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Discovery of novel series of benzoic acid derivatives containing biphenyl ether moiety as potent and selective human β_3-adrenergic receptor agonists: Part IV

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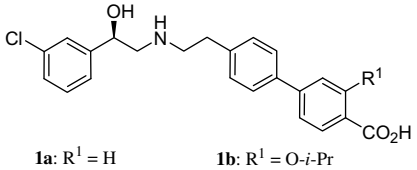
ABSTRACT

Identification and SAR study of novel series of β_3-AR agonists with benzoic acid are described. Conversion of ether linkage position of phenoxybenzoic acid derivative **2b** led to compound **7b** with moderate β_3-AR activity. Further modification in right, center and left parts of compound **7b** was investigated to improve the β_3-AR potency and selectivity. Compounds **7g** and **7k**, with the bulky aliphatic-substituted group at 2-position of benzoic acid moiety, were identified as potent and selective β_3-AR agonists. In addition, in vivo efficacy of compounds **7g** and **7k** was exhibited on dog OAB model.

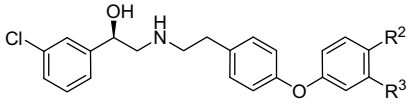
Overactive bladder (OAB) is defined as urinary urgency with or without urgency incontinence[1] and the number of patients with OAB is estimated to be about 16% of adult population in the United Sates and is increasing steadily worldwide.[2] Symptoms of OAB such as urinary frequency and nocturia affect quality of life (QOL) of the patients and the therapeutic agents of the disease are required. Although anti-muscarinic agents are today mainly used for the treatment of OAB, adverse effects such as dry mouth and constipation are unavoidable.[3] The β_3-adrenergic receptor (AR), which had identified as the third subtype of β-ARs in 1980s,[4] is present on the surface of both white and brown adipocytes, gall bladder, gastrointestinal tract, and urinary bladder detrusor tissues.[5] It was reported that stimulation of the β_3-AR induced a variety of pharmacological effects such as lipolysis and thermogenesis in adipocytes.[6] A number of β_3-AR agonists have been developed as anti-obesity or type II diabetes agents[7,8] and tested in clinical trials. Recently, in addition to the effects for metabolic diseases, it has been reported that β_3-AR is predominantly expressed in detrusor tissues in human[9] and activation of the β_3-AR induces relaxation of urinary bladder detrusor.[10] Therefore, β_3-AR agonist is expected to be a new therapeutic candidate for OAB.[11,12] For the development of β_3-AR agonists, the selectivity toward the β_1-AR is also important because stimulation of β_1-AR may induce severe side effect such as enhancement of heart rate.

In previous report,[13a] we investigated the structure–activity relationships (SARs) of novel series of biphenyl acid derivatives



1a: R^1 = H
β_3 EC_{50} = 6.7 nM
β_1 EC_{50} = 280 nM

1b: R^1 = O-*i*-Pr
β_3 EC_{50} = 1.1 nM
β_1 EC_{50} = 720 nM



2a: R^2 = CO_2H, R^3 = H
β_3 EC_{50} = 100 nM
β_1 EC_{50} = > 100 nM

2b: R^2 = H, R^3 = CO_2H
β_3 EC_{50} = 39 nM
β_1 EC_{50} = > 100 nM

Figure 1. Biphenyl acid and phenoxybenzoic acid derivatives.

