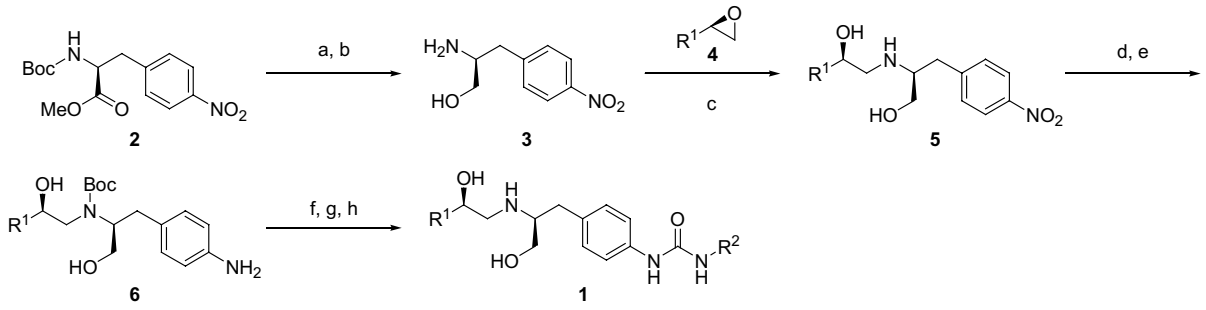


Scheme 1. Reagents: (a) $NaBH_4$, THF, MeOH; (b) HCl, dioxane; (c) **4**, EtOH; (d) Boc_2O, THF; (e) Fe, AcOH, EtOH, H_2O; (f) BSA, 1-methyl-2-pyrrolidinone; (g) R^2–NCO, DIEA, 1-methyl-2-pyrrolidinone; (h) TFA or HCl.

scaffold (part B) to attempt to improve β_3-AR selectivity, water solubility and pharmacokinetics. The left-wing (part A) represents the core pharmacophore of β_3-AR agonists, an aminoethanol moiety. The right-wing (part C) is considered critical for β_3-AR selectivity since isoproterenol is a non-selective β-AR agonist. We designed compounds **1** based on a urea scaffold to facilitate rapid synthesis and SAR evaluation. This paper describes these efforts and the discovery of a novel, potent analogue.

3. Synthesis

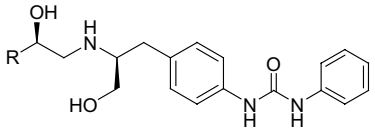
Our general synthetic approach to the compounds in this letter is illustrated in Scheme 1. Boc-protected *p*-nitrophenylalanine methyl ester **2** was reduced to the alcohol with $NaBH_4$. Removal of the Boc group under acidic conditions gave amine **3**. The first point of diversity was introduced by coupling of **3** with various epoxides **4**. The resulting amines **5** were protected as Boc and the nitro group was reduced to afford key amines **6**. The second point of diversity was introduced by parallel coupling of **6** with a variety of isocyanates. For efficient urea formation, pretreatment of **6** with *N*,*O*-bis(trimethylsilyl)-acetamide (BSA) to protect the hydroxyl groups, addition of isocyanate and finally acidic deprotection of the silyl and Boc protecting groups afford the desired urea derivatives **1** in good yield.

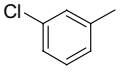
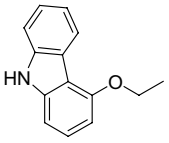
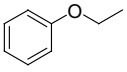
4. Results and discussion

All compounds were evaluated for their ability to produce cAMP in Chinese hamster ovary (CHO) cell lines expressing cloned human β_3-AR. Selected compounds were also evaluated for human β_1 or β_2-AR activity using a similar method.

We first aimed to determine whether a urea type structure would show potent β_3-AR agonist activity, by introducing representative left-wing structures to evaluate potential. Table 1 shows these results. Using 3-chlorophenyl, carbazoloxymethyl and phenoxymethyl as representatives, it can be seen that urea compounds have moderate to potent activity (**1a**, **1b** and **1c**). In particular, the phenoxymethyl compound **1c** displayed potent activity as compared to isoproterenol.

Table 1. Effect of conversion of left-wing part on β_3-AR activity

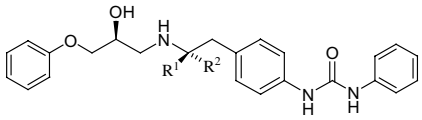


Compd	R	Human β_3 EC_{50} (nM)[a]
1a		74
1b		22
1c		0.5
Isoproterenol		0.97

[a] β_3-AR agonistic activity was assessed by measuring cAMP accumulation in CHO cell lines expressing cloned human β_3-AR.

