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Article

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Discovery of Novel Indazole Derivatives as Highly Potent and Selective Human β_3-Adrenergic Receptor Agonists with the Possibility of Having No Cardiovascular Side-Effects

Yasuhiro Wada*,[a,b] Hiromitsu Shirahashi,[a] Taisuke Iwanami,[a] Masami Ogawa,[a] Seiji Nakano,[a] Akifumi Morimoto,[a] Ken-ichi Kasahara,[a] Eiichi Tanaka,[a] Yoshio Takada,[a] Shigeki Ohashi,[a] Mutsuhiro Mori,[a] and Satoshi Shuto*[b,c]

[a]Pharmaceutical Research Center, Asahi Kasei Pharma Corporation, 632-1, Mifuku, Izunokuni-shi, Shizuoka 410-2321, Japan

[b]Faculty of Pharmaceutical Sciences and [c]Center for Research and Education on Drug Discovery, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

Running Title: Structurally Novel Human β3-Adrenergic Receptor Agonists

Corresponding Author:
Satoshi Shuto
Phone & Fax: +81-11-706-3769. E-mail: shu@pharm.hokudai.ac.jp
Yasuhiro Wada
Phone: +81-558-76-8494. Fax: +81-558-76-5755. E-mail: wada.yp@om.asahi-kasei.co.jp

Abstract

Novel indazole derivatives were prepared and evaluated for their biological activity and cardiovascular safety profile as human β_3-adrenergic receptor (AR) agonists. Although the initial hit compound **5** exhibited significant β_3-AR agonistic activity (EC_{50} = 21 nM), it also exhibited agonistic activity at the α_{1A}-AR (EC_{50} = 219 nM, selectivity:α_{1A}/β_3 = 10-fold). The major metabolite of **5**, which was an oxidative product at the indazole 3-methyl moiety, gave a clue to a strategy for improvement of the selectivity for β_3-AR agonistic activity versus α_{1A}-AR agonistic activity. Thus, modification of the 3-substituent of the indazole moiety effectively improved the selectivity to develop compound **11** with potent β_3-AR agonistic activity (EC_{50} = 13 nM) and high selectivity (α_{1A}/β_3 = >769-fold). Compound **11** was also inactive towards β_1 and β_2-ARs and showed dose dependent β_3-AR mediated relaxation of marmoset urinary bladder smooth muscle, while it did not obviously affect heart rate or blood pressure (iv, 3 mg/kg) in anesthetized rats.

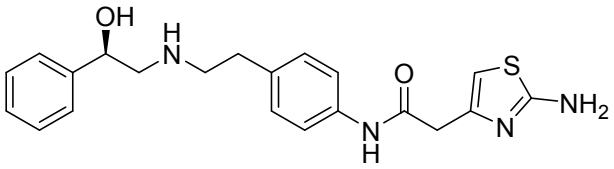
Introduction

Overactive bladder (OAB) is a urological disorder that is defined as denoting urgency with or without urge incontinence, usually with frequency and nocturia.[1] OAB symptoms can be highly distressing to the patient, and patients with OAB have been shown to have significantly lower quality-of-life.[2] Population-based estimates in five countries suggest that OAB affects 11.8% of adults.[3] Muscarinic receptor antagonists are widely used pharmacological agents for the symptoms of OAB.[4] These agents cause reductions in urinary frequency, urgency and urge urinary incontinence, all of which are primary symptoms of OAB.[5] However,

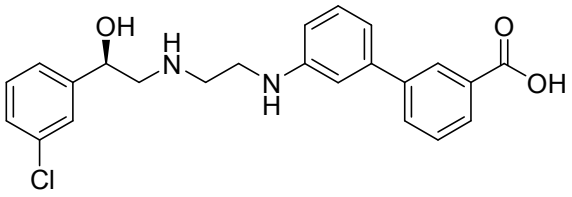
despite the proven efficacy of muscarinic receptor antagonists, their mechanistic side effects of dry mouth, constipation, blurred vision and occasional but rare urinary retention have been demonstrated.[5] A recent study found that 12 months after the initial prescription, the persistence rates of muscarinic receptor antagonists were ≦35% in patients with OAB.[6] Such adverse effects limit their tolerability and negatively affect long-term patient adherence and satisfaction in the clinical treatment of OAB symptoms.[7] In the search for alternative drugs for OAB treatment that have a novel target and that can improve the efficacy and tolerability profile, β_3-adrenergic receptor (AR) agonists have emerged as a promising class of drugs.[8]

β_3-AR, which is one of the three β-AR subtypes; β_1, β_2 and β_3-ARs,[9] is a G protein-coupled receptor (GPCR) that was identified by human genomic cloning in the late 1980s.[10] The β_3-AR mediates lipolysis in human brown adipose tissue and also mediates evoked muscle relaxation in gall bladder, stomach, small intestine, prostate, colon, and bladder.[11] The β_3-AR is vitally important for bladder smooth muscle, and 97% of the total β-AR messenger RNA in human bladder tissue is β_3-AR mRNA[12], which is likely to mediate mainly relaxation of the bladder smooth muscle in humans.[13] Because β_3-AR agonists increased bladder capacity in anesthetized rats,[14] pharmaceutical industries have been attempting to discover potent and selective β_3-AR agonists for the treatment of OAB, with the possibility that they might have fewer liabilities in terms of adverse effects compared to muscarinic receptor antagonists.[15] Several β_3-AR agonists such as **1** (mirabegron, YM-178)[16], **2** (solabegron, GW427353)[17], and **3** (ritobegron, KUC-7483)[18], which are shown in Figure 1, have been advanced into clinical studies, and **1** has been approved for the treatment of OAB. Treatment with mirabegron demonstrated significant efficacy for symptoms of OAB and resulted in less

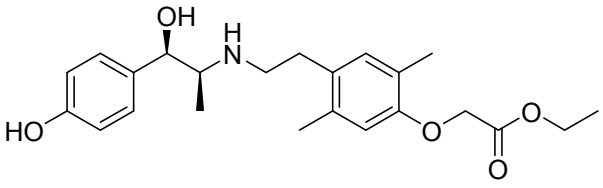
frequent incidence of dry mouth than muscarinic receptor antagonists.[8] However, mirabegron has been shown to increase the heart rate (HR) in animals and healthy subjects.[19] We hypothesized that, because the increased HR resulting from treatment with mirabegron is partly mediated by activation of the β_1-AR,[19a] highly selective novel β_3-AR agonists would be effective drugs for the treatment of OAB without cardiovascular side effects including an increased HR. We here describe identification of indazole derivatives as a new class of highly selective β_3-AR agonists with the possibility of having no cardiovascular side-effects.



1 (mirabegron, YM-178)



2 (solabegron, GW427353)



3 (ritobegron, KUC-7483)

Figure 1. Chemical Structure of **1**, **2** and **3**

Results and Discussion

Hit identification and issue of hit compound 5. A number of papers[15b, 15g, 20] and patents[21] have described potent β_3-AR agonists with either arylethanolamine or aryloxypropanolamine scaffolds. To discover a novel class of potent and selective β_3-AR agonists, we synthesized a series of phenylethanolamine analogs having a bicyclic heteroaromatic ring such as indazole, isoquinoline, 1H-pyrazolo[3,4-b]pyridine, benzoisoxazole, benzoisothiazole or benzoimidazole (Figure 2). Throughout screening of these compounds, we identified a novel indazole derivative **5** with significant and selective β_3-AR agonistic activity (EC_{50} = 21 nM), whose selectivity over the β_1- and β_2-ARs was >476-fold (Table 1). This increased selectivity of compound **5** was an advantage in terms of its use as a hit compound for the development of useful β_3-AR agonists, because the initial hit β_3-AR agonists reported in previous papers[15d, 22] had lower selectivity for β_3-AR versus the β_1- and β_2-ARs.