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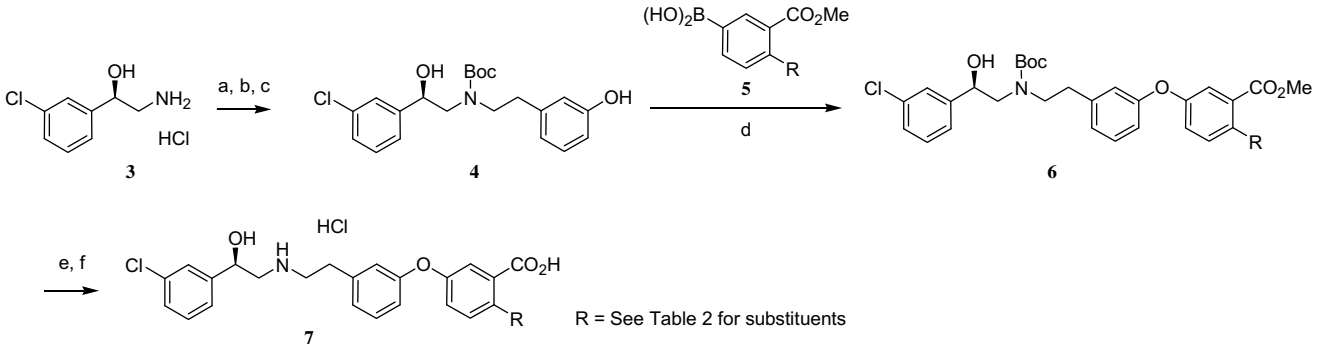


Scheme 1. Reagents: (a) 3-hydroxyphenylacetic acid, WSCD, HOBt, DMSO; (b) 1 N BH_3–THF complex, THF; (c) Boc_2O, 3 N NaOH aq; (d) $Cu(OAc)_2$, pyridine, MS 4 Å, CH_2Cl_2; (e) 1 N NaOH aq, MeOH; (f) 4 N HCl in dioxane.

such as compound **1a** and discovered compound **1b** with isopropoxy group in right phenyl ring as potent and selective β_3-AR agonists (Fig. 1). In the course of the SAR study, the phenoxybenzoic acid derivatives (compound **2a** and **2b**) were found to show equivalent PK profile with high C_{max} and AUC values compared to the biphenyl acid derivatives **1a** and **1b**, although the β_3-AR activity was low to moderate. In order to improve the insufficient β_3-AR potency, we investigated the SAR of right, center, and left parts in the phenoxybenzoic acid derivatives.

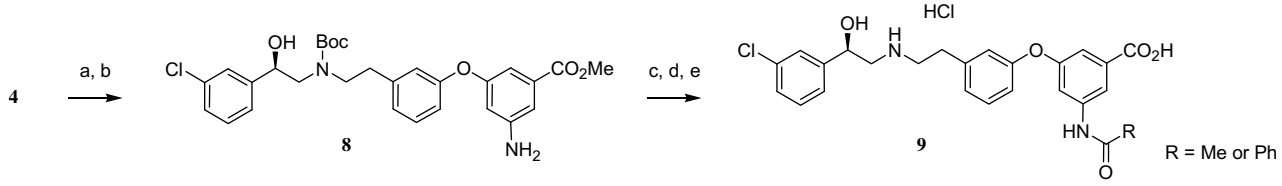
The general synthetic route of phenoxybenzoic acid derivatives is shown in Scheme 1. Key intermediate **4** was prepared by similar manner in our previous report.[13a] Commercially available (*R*)-2-amino-1-(3-chlorophenyl)ethanol **3** was coupled with 3-hydroxyphenylacetic acid, followed by the reduction of the amide moiety and protection by Boc group to afford the intermediate **4**. On the other hand, a variety of di-substituted phenyl boronic acids **5** were prepared from the corresponding phenyl bromides.[13a] The intermediate **4** was reacted with the boronic acids **5** in copper-catalyzed coupling condition[14] to yield the biphenyl ether compounds **6**. Hydrolysis and Boc-deprotection of compounds **6** afforded the desired phenoxybenzoic acid derivatives **7**.

Synthesis of analogues with 3-substituted group in benzoic acid moiety was shown in Scheme 2. Coupling reaction of the intermediate **4** with the boronic acid with nitro group and subsequent reduction by iron powder produced amine **8**. Acylation of the amino group of compound **8**, followed by deprotection of ester and Boc group afforded compounds **9**.