Next, we confirmed the importance of the hydroxymethyl group in the scaffold, as indicated in Table 2. Compound **1d** the stereoisomer of **1c**, showed a 26-fold decrease in β_3-AR activity. The stereochemistry of the hydroxymethyl group contributed to enhancement of β_3-AR activity. Compound **1e** with no substituent, was 3-fold less potent in β_3-AR activity. Therefore, it is suggested that the hydroxymethyl group may interact with the β_3-AR and the spatial configuration is important. Furthermore β_3-AR selectivity over β_1-AR of these compounds was evaluated. Compound **1c** showed 40-fold β_1/β_3 selectivity, whereas compound **1d** and **1e** resulted in more than 10-fold increase in β_1-AR activity and decrease in β_1/β_3 selectivity. Interestingly, compound **1d** and **1e** showed β_1-AR selectivity and non-selectivity, respectively. These results indicate that the presence and stereochemistry of the hydroxymethyl group enhanced the β_3-AR selectivity as well as the β_3-AR activity. In addition, compound **1c** was investigated for β_2/β_3 selectivity and was shown to be inactive towards the β_2-AR.

Table 2. Effect of hydroxymethyl group on β_3-AR activity and selectivity

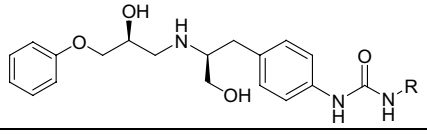


Compd	R^1	R^2	Human β_3 EC_{50} (nM)[a]	Human β_1 EC_{50} (nM)[a]	β_1/β_3 Selectivity	Human β_2 EC_{50} (nM)[a]	β_2/β_3 Selectivity
1c	CH_2OH	H	0.5	20	40	>100	>200
1d	H	CH_2OH	13	3.0	0.2	NT	—
1e	H	H	1.6	3.6	2.3	NT	—
Isoproterenol			0.97	0.084	0.087	2.0	2.1

[a] β-AR agonistic activity was assessed by measuring cAMP accumulation in CHO cell lines expressing cloned human β-ARs.

Table 3 shows the effect of modification of the right part. This moiety was expected to greatly affect the β_3-AR activity and selectivity. First, conversion of the terminal phenyl ring to an alkyl group was examined. For β_3-AR activity, while a bulky group such as cyclohexyl group was effective to maintain activity, the alkyl substituted derivatives, **1f**, **1g** and **1h**, were less potent than compound **1c**. This suggested that, in addition to bulkiness, aromaticity in the right portion was significant for β_3-AR activity. Next, we focused on introduction of substituents to the right terminal phenyl ring. The methoxy substituents, **1i**, **1j** and **1k**, maintained β_3-AR activity compared to lead compound **1c**, but increased β_1-AR activity and therefore lowered the β_1/β_3 selectivity of these compounds. While the position of the methoxy group affected β_1-AR activity and the β_1/β_3 selectivity was increased in the order *ortho* > *meta* > *para* substituent, these analogues showed less than 10-fold β_1/β_3 selectivity. Introduction of carboxylic acid (**1l**) and nitro group (**1m**) also resulted in an increase in β_1-AR activity and a decrease in β_1/β_3 selectivity. The effect of substituents appeared to influence β_1/β_3 selectivity and the functional group on the right terminal phenyl ring was suggested to contribute to interaction with the β_1-AR. The nonsubstituted phenyl analogue **1c** was consequently judged to be the most potent and selective.

Table 3. Effect of conversion of right-wing part on β_3-AR activity and selectivity



Compd	R	Human β_3 EC_{50} (nM)[a]	Human β_1 EC_{50} (nM)[a]	β_1/β_3 Selectivity
1c	Ph	0.5	20	40
1f	*c*-Hex	2.1	15	7.1
1g	*n*-Pr	8.3	51	6.1
1h	*i*-Pr	4.9	27	5.5
1i	2-OMe–Ph	1.1	12	11
1j	3-OMe–Ph	0.8	4.0	5.0
1k	4-OMe–Ph	2.5	2.2	0.9
1l	3-Carboxy–Ph	1.5	1.3	0.9
1m	3-NO_2–Ph	1.0	0.9	0.9

[a] β-AR agonistic activity was assessed by measuring cAMP accumulation in CHO cell lines expressing cloned human β-ARs.

The most potent and selective analogue **1c** was investigated in a pharmacokinetic (PK) study as shown in Table 4. Oral bioavailability of compound **1c** was low in rats and moderate in dogs ($F = 1.1\%$ and 20.3%, respectively). Values of clearance significantly differed in rats and dogs. These results would be attributed to species difference in metabolic stability. In dogs, however, compound **1c** showed high AUC and long half-life, indicating long duration of action.

5. Summary

In summary, we have discovered a number of novel and potent β_3-AR agonists. A SAR study revealed that the hydroxymethyl and phenylurea groups were important for β_3-AR activity and selectivity. Compound **1c** was identified as the most potent and selective in this series of β_3-AR agonists. In a PK study, compound **1c** showed prolonged plasma concentration and reasonable oral bioavailability in dogs.

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Table 4. Pharmacokinetic parameters of **1c** after po and iv administration to rats and dogs

	po[a]			iv[a]			*F* (%)
	Dose (mg/kg)	C_{max} (ng/mL)	AUC_{0-24} (ngh/mL)	Dose (mg/kg)	$t_{1/2}$ (h)	CL_{tot} (mL/min/kg)	
Rat	3.2	4.2	4.2	1.0	1.3	141.8	1.1
Dog	1.0	52.6	140.1	0.32	8.1	24.2	20.3

[a] $n = 3$.

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