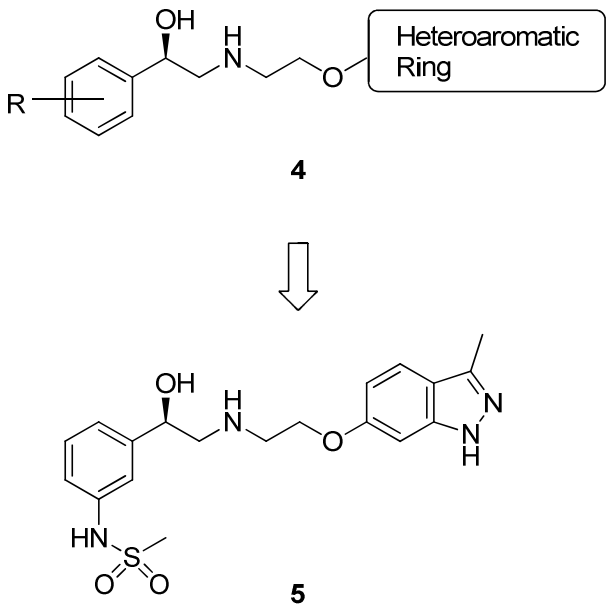


Figure 2. Hit identification strategy

Because we aimed to develop highly potent β_3-AR agonists without cardiovascular side effects, we evaluated the effects of compound **5** on the heart rate (HR) and blood pressure (BP) of anesthetized rats. As shown in Table 2, intravenous (iv) administration of compound **5** resulted in a transient biphasic change in mean blood pressure (MBP), i.e. there was a clear increase followed by a slight decrease. Within 5 minutes after dosing of compound **5**, MBP was restored to baseline. The maximum increase in MBP induced by **5** (3 mg/kg, iv) was 24.2%, and the maximum decrease was 8.5%. HR was slightly increased by 7.9% at 1 to 5 minutes after dosing of compound **5** (3 mg/kg, iv). To determine how **5** mediated this increase in MBP we therefore tested the agonistic profile of **5** on α-ARs, because not only a subfamily of β-ARs but also α-ARs are involved in the control of BP**.** α-ARs are sub-classified into six types, i.e., α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}-ARs.[9] Among these sub-classes, compound **5** showed significant α_{1A}-AR agonistic activity with an EC_{50} of 219 nM (Table 1). Three subtypes (α_{1A}, α_{1B}, α_{1D}) of the α_1-AR are widely distributed in the body and are highly expressed in the cardiovascular system, other smooth muscles and the brain.[23] The agonists of α_1-AR are known to increase blood pressure through vascular contraction in the peripheral tissues,[24] and the hypertension that occurred by treatment of compound **5** was prevented by the pre-dosed α_{1A}-AR antagonist Silodosin[25] (Table 2). Therefore, hypertension caused by compound **5** was suggested to occur via its α_{1A}-AR activation. Accordingly, we next aimed to obtain highly selective β_3-AR agonists without α_{1A}-AR activity.

Table 1. Human adrenergic receptor agonist activity of 5[a]

AR	EC_{50} (nM)[a]	I.A. (%)	Selectivity
β_3	21 ± 0.67	82[b]	
β_1	>10000	6.0[b]	$\beta_1 / \beta_3 > 476$
β_2	>10000	4.0[b]	$\beta_2 / \beta_3 > 476$
α_{1A}	219 ± 6.9	71[c]	$\alpha_{1A} / \beta_3 = 10$

[a]Data are shown as means ± SEM (n=3). 5[a] exhibited insignificant agonistic activity for α_{1B}, α_{1D}, α_{2A}, α_{2B} or α_{2C} (EC_{50} >10000 nM). [b]I.A. (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [c]I.A.: maximum response induced by norepinephrine was defined as 100%.

Table 2. Effect of 5 on HR and MBP in anesthetized rats[a]

		HR		MBP	
Dose (mg/kg)	n	Increase (%)	Decrease (%)	Increase (%)	Decrease (%)
Saline	5	1.8 ± 0.9	4.5 ± 0.8	1.0 ± 0.5	5.4 ± 1.9
1	3	4.2 ± 2.3	1.3 ± 0.7	7.5 ± 0.2	9.9 ± 0.2
3	4	7.9 ± 2.9	8.8 ± 4.5	24.2 ± 7.7	8.5 ± 1.1
1[b]	1	1.7	ND	ND	20.6

[a]Compound **5** was administered intravenously to rats, and, thereafter, HR and MBP were recorded for 30 min. Maximum increase or decrease of HR or MBP is shown as a value (%) relative to the baseline. Data are shown as means ± SEM. [b]Silodosin, a selective α_{1A}-AR antagonist, was administered intravenously at a dose of 0.01 mg/kg before administration of compound **5**. n: number, ND: not detected.

In order to obtain insight into the hypertension caused by compound **5** in vivo, we examined the metabolism of **5**. In this analysis, compound **6** was identified as a major oxidative metabolite in incubation of compound **5** with rat liver microsomes. This metabolite **6** provided a clue as to the strategy to be used for further structure activity relationship (SAR) study of **5**, because it showed more potent α_{1A}-AR agonist activity (EC_{50} = 33 nM, I.A. = 91%) than compound **5** (Figure 3). Introduction of a hydroxyl group to the 3-methyl group of the indazole moiety had brought about the dramatic change in α_{1A}-AR agonist activity. We therefore hypothesized that modifications of the 3-substituent of the indazole moiety would change the

α_{1A}-AR agonistic activity towards improving selectivity for β_3-AR versus α_{1A}-AR.

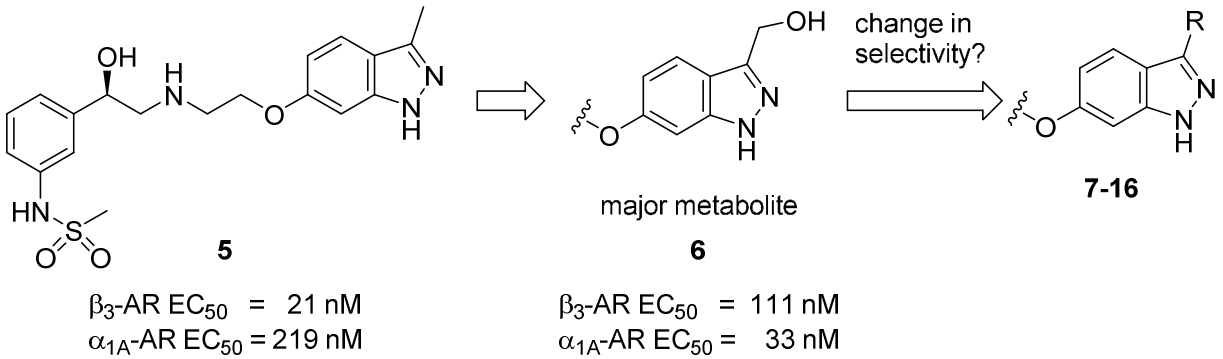


Figure 3. Design of indazole derivatives

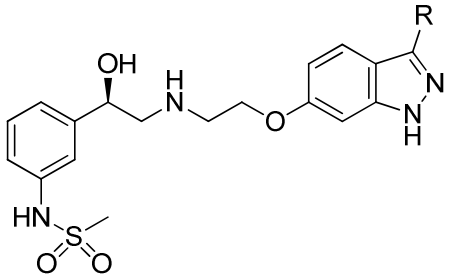
SAR of indazole analogs. Agonistic activity of derivatives of **5** that were modified at the indazole 3-moiety for the β_3-AR is shown in Table 3. Replacement of the methyl group (**5**) with a methoxy group (**7**) did not improve this activity (**5**, EC_{50} = 21 nM vs. **7**, EC_{50} = 40 nM), and compound **8** bearing an ethoxy substituent had further decreased activity (**8**, EC_{50} = 71 nM). Changing the methyl group to a chloro, ethyl, *iso*-propyl, or cyclopropyl group (**9**, **10**, **11**, **13**, respectively) did not significantly affect the EC_{50} value (**9**, EC_{50} = 43 nM; **10**, EC_{50} = 14 nM; **11**, EC_{50} = 13 nM; **13**, EC_{50} = 17 nM). However, replacement with a *tert*-butyl or phenyl groups (**12** or **16**) hugely decreased the β_3-AR agonistic activity (**12**, EC_{50} = 338 nM, **16**, EC_{50} = >10000 nM). Cyclobutyl (**14**) and trifluoromethyl (**15**) derivatives displayed slightly lower β_3-AR agonistic activity than **5** (**14**, EC_{50} = 66 nM, **15**, EC_{50} = 64 nM vs. **5**, EC_{50} = 21 nM). Thus, in order of their β_3-AR agonist activity, the compounds were ranked as **5**, **7**, **9**, **10**, **11**, **13** > **8**, **14**, **15** > **12** > **16**.

Agonistic activity of these compounds for the α_{1A}-AR is also presented in Table 3. Replacement with a

methoxy or ethoxy group (**7** or **8**) did not change this activity (**5**, EC_{50} = 219 nM vs. **7**, EC_{50} = 252 nM, **8**, EC_{50} = 277 nM). A chloro derivative (**9**) showed higher potency than **5** (**9**, EC_{50} = 61 nM vs. **5**, EC_{50} = 219 nM). On the other hand, ethyl (**10**), cyclopropyl (**13**) and trifluoromethyl (**15**) derivatives displayed remarkably attenuated agonistic potency for the α_{1A}-AR (**10**, EC_{50} = 857 nM; **13**, EC_{50} = 1548 nM; **15**, EC_{50} = 812 nM). Finally, changing the methyl group to an *iso*-propyl, *tert*-butyl, cyclobutyl or phenyl group (**11**, **12**, **14**, **16**, respectively) made these compounds inactive in terms of α_{1A}-AR activity (EC_{50} = >10000 nM). Thus, in order of α_{1A}-AR agonist activity, the compounds were ranked as **9** > **5**, **7**, **8 > 10**, **13**, **15** >> **11**, **12**, **14**, **16**.

Based on these agonistic activities for β_3-AR and α_{1A}-AR, these compounds were ranked in terms of their selectivity for β_3-AR over α_{1A}-AR as: **11** > **14** > **13** > **10** > **12** > **15** > **5** > **7** > **8** > **9**. Thus, compounds **10**, **11**, and **13** were identified as potent and highly selective β_3-AR agonists, and were next subjected to further pharmacological evaluation.