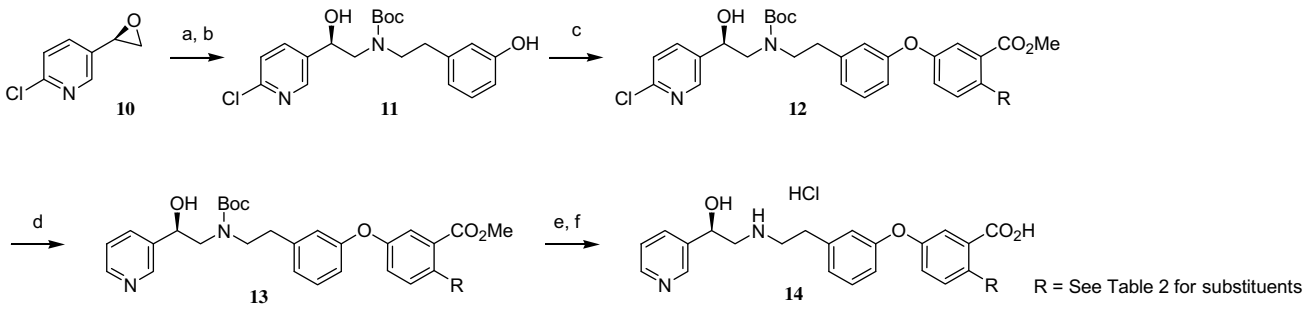
Scheme 3 shows the synthesis of pyridine derivatives. The optically active chloro-pyridyl epoxide **10** was prepared by the reported manner by Naylor et al.[15] Coupling of the epoxide **10** with 3-(2-aminoethyl)phenol, followed by protection with Boc group afforded intermediate **11**. Compound **11** was coupled with the boronic acids to yield the biphenyl ether intermediates **12**. Reduction of chlorine group of compounds **12,** and following deprotection with ester and Boc group provided the pyridine derivatives **14**.

All compounds synthesized above were evaluated for ability to produce cAMP in Chinese hamster ovary (CHO) cell lines expressing cloned human β_3 and β_1-ARs.

In our laboratory,[13] the chlorophenyl ethanolamine derivatives with benzoic acid are mainly focused and we examined the conversion of biphenyl junction moiety in the right part. It was found that compound **2a** and **2b** with biphenyl ether structure showed good

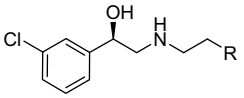


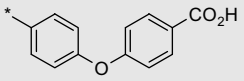
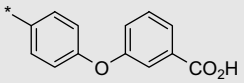
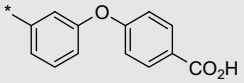
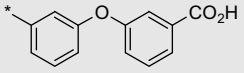
Scheme 2. Reagents: (a) 3-methoxycarbonyl-5-nitrophenylboronic acid, $Cu(OAc)_2$, pyridine, MS 4 Å, CH_2Cl_2; (b) Fe, NH_4Cl, EtOH, H_2O; (c) RC(O)Cl, Et_3N, CH_2Cl_2; (d) 1 N NaOH aq, MeOH; (e) 4 N HCl in dioxane.



Scheme 3. Reagents: (a) 3-(2-aminoethyl)phenol, EtOH; (b) Boc_2O, THF; (c) boronic acid, $Cu(OAc)_2$, pyridine, MS 4 Å, CH_2Cl_2; (d) $HC(O)NH_4$, Pd-C, MeOH, H_2O; (e) 1 N NaOH aq, MeOH; (f) 4 N HCl in dioxane.

Table 1
Effect of conversion of ether linkage position



Compound	R	Human β_3 EC_{50}[a] (nM)	Human β_1 EC_{50}[a] (nM)
2a		100	>100
2b		39	>100
7a		60	>100
7b		14	>100

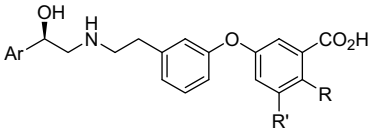
[a] β-AR agonistic activity was assessed by measuring cAMP accumulation in CHO cell lines expressing cloned human β-ARs.

oral absorption in dogs, although the β_3-AR potency of these compounds was insufficient (EC_{50} = 100 and 39 nM, respectively). Due to the attractive PK profile of these compounds, we explored the biphenyl ether derivatives in order to enhance the β_3-AR activity.