Table 3. Human β_3- and α_{1A}-AR agonistic activity of indazole derivatives[a]



Compound	R	β_3		α_{1A}		Selectivity
		EC_{50} (nM)	I.A. (%)[b]	EC_{50} (nM)	I.A. (%)[c]	α_{1A} / β_3
5	-Me	21 ± 0.67	82	219 ± 6.9	71	10
7	-OMe	40 ± 1.9	70	252	60	6.3

8	-OEt	71 ± 6.1	69	277	59	3.9
9	-Cl	43 ± 2.9	89	61	91	1.4
10	-Et	14 ± 0.58	78	857 ± 164	31	61
11	-*i*-Pr	13 ± 1.5	69	>10000	9.1	>769
12	-*t*-Bu	338 ± 159	62	>10000	19	>30
13		17 ± 3.8	72	1548 ± 419	19	91
14		66	68	>10000	4	>151
15	-CF_3	64 ± 11	86	812 ± 179	31	13
16	-Ph	>10000	29	>10000	6	
isoproterenol		86 ± 3.7	100	NT		
norepinephrine		NT		9.1 ± 0.52	100	

[a]Data are shown as means ±SEM (n≧3) or are presented as the average of two experiments. [b]I.A. (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [c]I.A.: maximum response induced by norepinephrine was defined as 100%. NT: not tested.

The β_1-AR and β_2-AR agonistic activities of **10**, **11**, and **13** were investigated. All of these compounds were inactive towards these two AR subtypes (β_1-AR EC_{50} = >10000 nM; β_2-AR EC_{50} = >10000 nM). Thus, these three compounds were identified as highly selective agonists of the β_3-AR.

Cardiovascular safety profiles and Pharmacological data. We evaluated the cardiovascular safety profiles of the highly selective β_3-AR agonists **10**, **11** and **13** on HR and MBP, and compared them with those of the hit compound **5** and the clinically useful β_3-AR agonist mirabegron (**1**). As summarized in

Table 4, none of the three optimized compounds (iv, 3 mg/kg) affected MBP in anesthetized rats, while the prototype hit compound **5** clearly raised the MBP (24.2%). In the same evaluation, mirabegron (**1**) caused a significant decrease in MBP (38.3%). Compound **11** did not affect the HR (iv, 3 mg/kg), while mirabegron (**1**) clearly increased the HR (10.0%). These results can be attributed to the fact that compounds **10**, **11** and **13** are highly active only towards the β_3-AR and are inactive towards all of the other α-and β-ARs.

Next, we examined the effect of compound **11** on the relaxation of urinary bladder smooth muscle. Rat models have been used for the evaluation of compounds in terms of their relaxation effect on urinary bladder smooth muscle.[16] However, because compound **11** showed only weak agonistic activity on the rat β_3-AR (EC_{50} = 535 nM, I.A. = 95%), other animal species were required to examine its relaxation effect on urinary bladder smooth muscle. We found that compound **11** acted as a strong agonist for the marmoset β_3-AR (EC_{50} = 15 nM, I.A. = 75%), and therefore compound **11** was evaluated for its effects on the relaxation response using urinary bladder smooth muscle strips from marmosets. In this evaluation with isolated marmoset urinary bladder smooth muscle that was pre-contracted with KCl, compound **11** showed full relaxant activity on the smooth muscle, and this activity was similar to that of a potent but non-selective β-AR agonist isoproterenol (Figure 4).

Based on the excellent β_3-AR selectivity profile, as well as the favorable relaxation effect on marmoset urinary bladder smooth muscle, the optimized compound **11** would be useful for the treatment of OAB with the possibility of having no cardiovascular side-effects.

Table 4. Effects of Intravenous Administration of Indazole derivatives on HR and MBP in Anaesthetized Rats[a]

		HR		MBP	
Compound	N	Increase (%)	Decrease (%)	Increase (%)	Decrease (%)
Saline	5	1.8 ± 0.9	4.5 ± 0.8	1.0 ± 0.5	5.4 ± 1.9
mirabegron (**1**)	3	10.0 ± 2.5	ND	3.5 ± 3.5	38.3 ± 3.4
5	4	7.9 ± 2.9	8.8 ± 4.5	24.2 ± 7.7	8.5 ± 1.1
10	6	9.6 ± 1.3	1.6 ± 2.0	3.1 ± 0.2	10.8 ± 1.1
11	6	3.3 ± 0.6	1.8 ± 0.5	4.7 ± 0.9	7.6 ± 1.7
13	3	5.7 ± 1.5	3.3 ± 0.7	2.7 ± 0.3	11.0 ± 3.0

[a]Compounds were administered intravenously to rats (3 mg/kg), and, thereafter, HR and MBP were recorded for 30 min. Maximum increase or decrease of HR or MBP is shown as a value (%) relative to the baseline. Data are shown as means ± SEM. N: Numbers of rats used. ND: Not detected.

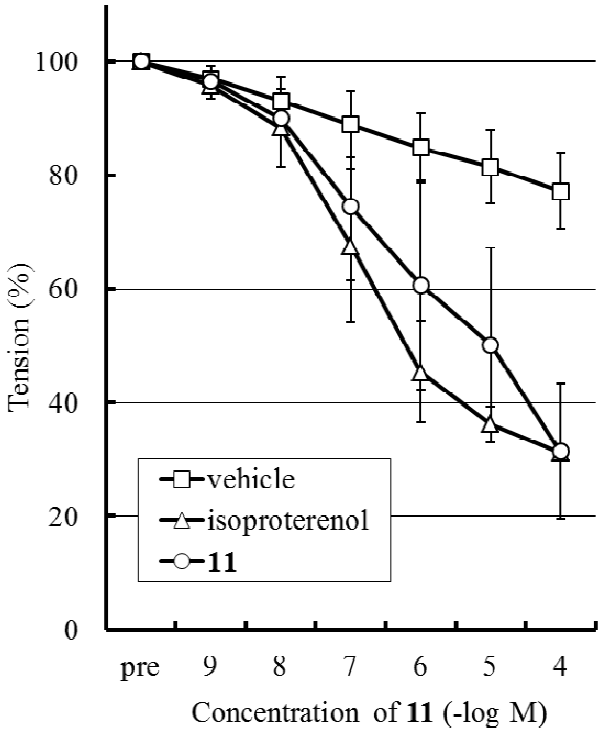


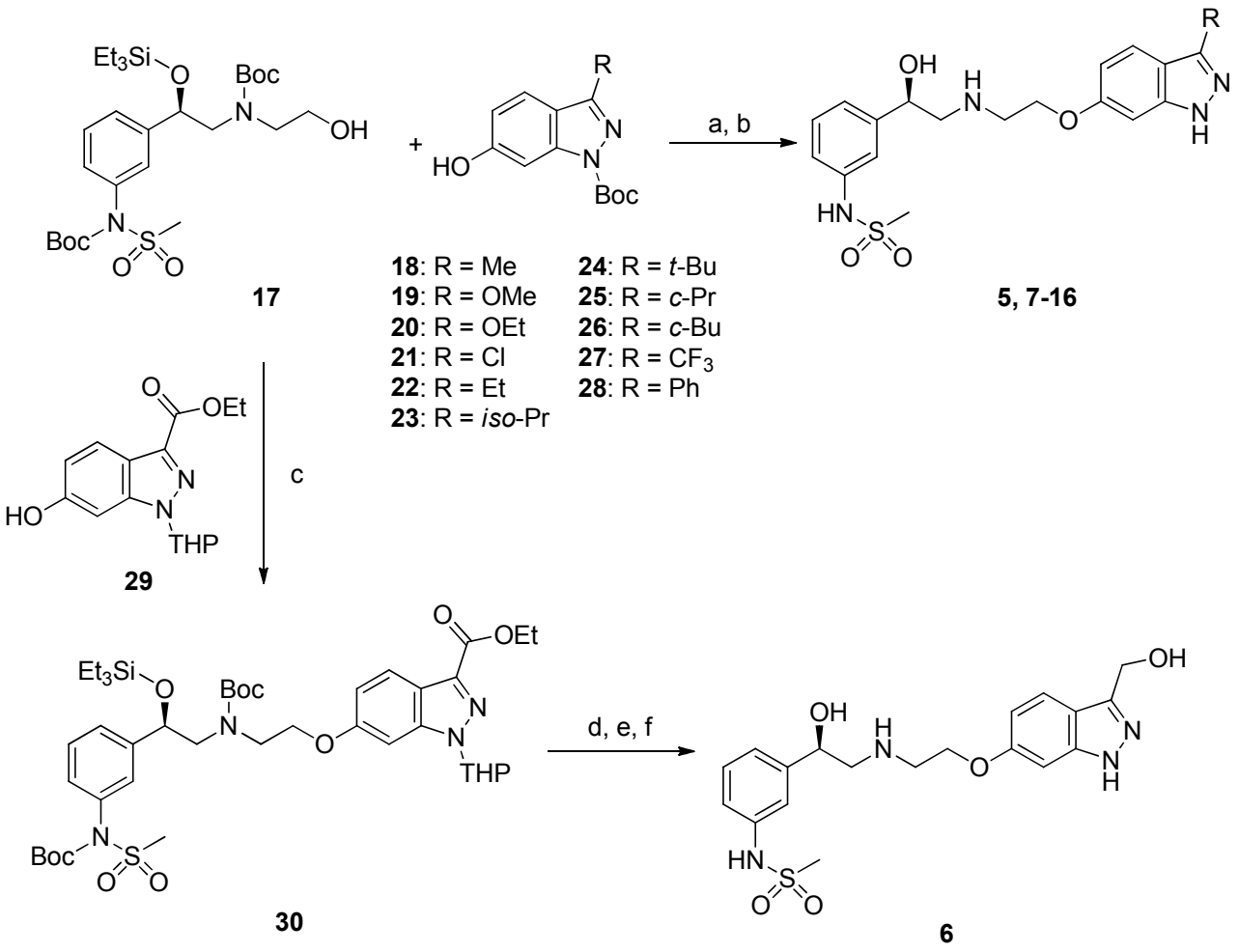
Figure 4. Relaxation of marmoset urinary bladder smooth muscle by **11**. Each point represents the mean ± SD (n=3). The marmoset urinary bladder was cut by a midline incision and 6 muscle strips of the bladder body were prepared. The strips were pre-contracted with 40 mmol/L KCl, and, after the tension had stabilized, test compounds were cumulatively added. When the relaxation response at the maximum concentration of the test compound was completed, papaverine was added at a final concentration of 10^{-4} mol/L, and the maximum relaxation response of each strip was determined. See Experimental Section for

further details.

Chemistry

Synthesis of the indazole analogs **5-16** is shown in Scheme 1. Mitsunobu reaction of **17** and **18**-**28** proceeded to give the corresponding indazole ether products, of which deprotection with 4 M HCl in 1,4-dioxane or ethyl acetate gave the target compounds (**5, 7-16**). Similarly, Mitsunobu reaction of **17** and **29** proceeded to give **30**. After hydrolysis of the ethyl ether group of **30,** reduction of its carboxylic acid group with borane tetrahydrofuran complex and subsequent deprotection with HCl in ethanol gave the target compound **6**.

Scheme 1. Synthesis of Targets **5-16**[a]



[a]Reagents and conditions: (a) TMAD, PPh_3, toluene, rt; (b) 4 M HCl in EtOAc or 4 M HCl in 1,4-dioxane, rt; (c) TMAD, PPh_3, THF, rt; (d) 2 M NaOH, MeOH, 40 °C; (e) BH_3·THF, THF, rt; (f) conc. HCl aq, EtOH, rt.

Conclusion

We identified a new series of indazole derivatives as β_3-AR agonists. Among the developed β_3-AR agonists, compound **11** had a high selectivity for β_3-AR over β_1, β_2 and α_{1A}-ARs and showed dose dependent β_3-AR mediated responses in marmoset urinary bladder smooth muscle. Compound **11** did not obviously affect HR or MBP (iv, 3 mg/kg) in anesthetized rats. Thus, we were successful in identification of **11**, a highly selective β_3-AR agonist with the possibility of having no cardiovascular side-effects.

Experimental Section

General Methods. All reagents and solvents were purchased from commercial sources and were used as received. Anhydrous solvents were obtained from commercial sources. Thin layer chromatography (TLC) was carried out using Merck GmbH Precoated silica gel 60 F254. Chromatography on silica gel was carried out using prepacked silica gel cartridges (Yamazen Hi-Flash Column Silicagel or Purifpack-Si series). Chemical shifts in ^{1}H NMR spectra were reported in δ values (ppm) relative to trimethylsilane. Ion chromatographic analyses were performed under the following conditions: (Anion) ICS-1000 (Dionex), Dionex IonPac AS14 (Dionex or Thermo Scientific), 30 °C column temperature, 1.2 mL/min flow rate, electrical conductivity detection, mobile phase of 1.0 mmol/L sodium hydrogen carbonate and 3.5 mmol/L sodium carbonate in water, standard solution of Mixed Anion Standard Solution IV (Kanto Chemical Industry Co., Ltd.); (Cation) DX500 (Dionex), Dionex IonPac CS14 (Dionex), 30 °C column temperature, 1.0 mL/min flow rate, electrical conductivity detection, mobile phase of 10 mmol/L methanesulfonic acid in water, standard solution of Mixed Cation Standard Solution II (Kanto Chemical Industry Co., Ltd.). HPLC analyses were performed following conditions: (Method A) Shiseido CAPCELL CORE ADME column (2.7 μm, 2.1 x 50 mm), 40 °C column temperature, 1.0 mL/min flow rate, photodiode array detection (254 nm), linear mobile phase gradient of 10-95% B over 2 min, holding 1.5 min at 95% B, holding 1.5 min at 10% B (mobile phase A: 10 mM ammonium acetate in water; mobile phase B: acetonitrile), (Method B) YMC Meteoric Core C18 column (2.7 μm, 3.0 x 50 mm), 40 °C column temperature, 1.0 mL/min flow rate,

photodiode array detection (254 nm), linear mobile phase gradient of 10-95% B over 2 min, holding 2 min at 95% B, holding 2 min at 10% B (mobile phase A: 10 mM ammonium acetate in water; mobile phase B: acetonitrile).

***N*-(3-((1*R*)-1-Hydroxy-2-(2-((3-methyl-1*H*-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methanesulfonamide (5).** To a stirred solution of **18** (128 mg, 0.52 mmol), **17** (2 mL, 1.0 mmol, 0.5 M toluene solution), and triphenylphosphine (261 mg, 1.0 mmol) in anhydrous toluene (5 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (195 mg, 1.1 mmol) at room temperature, and the solution was stirred for 4 days. The reaction solution was then purified by flash column chromatography on silica gel (74:26 to 53:47 n-hexane/ethyl acetate) to give 371 mg (90% yield) of the coupling product. ^{1}H-NMR (400 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 8.0), 0.89 (9H, t, *J* = 8.0), 1.44 (9H, s), 1.48 and 1.52 (9H, each s), 1.69 and 1.70 (9H, each s), 2.52 and 2.53 (3H, each s), 3.24-3.26 (7H, m), 4.03-4.11 (2H, m), 4.94-4.97 and 5.10-5.13 (1H, each m), 6.86 (1H, dd, *J* = 1.7, 8.6), 7.12-7.16 (1H, m), 7.21-7.47 (4H, m), 7.54 (1H, s); LC/MS (ESI, $[M+H]^+$, *m/z*) 819. To the solution of the obtained product (287 mg, 0.35 mmol) in 1,4-dioxane (0.4 mL) was added 4 M HCl in 1,4-dioxane (4 mL), and the mixture was stirred at room temperature for 6.5 h. The resultant solid was collected by suction filtration and dried. The crude solid (170 mg) was treated with water (0.5 mL) and ethanol (2.0 mL) to promote crystallization. The crystals were filtered, and washed with ethanol to afford 61 mg (37% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.46 (3H, s), 3.00 (3H, s), 3.04-3.10 (1H, m), 3.24-3.28 (1H, m),

3.44-3.47 (2H, m), 4.34-4.41 (2H, m), 5.02 (1H, dd, *J* = 2.1, 10.1), 6.80 (1H, dd, *J* = 2.1, 8.8), 6.92 (1H, d, *J* = 2.1), 7.12-7.17 (2H, m), 7.31 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.63 (1H, d, *J* = 8.8), 9.05 (1H, brs), 9.38 (1H, brs), 9.86 (1H, brs); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 11.3, 39.1, 45.9, 53.6, 63.4, 67.9, 92.1, 111.9, 116.9, 117.0, 118.9, 121.0, 121.1, 129.3, 138.5, 140.7, 141.6, 143.0, 157.4; HRMS calculated for $C_{19}H_{24}N_4O_4S$ + H^+ 405.1591, found (ESI, $[M+H]^+$) 405.1588; LC/MS (ESI, $[M+H]^+$, *m/z*) 405; HPLC (Method A): purity 100% R_T 1.7 min.

***N*-(3-((1*R*)-1-Hydroxy-2-(2-((3-(hydroxymethyl)-1*H*-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)metha nesulfonamide (6).** To a stirred solution of **30** (139 mg, 0.16 mmol) in THF (1.2 mL) and methanol (2.0 mL) was added 2 M NaOH (1.6 mL, 3.2 mmol) at 40 °C, and the solution was stirred for 5 h. The reaction mixture was poured into water (50 mL) and washed twice with diethyl ether. The aqueous layer was added 5 M HCl (5 mL) and extracted three times with ethyl acetate. The combined organic layers were dried over magnesium sulfate and filtered. The solution was concentrated to give carboxylic acid (95 mg), which was used without further purification. This material (77 mg, 0.11 mmol) was dissolved in anhydrous THF (4.3 mL) under nitrogen and the solution was added borane tetrahydrofuran complex (0.5 mL, 0.6 mmol, 1.2 M in THF) at 0 °C. The resulting solution was allowed to warm to room temperature and was then stirred overnight. The reaction mixture was added methanol (0.5 mL) at 0 °C. The solution was poured into water and extracted twice with ethyl acetate. The combined organic layers were washed twice with water, dried over sodium sulfate, and filtered. The organic layer was concentrated to give alcohol (95 mg), which was

used without further purification. This material (68 mg, 0.1 mmol) was dissolved in ethanol (0.5 mL), hydrochloric acid (0.17 mL) was then added at room temperature and the mixture was stirred for 24 h. The resultant solid was collected by filtration and dried to give 39 mg (80% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (3H, s), 3.04-3.11 (1H, m), 3.24-3.28 (1H, m), 3.41-3.46 (2H, m), 4.32-4.41 (2H, m), 4.73 (2H, s), 5.02 (1H, dd, J = 2.1, 10.2), 6.79 (1H, dd, J = 2.1, 8.8), 6.93 (1H, d, J = 2.1), 7.12-7.17 (2H, m), 7.31 (1H, s), 7.35 (1H, t, J = 7.8), 7.74 (1H, d, J = 8.8), 9.02 (1H, brs), 9.32 (1H, brs), 9.86 (1H, s), 12.65 (1H, brs); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 39.1, 45.9, 53.6, 56.6, 63.3, 67.9, 92.1, 111.6, 116.5, 116.9, 118.9, 121.2, 121.5, 129.3, 138.5, 141.9, 143.0, 145.4, 156.9; HRMS calculated for $C_{19}H_{24}N_4O_5S + H^+$ 421.1540, found (ESI, $[M+H]^+$) 421.1537; LC/MS (ESI, $[M+H]^+$, *m/z*) 421; HPLC (Method A): purity 100% R_T 1.5 min.