The conversion of disposition of the ether linkage from *para* to *meta* position was examined as shown in Table 1. The *meta*-linked analogues **7a** and **7b** showed improved β_3-AR activity compared to the corresponding *para* linked analogues **2a** and **2b**, suggesting that the conversion of ether linkage position effectively orientated benzoic acid moiety in right part toward the β_3-AR pharmacophore. In particular, compound **7b** showed moderate β_3-AR activity with EC_{50} value of 14 nM and maintained weak β_1-AR activity with EC_{50} value of above 100 nM. The indication encouraged us to select the compound **7b** as lead compound and investigate further optimization of the phenoxybenzoic acid derivatives.

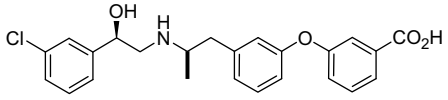
The effect of introduction of substituted group to right terminal phenyl ring was investigated as shown in Table 2. First, we examined to introduce a number of substituted groups to 2-position of benzoic acid moiety. Introduction of halogen group improved β_3-AR activity, and fluorine analogue **7c** and chlorine analogue **7d** showed the nanomole order EC_{50} value. However, β_1/β_3 selectivity of the halogen analogues was low due to the potent β_1-AR activity. On the other hand, methyl analogue **7e** and methoxy analogue **7f** maintained weak β_1-AR activity, although β_3-AR activity was moderate (EC_{50} = 13 and 11 nM, respectively). Therefore, we examined to introduce isopropoxy group, which was effective to increase β_3-AR potency and selectivity in the case of the biphenyl acid derivatives **1**. As a result, isopropoxy analogue **7g** showed excellent profile in β_3-AR activity and selectivity with EC_{50} value of 3.4 nM and 240-fold β_1/β_3 selectivity. Furthermore, introduction of acylamine group was investigated. Acetylamine analogue **7h** showed twofold increase in β_3-AR activity compared to **7b** (EC_{50} = 7.2 nM) and 53-fold β_1/β_3 selectivity. Benzoylamine analogue **7i** maintained potent β_3-AR activity (EC_{50} = 8.6 nM) but the β_1/β_3 selectivity was decreased to 15-fold. Due to the potent β_3-AR activity of the 2-acylamine analogue **7h** and **7i**, we investigated to introduce other acylamine group to the 2-position of right benzoic acid moiety. The pivaloylamine analogue **7j** showed further improved β_3-AR activity (EC_{50} = 4.6 nM) and less β_1-AR activity (EC_{50} = 620 nM), resulting in excellent β_3-AR selectivity over β_1-AR (β_1/β_3 = 135). Furthermore, introduction of pyrrolidinone ring led to the potent

Table 2
Effect of conversion of right, left and center parts of the phenoxybenzoic acid derivatives



Compound	Ar	R	R′	Human β_3 EC_{50}[a] (nM)	Human β_1 EC_{50}[a] (nM)	β_1/β_3 selectivity
7b	3-Cl-Ph	H	H	14	>100	>7.1
7c	3-Cl-Ph	F	H	9.4	140	15
7d	3-Cl-Ph	Cl	H	9.5	64	6.7
7e	3-Cl-Ph	Me	H	13	550	42
7f	3-Cl-Ph	OMe	H	11	510	46
7g	3-Cl-Ph	O-*i*-Pr	H	3.4	810	238
7h	3-Cl-Ph	NHAc	H	7.2	380	53
7i	3-Cl-Ph	NHC(O)Ph	H	8.6	130	15
7j	3-Cl-Ph	NHC(O)-*t*-Bu	H	4.6	620	135
7k	3-Cl-Ph		H	6.6	>1000	>152
9a	3-Cl-Ph	H	NHAc	6.5	240	37
9b	3-Cl-Ph	H	NHC(O)Ph	21	190	9.0
14a	3-Py	F	H	31	840	27
14b	3-Py	OMe	H	56	690	12
14c	3-Py	NHAc	H	12	190	16
15[b]				1.9	28	15
ISP[c]				0.97	0.084	0.087

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

[b] **15**: 

[c] ISP = isoproterenol; non-selective β-AR agonist.

and selective compound **7k** with EC_{50} value of 6.6 nM and above 150-fold β_1/β_3 selectivity. The results indicate that the bulky aliphatic group at the 2-position of benzoic acid is significantly important to β_3-AR potency and selectivity.