***N*-(3-((1*R*)-1-Hydroxy-2-(2-((3-methoxy-1*H*-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methanesulfonamide (7).** To a stirred solution of **19** (215 mg, 0.8 mmol), **17** (942 mg, 1.6 mmol), and triphenylphosphine (448 mg, 1.7 mmol) in anhydrous toluene (12 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (288 mg, 1.7 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (100:0 to 74:26 n-hexane/ethyl acetate) to give 587 mg (87% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.53 (6H, q, J = 7.9), 0.87 (9H, t, J = 7.9), 1.42 (9H, s), 1.46 and 1.51 (9H, each s), 1.67 (9H, s), 3.20-3.60 (7H, m), 3.99-4.04 (m, 2H), 4.12 (3H, s), 4.92-4.96 and 5.08-5.12 (1H, each m), 6.80 (1H, dd, J = 2.1, 8.7), 7.11-7.47 (6H, m);

LC/MS (ESI, $[M+H]^+$, *m/z*) 835. To the solution of the obtained product (580 mg, 0.7 mmol) in *tert*-butyl methyl ether (1 mL) was added 4 M HCl in 1,4-dioxane (7 mL), and the mixture was stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with *tert*-butyl methyl ether, and dried to afford 321 mg (93% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (3H, s), 3.03-3.10 (1H, m), 3.23-3.27 (1H, m), 3.44-3.46 (2H, m), 3.96 (3H, s), 4.33-4.41 (2H, m), 5.04 (1H, dd, J = 2.1, 10.2), 6.70 (1H, dd, J = 2.0, 8.8), 6.81 (1H, d, J = 2.0), 7.13 (1H, d, J = 7.8), 7.16 (1H, dd, J = 1.3, 7.8), 7.30 (1H, s), 7.35 (1H, t, J = 7.8), 7.48 (1H, d, J = 8.8), 9.09 (1H, brs), 9.47 (1H, brs), 9.87 (1H, s), 11.83 (1H, brs); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 39.7, 46.4, 54.2, 56.1, 63.9, 66.8, 92.8, 106.5, 111.4, 117.5, 119.5, 120.5, 121.8, 129.8, 139.1, 143.5, 143.6, 156.7, 158.4; HRMS calculated for $C_{19}H_{24}N_4O_5S + H^+$ 421.1540, found (ESI, $[M+H]^+$) 421.1538; LC/MS (ESI, $[M+H]^+$, *m/z*) 421; HPLC (Method A): purity 100% R_T 1.8 min.

***N*-(3-((1*R*)-2-(2-((3-Ethoxy-1H-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (8).** To a stirred solution of **20** (51 mg, 0.18 mmol), **17** (212 mg, 0.36 mmol), and triphenylphosphine (94 mg, 0.36 mmol) in anhydrous toluene (2.7 mL) was added *N,N,N',N'*-tetramethylazodicarboxamide (62 mg, 0.36 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (88:12 to 67:33 n-hexane/ethyl acetate) to give 137 mg (89% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, t, J = 7.9), 0.89 (9H, q, J = 7.9), 1.44-1.47 (12H, m), 1.48 and 1.52 (9H, each s), 1.68 (9H, s), 3.22-3.61 (7H, m),

4.01-4.11 (2H, m), 4.51 (2H, q, *J* = 7.0), 4.93-4.98 and 5.09-5.13 (1H, each m), 6.80 (1H, dd, *J* = 2.1, 8.7), 7.13-7.50 (6H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 849. To the solution of the obtained product (130 mg, 0.15 mmol) in *tert*-butyl methyl ether (0.2 mL) was added 4 M HCl in 1,4-dioxane (1.5 mL), and the mixture was shaken (600 min^{-1}) for overnight at room temperature. Nitrogen gas was blown into the reaction solution to evaporate the solvent. Subsequently, water was added to dissolve the residue and the solution was freeze-dried to give 76 mg (98 % yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.39 (3H, t, *J* = 7.0), 3.00 (3H, s), 3.03-3.10 (1H, m), 3.23-3.27 (1H, m), 3.43-3.49 (2H, m), 4.33 (2H, q, *J* = 7.0), 4.34-4.41 (2H, m), 5.03 (1H, dd, *J* = 2.1, 10.2), 6.70 (1H, dd, *J* = 2.0, 8.8), 6.81 (1H, d, *J* = 2.0), 7.13 (1H, d, *J* = 7.7), 7.15-7.18 (1H, m), 7.30 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.47 (1H, d, *J* = 8.8), 9.07 (1H, brs), 9.42 (1H, brs), 9.87 (1H, s), 11.78 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 14.7, 39.1, 45.8, 53.6, 63.3, 63.8, 67.9, 92.2, 106.1, 110.8, 117.0, 119.0, 120.0, 121.2, 129.2, 138.5, 142.7, 143.0, 155.5, 157.7; HRMS calculated for $C_{20}H_{26}N_4O_5S + H^+$ 435.1967, found (ESI, $[M+H]^+$) 435.1696; LC/MS (ESI, $[M+H]^+$, *m/z*) 435; HPLC (Method A): purity 100% R_T 1.9 min.

***N*-(3-((1*R*)-2-(2-((3-Chloro-1*H*-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (9).** To a stirred solution of **21** (26 mg, 0.1 mmol), **17** (118 mg, 0.2 mmol), and triphenylphosphine (55 mg, 0.2 mmol) in anhydrous toluene (1.5 mL) was added *N,N,N',N'*-tetramethylazodicarboxamide (35 mg, 0.2 mmol) at room temperature, and the solution was stirred overnight. Then the reaction solution was then purified by flash column chromatography on silica gel (81:19 to 60:40 n-hexane/ethyl acetate) to give 71

mg (85% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.44 (9H, s), 1.49 and 1.52 (9H, each s), 1.69 (9H, s), 3.25-3.63 (7H, m), 4.03-4.11 (2H, m), 4.94-4.98 and 5.10-5.14 (1H, each m), 6.93 (1H, dd, *J* = 2.0, 8.8), 7.12-7.44 (4H, m), 7.50 (1H, dd, *J* = 3.6, 8.8), 7.57 (1H, d, *J* = 1.5); LC/MS (ESI, $[M+H]^+$, *m/z*) 839. To the solution of the obtained product (71 mg, 0.085 mmol) in *tert*-butyl methyl ether (0.2 mL) was added 4 M HCl in 1,4-dioxane (1.2 mL), and the mixture was shaken (600 min^{-1}) overnight at room temperature. The resultant solid was collected by suction filtration, washed with *tert*-butyl methyl ether, and dried to afford 41 mg (96% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (3H, s), 3.04-3.10 (1H, m), 3.24-3.28 (1H, m), 3.46-3.48 (2H, m), 4.38-4.43 (2H, m), 5.03 (1H, dd, *J* = 2.1, 10.2), 6.91 (1H, dd, *J* = 2.0, 8.9), 7.01 (1H, d, *J* = 2.0), 7.12-7.17 (2H, m), 7.31 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.56 (1H, d, *J* = 8.9), 9.08 (1H, brs), 9.43 (1H, brs), 9.86 (1H, s), 13.21 (1H, brs); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 39.1, 45.8, 53.6, 63.5, 67.9, 92.6, 113.8, 114.4, 116.9, 118.9, 119.5, 121.2, 129.2, 132.0, 138.5, 142.1, 143.0, 157.9; HRMS calculated for $C_{18}H_{21}ClN_4O_4S$ + H^+ 425.1045, found (ESI, $[M+H]^+$) 425.1045; LC/MS (ESI, $[M+H]^+$, *m/z*) 425; HPLC (Method A): purity 100% R_T 1.9 min.

***N*-(3-((1*R*)-2-(2-((3-Ethyl-1*H*-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (10).** To a stirred solution of **22** (1.326 g, 5 mmol), **17** (10 mL, 10 mmol, 1 M toluene solution), and triphenylphosphine (2.905 g, 11 mmol) in anhydrous toluene (15 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (1.953 g, 11 mmol) at room temperature, and the solution was

stirred overnight. Triphenylphosphine (1.344 g) and *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (0.973 g) were further added to the reaction solution, and the mixture was stirred for 2 h at room temperature. Triphenylphosphine (1.24 g) and *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (0.923 g) were further added to the reaction solution, and the mixture was stirred for 0.5 h at room temperature. The reaction solution was then purified by flash column chromatography on silica gel (88:12 to 67:33 n-hexane/ethyl acetate) to give 3.71 g (88% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.38 (3H, t, *J* = 7.6), 1.43 (9H, s), 1.48 and 1.52 (9H, each s), 1.70 (9H, s), 2.95 (2H, q, *J* = 7.6), 3.22-3.62 (7H, m), 4.02-4.11 (2H, m), 4.93-4.97 and 5.09-5.13 (1H, each m), 6.77 (1H, dd, *J* = 2.0, 8.7), 7.13-7.60 (6H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 833. The obtained product (3.56 g, 4.3 mmol) and 4 M HCl in ethyl acetate (70 mL) were stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with diethyl ether, and dried to afford 2.056 g (97% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.31 (3H, t, *J* = 7.6), 2.93 (2H, q, *J* = 7.6), 3.00 (3H, s), 3.04-3.11 (1H, m), 3.23-3.27 (1H, m), 3.43-3.48 (2H, m), 4.37-4.43 (2H, m), 5.05 (1H, dd, *J* = 2.1, 10.2), 6.83 (1H, dd, *J* = 2.1, 8.8), 6.95 (1H, d, *J* = 2.1), 7.13 (1H, d, *J* = 7.8), 7.16-7.18 (1H, m), 7.31 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.70 (1H, d, *J* = 8.8), 9.13 (1H, brs), 9.54 (1H, brs), 9.88 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.3, 19.2, 39.1, 45.8, 53.7, 63.4, 67.9, 92.1, 112.4, 115.8, 117.0, 119.0, 121.2, 121.3, 129.2, 138.5, 141.6, 143.0, 145.9, 157.8; HRMS calculated for $C_{20}H_{26}N_4O_4S + H^+$ 419.1748, found (ESI, $[M+H]^+$) 419.1747; LC/MS (ESI, $[M+H]^+$, *m/z*) 419; HPLC (Method A): purity 100% R_T 1.8 min.

_N_-(3-((1_R_)-1-Hydroxy-2-(2-((3-isopropyl-1_H_-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methanesulfonamide (11). To a stirred solution of **23** (146 mg, 0.5 mmol), **17** (1.19 g, 2.0 mmol), and triphenylphosphine (574 mg, 2.2 mmol) in anhydrous toluene (5 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (371 mg, 2.1 mmol) at room temperature and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (100:0 to 67:33 n-hexane/ethyl acetate) to give 400 mg (95% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CHCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.43-1.45 (15H, m), 1.47 and 1.52 (9H, each s), 1.70 (9H, s), 3.22-3.62 (8H, m), 4.02-4.11 (2H, m), 4.94-4.98 and 5.10-5.14 (1H, each m), 6.85 (1H, dd, *J* = 1.6, 8.8), 7.12-7.44 (4H, m), 7.51 (1H, s), 7.57 (1H, dd, *J* = 2.4, 8.8); LC/MS (ESI, $[M+H]^+$, *m/z*) 847. To the solution of the obtained product (400 mg, 0.47 mmol) in THF (2.4 mL) was added 4 M HCl in 1,4-dioxane (1.5 mL) and the mixture was stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with diethyl ether, and dried under reduced pressure. The solid was dissolved with water, and the solution was freeze-dried to give 191 mg (93 % yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.38 (6H, d, *J* = 7.0), 3.00 (3H, s), 3.05-3.12 (1H, m), 3.24-3.28 (1H, m), 3.40 (1H, septet, *J* = 7.0), 3.45-3.49 (2H, m), 4.37-4.47 (2H, m), 5.07 (1H, dd, *J* = 2.0, 10.1), 6.86 (1H, dd, *J* = 2.0, 8.9), 6.97 (1H, d, *J* = 2.0), 7.15 (1H, d, *J* = 7.8), 7.17-7.19 (1H, m), 7.32 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.78 (1H, d, *J* = 8.9), 9.20 (1H, brs), 9.62 (1H, brs), 9.90 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 22.1, 26.6, 39.3, 45.9, 53.9, 63.6, 68.1, 92.4, 113.0, 115.0, 117.2, 119.2, 121.4, 121.9, 129.4, 138.7, 141.9, 143.2, 149.8,

158.1; HRMS calculated for $C_{21}H_{28}N_4O_4S + H^+$ 433.1904, found (ESI, $[M+H]^+$) 433.1903; LC/MS (ESI, $[M+H]^+$, *m/z*) 433; HPLC (Method A): purity 100% R_T 1.9 min.

***N*-(3-((1*R*)-2-(2-((3-*tert*-Butyl-1*H*-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (12).** To a stirred solution of **24** (141 mg, 0.5 mmol), **17** (0.92 mL, 0.9 mmol, 1 M toluene solution), and triphenylphosphine (401 mg, 1.5 mmol) in anhydrous toluene (5 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (284 mg, 1.6 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (95:5 to 74:26 n-hexane/ethyl acetate) to give 354 mg (82% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, t, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.43 (9H, s), 1.47 and 1.51 (9H, each s), 1.50 (9H, s), 1.69 (9H, s), 3.22-3.62 (7H, m), 4.01-4.10 (2H, m), 4.93-4.97 and 5.1-5.13 (1H, each m), 6.83 (1H, d, *J* = 8.8), 7.13-7.44 (4H, m), 7.51 (1H, d, *J* = 1.7), 7.67 (1H, dd, *J* = 2.4, 8.8); LC/MS (ESI, $[M+H]^+$, *m/z*) 861. To the solution of the obtained product (232 mg, 0.27 mmol) in 1,4-dioxane (0.45 mL) was added 4 M HCl in 1,4-dioxane (1.5 mL), and the mixture was shaken (600 min^{-1}) overnight at room temperature. The reaction mixture was added ethanol and was shaken (600 min^{-1}) for 4 h at room temperature. Nitrogen gas was blown into the reaction solution to evaporate the solvent. Subsequently, water was added to dissolve the residue and the solution was freeze-dried to give 140 mg (quantitative yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.44 (9H, s), 3.00 (3H, s), 3.06-3.08 (1H, m), 3.24-3.28 (1H, m), 3.45-3.49 (2H, m), 4.36-4.39 (2H, m), 5.03 (1H, dd, *J* = 2.0,

10.1), 6.77 (1H, dd, *J* = 2.0, 8.9), 6.93 (1H, d, *J* = 2.0), 7.13 (1H, d, *J* = 7.8), 7.15-7.17 (1H, m), 7.31 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.80 (1H, d, *J* = 8.9), 9.08 (1H, brs), 9.45 (1H, brs), 9.87 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 29.9, 33.1, 39.1, 45.8, 53.6, 63.4, 67.9, 92.4, 111.3, 114.7, 117.0, 119.0, 121.2, 122.4, 129.2, 138.5, 142.5, 143.0, 152.1, 156.6; HRMS calculated for $C_{22}H_{30}N_4O_4S + H^+$ 447.2061, found (ESI, $[M+H]^+$) 447.2061; LC/MS (ESI, $[M+H]^+$, *m/z*) 447; HPLC (Method A): purity 100% R_T 2.0 min.

***N*-(3-((1*R*)-2-(2-((3-Cyclopropyl-1*H*-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (13).** To a stirred solution of **25** (1.415 g, 5 mmol), **17** (10 mL, 10 mmol, 1 M toluene solution), and triphenylphosphine (2.910 g, 11 mmol) in anhydrous toluene (50 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (1.915 g, 11 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was purified by flash column chromatography on silica gel (95:5 to 74:26 n-hexane/ethyl acetate) to give 3.779 g (89% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.03-1.05 (2H, m), 1.15-1.20 (2H, m), 1.44 (9H, s), 1.48 and 1.52 (9H, each s), 1.68 (9H, s), 2.13-2.21 (1H, m), 3.22-3.63 (7H, m), 4.02-4.13 (2H, m), 4.94-4.98 and 5.10-5.14 (1H, each m), 6.84 (1H, dd, *J* = 2.0, 8.7), 7.13-7.53 (6H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 845. To the solution of the obtained product (3.770 g, 4.5 mmol) in 1,4-dioxane (9 mL) was added 4 M HCl in 1,4-dioxane (20 mL), and the mixture was stirred overnight at room temperature. Then, 4 M HCl in 1,4-dioxane (14 mL) was further added to the reaction solution, and the mixture was stirred for 2 h at room temperature. The resultant solid was collected by suction filtration, washed with diethyl ether, and

dried under reduced pressure. The solid was dissolved with water, and the solution was freeze-dried to give 2.033 g (90 % yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.96-1.02 (4H, m), 2.23-2.30 (1H, m), 3.00 (3H, s), 3.04-3.10 (1H, m), 3.24-3.28 (1H, m), 3.44-3.47 (2H, m), 4.34-4.42 (2H, m), 5.04 (1H, dd, *J* = 2.1, 10.2), 6.80 (1H, dd, *J* = 2.1, 8.8), 6.91 (1H, d, *J* = 2.1), 7.13 (1H, d, *J* = 8.0), 7.17 (1H, dd, *J* = 1.3, 8.0), 7.31 (1H, s), 7.34 (1H, t, *J* = 8.0), 7.70 (1H, d, *J* = 8.8), 9.11 (1H, brs), 9.50 (1H, brs), 9.87 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 7.6, 7.7, 39.1, 45.8, 53.7, 63.4, 67.9, 92.2, 112.1, 116.2, 117.0, 119.0, 121.0, 121.2, 129.2, 138.5, 141.7, 143.0, 146.1, 157.6; HRMS calculated for $C_{21}H_{26}N_4O_4S$ + H^+ 431.1748, found (ESI, $[M+H]^+$) 431.1743; LC/MS (ESI, $[M+H]^+$, *m/z*) 431; HPLC (Method B): purity 96% R_T 1.9 min.