Next, the conversion of substituted group from 2-position to 3-position at the benzoic acid moiety was investigated. Compound **9a** with 3-acetylamine group was equipotent to compound **7h** (EC_{50} = 6.5 nM), whereas compound **9b** with 3-benzoylamine group decreased the β_3-AR activity (EC_{50} = 21 nM) compared to compound **7i**, suggesting that bulky substituted group was not tolerated spatially at the 3-position.

In previous findings,[8,13] conversion to pyridine ring on the left part and introduction of a methyl group to the center part were advantageous for β_3-AR activity. Therefore, we investigated the replacement of chlorophenyl ring to pyridine (Table 2). Introduction of representative substituted groups such as fluorine (**14a**), methoxy (**14b**), and acetylamine (**14c**) to right part resulted in less potent β_3-AR activity compared to the corresponding chlorophenyl analogues (**7c**, **7f**, and **7h**, respectively), indicating that the pyridine ring was detrimental in the phenoxybenzoic acid derivatives. Furthermore, introduction of a methyl group to the α-position of the phenethylamine moiety was examined. Our previous study and others indicated that (*R*)-configuration of the methyl group was important for enhancing β_3-AR activity.[8a–c,13b] Compound **15** showed sevenfold increase in β_3-AR activity (EC_{50} = 1.9 nM) compared to the non-substituted analogue **7b**. However, β_1-AR activity was also increased and β_1/β_3 selectivity was insufficient (β_1/β_3 = 15).

Last, we examined in vitro dog β_3-AR activity and the inhibitory effect of compounds **7g**, **7j**, and **7k** on carbachol-induced increase

Table 3
Dog β_3-AR activity and in vivo efficacy of compounds **7g**, **7j** and **7k**

Compound	In vitro Dog β_3 EC_{50}[a] (nM)	In vivo Inhibition % after iv injection[b]
7g	3.2	43% (@ 10 μg/kg)
7j	1.6	30% (@ 32 μg/kg)
7k	1.9	84% (@ 32 μg/kg)

[a] β_3-AR agonistic activity was assessed by measuring cAMP accumulation in CHO cell lines expressing cloned dog β_3-AR.
[b] Inhibitory effect on increase in IVP, induced by carbachol in anesthetized dogs.

of intravesical pressure (IVP) in anesthetized dogs for OAB model[13] as shown in Table 3. The oral bioavailability of these compounds was not investigated and we therefore confirmed the in vivo efficacy by intravenous administration test. All of these compounds showed equivalent potent activity toward dog β_3-AR as well as human β_3-AR. In in vivo study, compounds **7g** and **7k** showed significant efficacy to inhibit the IVP increase after intravenous administration at doses of 10 or 32 μg/kg, supporting the validation of a series of the phenoxybenzoic acid derivatives as the therapeutic candidate for OAB.

In summary, we investigated the SAR of the phenoxybenzoic acid derivatives in this letter. Conversion of the ether junction from *para* to *meta* position improved the β_3-AR activity and moderate potent analogue **7b** was found as lead compound. Further modification of compound **7b** revealed that the bulky aliphatic-substituted group at 2-position of benzoic acid moiety was significantly effective to β_3-AR activity and selectivity, leading to a number of potent and selective β_3-AR agonists such as compounds **7g**, **7j**, and **7k**. Furthermore, in vivo efficacy of compounds **7g** and **7k** were indicated on our OAB model. These compounds are attractive and expected for therapeutic application in the treatment of OAB.

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