_N_-(3-((1_R_)-2-(2-((3-Cyclobutyl-1_H_-indazol-6-yl)oxy)ethylamino)-1-hydroxy-ethyl)phenyl)methanesulfonamide (14). To a stirred solution of **26** (1.426 g, 5 mmol), **17** (10 mL, 10 mmol, 1 M toluene solution), and triphenylphosphine (2.914 g, 11 mmol) in anhydrous toluene (25 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (1.911 g, 11 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was purified by flash column chromatography on silica gel (88:12 to 67:33 n-hexane/ethyl acetate) to give 3.848 g (90% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.44 (9H, s), 1.48 and 1.52 (9H, each s), 1.70 (9H, s), 1.95-2.02 (1H, m), 2.11-2.20 (1H, m), 2.39-2.59 (4H, m), 3.22-3.63 (7H, m), 3.87 (1H, qu, *J* = 8.8), 4.02-4.08 (2H, m), 4.94-4.98 and 5.10-5.14 (1H, each m), 6.84 (1H, dd, *J* = 1.7, 8.6), 7.13-7.54 (6H,

m); LC/MS (ESI, $[M+H]^+$, *m/z*) 859. The obtained product (3.840 g, 4.3 mmol) and 4 M HCl in ethyl acetate (80 mL) were stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with diethyl ether, and dried to afford 2.166 g (93% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.90-1.98 (1H, m), 2.02-2.13 (1H, m), 2.35-2.43 (4H, m), 3.00 (3H, s), 3.04-3.11 (1H, m), 3.23-3.28 (1H, m), 3.45-3.48 (2H, m), 3.90 (1H, qu, *J* = 8.7), 4.37-4.44 (2H, m), 5.05 (1H, dd, *J* = 2.0, 10.2), 6.82 (1H, dd, *J* = 2.1, 8.8), 6.95 (1H, d, *J* = 2.1), 7.14 (1H, d, *J* = 7.8), 7.16-7.18 (1H, m), 7.31 (1H, s), 7.35 (1H, t, *J* = 7.8), 7.69 (1H, d, *J* = 8.8), 9.15 (1H, brs), 9.56 (1H, brs), 9.89 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 18.4, 27.9, 32.0, 39.1, 45.8, 53.7, 63.4, 67.9, 92.2, 112.4, 115.3, 117.0, 119.0, 121.2, 121.3, 129.2, 138.5, 141.8, 143.0, 147.6, 157.7; HRMS calculated for $C_{22}H_{28}N_4O_4S + H^+$ 445.1904, found (ESI, $[M+H]^+$) 445.1897; LC/MS (ESI, $[M+H]^+$, *m/z*) 445; HPLC (Method A): purity 96% R_T 2.0 min.

_N_-(3-((1_R_)-1-Hydroxy-2-(2-((3-(trifluoromethyl)-1_H_-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methanesulfonamide (15). To a stirred solution of **27** (1.511 g, 5 mmol), **17** (10 mL, 10 mmol, 1 M toluene solution), and triphenylphosphine (2.600 g, 9.9 mmol) in anhydrous toluene (15 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (1.733 g, 10 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (82:18 to 61:39 n-hexane/ethyl acetate) to give 3.544 g (81% yield) of the coupling product. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers): δ 0.54 (6H, q, *J* = 8.1), 0.89 (9H, t, *J* = 8.1), 1.44 (9H, s), 1.49 and 1.53

(9H,each s), 1.71 (9H, s), 3.22-3.64 (7H, m), 4.04-4.13 (2H, m), 4.94-4.99 and 5.10-5.14 (1H, each m), 6.98 (1H, dd, J = 2.2, 8.8), 7.14-7.45 (4H, m) 7.59 (1H, d, J = 1.8), 7.64(1H, dd, J = 4.0, 8.8); LC/MS (ESI, $[M+H]^+$, *m/z*) 873. The obtained product (3.514 g, 4 mmol) and 4 M HCl in ethyl acetate (80 mL) were stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with *tert*-butyl methyl ether, and dried to afford 1.930 g (93% yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (3H, s), 3.05-3.12 (1H, m), 3.24-3.28 (1H, m), 3.49 (2H, brs), 4.40-4.47 (2H, m), 5.02 (1H, d, J = 9.8), 6.28 (1H, brs), 7.03 (1H, dd, J = 2.1, 8.8), 7.13-7.18 (3H, m) 7.31 (1H, s), 7.35 (1H, t, J = 7.8), 7.71 (1H, d, J = 8.8), 9.11 (1H, brs), 9.46 (1H, brs), 9.87 (1H, s), 13.96 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 39.2, 45.8, 53.6, 63.6, 67.9, 92.8, 113.7, 115.4, 117.0, 119.0, 119.6, 121.2, 122.2 (q, J = 268), 129.2, 133.0 (q, J = 36), 138.5, 141.9, 143.0, 157.7; HRMS calculated for $C_{19}H_{21}F_3N_4O_4S$ + H^+ 459.1308, found (ESI, $[M+H]^+$) 459.1307; LC/MS (ESI, $[M+H]^+$, *m/z*) 459; HPLC (Method A): purity 99% R_T 2.0 min.

_N_-(3-((1_R_)-1-Hydroxy-2-(2-((3-phenyl-1_H_-indazol-6-yl)oxy)ethylamino)ethyl)phenyl)methanesulfonamide (16). To a stirred solution of **28** (34 mg, 0.13 mmol), **17** (0.5 mL, 0.20 mmol, 0.4 M toluene solution), and triphenylphosphine (56 mg, 0.21 mmol) in anhydrous toluene (1 mL) was added *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide (40 mg, 0.23 mmol) at room temperature, and the solution was stirred overnight. The reaction solution was then purified by flash column chromatography on silica gel (88:12 to 67:33 n-hexane/ethyl acetate) to give 77 mg (87% yield) of the coupling product. ^{1}H-NMR (300

MHz, $CDCl_3$, 1:1 rotamers): δ 0.55 (6H, q, *J* = 7.9), 0.89 (9H, t, *J* = 7.9), 1.44 (9H, s), 1.46 and 1.49 (9H, each s), 1.73 (9H, s), 3.24-3.64 (7H, m), 4.06-4.15 (2H, m), 4.95-4.99 and 5.10-5.15 (1H, each m), 6.93 (1H, dd, *J* = 1.9, 8.8), 7.13-7.52 (7H, m), 7.64 (1H, d, *J* = 1.9), 7.79 (1H, dd, *J* = 3.0, 8.8), 7.96 (2H, dd, *J* = 1.5, 8.0); LC/MS (ESI, $[M+H]^+$, *m/z*) 881. To the solution of the obtained product (75 mg, 0.09 mmol) in *tert*-butyl methyl ether (0.2 mL) was added 4 M HCl in 1,4-dioxane (1 mL) and the mixture was stirred overnight at room temperature. The resultant solid was collected by suction filtration, washed with *tert*-butyl methyl ether, and dried to afford 48 mg (quantitative yield) of the title compound as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.00 (s, 3H), 3.08-3.12 (1H, m), 3.25-3.30 (1H, m), 3.47-3.50 (2H, m), 4.42-4.44 (2H, m), 5.04 (1H, dd, *J* = 2.0, 10.2), 6.91 (1H, dd, *J* = 2.0, 8.9), 7.05 (1H, d, *J* = 2.0), 7.13-7.18 (2H, m), 7.32 (1H, s), 7.35 (1H, t, *J* = 7.9), 7.38-7.42 (1H, m), 7.49-7.53 (2H, m), 7.96-7.99 (3H, m), 9.08 (1H, brs), 9.41 (1H, brs), 9.87 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 39.1, 45.9, 53.6, 63.4, 67.9, 92.5, 113.0, 115.1, 116.9, 118.9, 121.2, 121.5, 126.5, 127.6, 128.7, 129.2, 133.6, 138.5, 142.6, 142.9, 143.0, 156.9; HRMS calculated for $C_{24}H_{26}N_4O_4S + H^+$ 467.1748, found (ESI, $[M+H]^+$) 467.1746; LC/MS (ESI, $[M+H]^+$, *m/z*) 467; HPLC (Method A): purity 99% R_T 2.0 min.

Ethyl 6-(2-(*tert*-butoxycarbonyl-((2*R*)-2-(3-(*tert*-butoxycarbonyl(methylsulfonyl)amino)phenyl)-2-triethylsilyloxy-ethyl)amino)ethoxy)-1-tetrahydropyran-2-yl-indazole-3-carboxylate (30). To a stirred solution of **17** (506 mg, 0.86 mmol), **29** (124 mg, 0.43 mmol), and triphenylphosphine (229 mg, 0.87 mmol) in

anhydrous THF (4.3 mL) under nitrogen was added diethyl azodicarboxylate (0.39 mL, 0.86 mmol, 2.2 M toluene solution) at 0 °C. The resulting solution was allowed to warm to room temperature and was then stirred overnight. The solution was concentrated and purified by flash column chromatography on silica gel (85:15 to 64:36 n-hexane/ethyl acetate) to afford 238 mg (64% yield) of the title compound. ^{1}H-NMR (300 MHz, $CDCl_3$, 2:3 rotamers): δ 0.48-0.61 (6H, m), 0.87-0.97 (12H, m), 1.22-1.33 (2H, m), 1.43-1.52 (18H, m), 1.64-1.78 (3H, m), 2.04-2.14 (2H, m), 2.47-2.59 (1H, m), 3.22-3.62 (6H, m), 3.71-3.78 (1H, m), 4.01-4.07 (2H, m), 4.48 (2H, q, J = 7.1), 4.95-4.99 and 5.10-5.15 (1H, each m), 5.72-5.77 (1H, m), 6.89-7.00 (2H, m), 7.12-7.44 (4H, m), 7.98-8.04 (1H, m); LC/MS (ESI, $[M+H]^+$, *m/z*) 861.

Biological Methods.

Human β_1, β_2 or β_3, or marmoset β_3 adrenergic receptor agonist assay. The measurement of human β_1, β_2 or β_3, or marmoset β_3 adrenergic receptor agonist activity was carried out using stably transfected Chinese Hamster Ovary (CHO) cells expressing recombinant human β_1, β_2 or β_3, or marmoset β_3 adrenergic receptor. The cells were cultured in Ham's F-12 medium containing 10% fetal bovine serum, 400 μg/ml geneticin (Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin. These cells were seeded on a 96-well plate at a density of 2×10^4 cells/well, and were then cultured for about 20 hours. The medium was then aspirated from each well and replaced with 80 μL of serum-free Ham's F-12 medium, and the cells were incubated for a further 15 minutes. A test compound was initially dissolved in DMSO and was then diluted with Ham's F-12 containing 100 mmol/L HEPES and 1 mmol/L isobutylmethylxanthine. The diluted

sample (20 μL) was added to the cells and the cells were cultured for 30 minutes. The medium was then removed and 0.1 mL of the Assay/lysis Buffer that was included in the cAMP-Screen kit (Applied Biosystems) was added to the cells. These cells were then incubated at 37 °C for 30 minutes. The cAMP level in the resulting cell lysate was quantified using the cAMP-Screen kit. The maximum response to the positive control, isoproterenol, was taken as 100%. The maximum response of each test compound was calculated as a percentage of the maximum response of isoproterenol, and is termed the Intrinsic Activity [I.A. (%)]. The concentration of the compound solution that resulted in a response that was 50% of the maximum for that compound (EC_{50}) was also determined.

Human α_{1A} adrenergic receptor agonist assay. Human α_{1A} adrenergic receptor agonist activity was measured using stably transfected HEK293 cells expressing recombinant the human α_{1A}-adrenergic receptor. The cells were cultured in DMEM medium containing 10% fetal bovine serum, 400 μg/ml hygromycin B (Gibco BRL), 100 U/ml penicillin and 100 μg/ml streptomycin. Subsequently, the cells were prepared at a density of 5×10^6 cells/ml using Assay Buffer (20 mmol/L HEPES-KOH (pH 7.4), 115 mmol/L NaCl, 5.4 mmol/L KCl, 0.8 mmol/L $MgCl_2$, 1.8 mmol/L $CaCl_2$, 13.8 mmol/L D-glucose, and 0.1% bovine serum albumin) containing 0.2% Pluronic F-127 (Invitrogen) and 20 μmol/L Fura-2AM (Dojin). The cells were incubated in a CO_2 incubator for 30 minutes, washed twice with the Assay Buffer to remove excess Fura-2AM, and were then resuspended at a density of 5×10^6 cells/ml with the Assay Buffer. Subsequently, the cells were dispensed on a 96-well plate at a volume of 80 μl/well. This plate was used as the cell plate. In addition to the cell plate, a sample plate was provided in which wells contained a test compound that had

been diluted 10 times with the Assay Buffer to concentrations ranging from 10^{-5} to 10^{-12} M. The plates were set up in an FDSS4000 kinetic plate reader (Hamamatsu Photonics K.K.), and were pre-incubated for 180 seconds at 37 °C. Subsequently, measurement of fluorescence intensity (excitation wavelengths 340 nm and 380 nm, measurement wavelength 500 nm) was initiated and proceeded at intervals of 2 seconds. After measurements were taken over about 30 seconds, 20 μl of the test sample from the sample plate was added to the cell plate, and measurements were continued for a further 270 seconds. The Ca flux caused by the test compound was calculated utilizing the difference between the maximum value of the fluorescence intensity ratio at wavelengths 340 nm and 380 nm after addition of the test compound, and the fluorescence intensity ratio before addition of the test compound, as the peak height. Maximum response to norepinephrine that was used as a positive control was taken as 100%. The maximum response to each test compound was calculated as a percentage of the maximum response to epinephrine, and is termed the Intrinsic Activity [I.A. (%)]. The concentration of a compound solution that resulted in a response that was 50% of the maximum for that compound (EC_{50}) was also determined.

Effects on blood pressure and heart rate in rats. Male Sprague-Dawley rats (Japan SLC, Inc., Shizuoka, Japan) weighing 200-300 g were used. Rats were housed in an air-conditioned room (20-26 °C and 30-75% relative humidity with a 12 h light-dark cycle), were fed a standard laboratory diet (CRF-1, Oriental Yeast, Tokyo, Japan), and were given water ad libitum. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p. and 25 mg/kg, s.c.). The left femoral vein and artery were exposed by a small incision. A polyethylene tube (SP10 Natsume Seisakusyo Co., Ltd., Tokyo, Japan) was inserted into the vein for

compound injection. A polyethylene tube (SP31 Natsume Seisakusyo Co., Ltd.) was inserted into the artery, and was connected to a pressure transducer. Blood pressure was measured from the pressure transducer through a pressure amplifier (AP-641G, Nihon Koden Corp.). Heart rate was measured by the Heart Rate Counter (AT-601G, Nihon Koden Corp.), using the pulse wave of the blood pressure as the trigger. The blood pressure, mean blood pressure and heart rate were recorded. Once the blood pressure and heart rate were almost constant, the test compound was administered through the left femoral vein and the parameters were recorded for 30 min. Maximum increase or decrease of HR and MBP is shown as a value (%) relative to the baseline.

Relaxant activity on isolated marmoset urinary bladder smooth muscle. Three female common marmosets (*Callithrix jacchus*) aged 11 to 14 months were purchased from CLEA Japan Inc. (Tokyo, Japan) and were used at 19 to 20 months. Marmosets were housed in an air-conditioned room (27 ± 3 °C, 45 ± 15% humidity, 12 h light-dark cycle), were fed 30 g of balanced marmoset food pellets (CMS-1, CLEA Japan, Tokyo, Japan) once a day, and were given water ad libitum. Marmosets were anesthetized with an intramuscular injection of ketamine hydrochloride (15 mg/kg), and were sacrificed by exsanguination. The urinary bladder was carefully isolated and immersed in ice-cold Krebs-Henseleit solution that was sufficiently gassed with 95% O_2 and 5% CO_2. The bladder was cut by a midline incision and 6 muscle strips of the bladder body, approximately 10-15 mm long and 2-5 mm wide were prepared. The strips were suspended in a 10 mL glass organ bath filled with Krebs-Henseleit solution that was aerated with 95% O_2 and 5% CO_2 at 37 °C, and were allowed to equilibrate for over 30 min under a resting tension of 1 g. Strip

tension was measured isometrically by using a TB-612T force displacement transducer (Nihon Koden, Tokyo, Japan). Following the equilibration period, the strips were pre-contracted with KCl (40 mmol/L) three times and strips that exhibited three equivalent contractions were used. These strips were then again pre-contracted with 40 mmol/L KCl, and, after the tension had stabilized, a test compound was cumulatively added (at an interval of 20 minutes). When the relaxation response at the maximum concentration of the test compound was completed, papaverine at a final concentration of 10^{-4} mol/L was added, and the maximum relaxation response of each strip was determined.

Supporting Information Available

Experimental details of intermediates **17**-**29**; the rat β_3-AR agonist assay method; summary of human β and α_{1A}-AR agonistic activity of indazole derivatives **10**, **11** and **13**; a csv file containing molecular formula strings. This material is available free of charge via the Internet at http://pubs.acs.org.

Corresponding author Information

* Yasuhiro Wada

E-mail address: wada.yp@om.asahi-kasei.co.jp

Pharmaceutical Research Center, Asahi Kasei Pharma Corporation, 632-1, Mifuku, Izunokuni-shi, Shizuoka 410-2321, Japan

Telephone number: +81-558-76-8494

Fax number: +81-558-76-5755

* Satoshi Shuto

E-mail address: shu@pharm.hokudai.ac.jp

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

Telephone & fax number: +81-11-706-3769

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Abbreviations Used

OAB, Overactive bladder; AR, adrenergic receptor; HR, heart rate; BP, blood pressure; MBP, mean blood pressure; I.A., intrinsic activity; TMAD, *N*,*N*,*N'*,*N'*-tetramethylazodicarboxamide; TBSCl, chloro

tert-butyldimethylsilane; TBDPSCl, chloro *tert*-butyldiphenylsilane.

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Table of Contents graphic.