Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.6b01197 • Publication Date (Web): 29 Mar 2017

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Discovery of Novel Indazole Derivatives as Orally Available β_3-Adrenergic Receptor Agonists Lacking Off-Target-Based Cardiovascular Side Effects

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Running Title: Structurally Novel Human β_3-Adrenergic Receptor Agonists

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Abstract

We previously discovered that indazole derivative **8** was a highly selective β_3-adrenergic receptor (β_3-AR) agonist, but it appeared to be metabolically unstable. To improve metabolic stability, further optimization of this scaffold was carried out. We focused on the sulfonamide moiety of this scaffold, which resulted in the discovery of compound **15** as a highly potent β_3-AR agonist (EC_{50} = 18 nM) being inactive to β_1-, β_2-, and α_{1A}-AR (β_1/β_3, β_2/β_3, and α_{1A}/β_3 >556-fold). Compound **15** showed dose-dependent β_3-AR-mediated responses in marmoset urinary bladder smooth muscle, had a desirable metabolic stability and pharmacokinetic profile (C_{max} and AUC), and did not obviously affect heart rate or mean blood pressure when administered intravenously (3 mg/kg) to anesthetized rats. Thus, compound **15** is a highly potent, selective, and orally available β_3-AR agonist, which may serve as a candidate drug for the treatment of overactive bladder without off-target-based cardiovascular side effects.

Introduction

The International Union of Pharmacology recognizes three adrenergic receptor (AR) subfamilies, the α_1-, α_2-, and β-AR subfamilies, with β_1-, β_2-, and β_3-ARs as members of the latter.[1] The β_1-AR is the dominant receptor in heart and adipose tissue, whereas the β_2-AR is responsible for relaxation of vascular, uterine, and airway smooth muscle.[2] The β_3-AR mediates metabolic effects such as lipolysis and thermogenesis in adipose tissues.[3] Given that β_3-AR mRNA is present in human white fat, gall bladder, stomach, small intestine, prostate, and urinary bladder detrusor,[4] this particular receptor represents an attractive target for drug discovery efforts that may culminate in the development of potent and selective β_3-AR agonists.[5] Because many β_3-AR agonists induce weight loss in obese animals and exert anti-diabetic effects in rodent models of type 2 diabetes, these agonists were investigated in clinical trials for the treatment of obesity

and/or diabetes.[6] However, these agonists failed during early clinical investigations, likely because the expression pattern of β_3-AR in most animal species differs considerably from that in humans, particularly in adipose tissues.[7] Moreover, many of the agonists also showed either poor pharmacokinetic (PK) profiles and/or cardiovascular adverse effects due to a lack of selectivity for the β_3-AR over β_1- and β_2-ARs.[8] However, these earlier β_3-AR agonists were observed to relax isolated rat and human detrusor muscle strips, and to increase bladder capacity in rats.[9] Therefore, pharmaceutical companies have recently been endeavoring to discover potent, selective, and orally bioavailable β_3-AR agonists for the treatment of overactive bladder (OAB).[10]

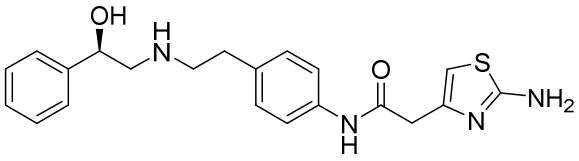
OAB is defined as urgency with or without urinary incontinence, usually associated with frequency and nocturia.[11] Population-based estimations demonstrated that OAB affects 11.8% of adults, and that its rates are similar between men and women and increase with age.[12] OAB can be extremely distressing to the patient and has been shown to negatively affect quality of life.[13] OAB is associated with enhanced voiding frequency, increased risk of depression, sleep disorders, skin infections, and falls with fractures.[13] Detrusor overactivity is a urodynamic observation characterized by involuntary detrusor contractions during the filling phase that may be spontaneous or provoked.[14] Muscarinic antagonists have been used as first-line treatment drugs for OAB for many years.[15] These drugs demonstrate improvement in continence, number of micturitions, and urgency.[16] However, they are associated with mechanistic side effects such as dry mouth, constipation, and blurred vision, and in rare instances with urinary retention.[16] A recent study found that the persistence rates with pharmacotherapy for OAB were $\leq$35% for twelve months after the initial prescription of muscarinic antagonists.[17] The side effects can be related to the limitation of their long-term adherence in the pharmacotherapy of OAB, at least to some extent.[18] Therefore, alternative drugs are sought to both avoid adverse effects and provide more potent therapeutic options. The β_3-AR agonists have emerged as a promising class of such

drugs, because this class may be less prone towards inducing adverse effects in comparison with muscarinic antagonists.[19]

Clinical studies have examined several β_3-AR agonists such as **1** (mirabegron, YM-178),[20] **2** (solabegron, GW427353),[21] **3** (ritobegron, KUC-7483),[22] and **4** (vibegron, MK-4618),[10a] which are shown in Figure 1. In 2012 Mirabegron was approved for the treatment of OAB,[23] and its treatment showed significant efficacy for OAB and resulted in a lower dry mouth incidence compared to muscarinic antagonists.[19] However, treatment with mirabegron caused hypertension and increased heart rate (HR) in both animals and healthy individuals.[19, 24] We hypothesize that, the increased HR resulting from treatment with mirabegron is at least partially induced via activation of the β_1-AR,[24a] and accordingly, highly selective novel β_3-AR agonists would be effective drugs for the treatment of OAB without the risk of such cardiovascular side effects as increased HR. In contrast to mirabegron, which inhibits CYP2D6 and generally should not be coadministered with CYP2D6 substrates,[25] highly selective novel β_3-AR agonists should be inactive to CYP enzymes.

In our previous publication, we reported on novel indazole derivatives, represented by compound **8**, which exhibited a highly selective β_3-AR agonistic activity without cardiovascular side effects.[10c] However, PK studies of **8** in rats indicated that it was metabolically unstable and had poor PK properties (C_{max} and AUC). Based on these findings, we performed a structure–activity relationship (SAR) study of the sulfonamide moiety of the indazole derivatives to improve the metabolic stability whilst maintaining the excellent selectivity over α_{1A}-AR, and obtained the optimized compound **15**, which had more desirable PK properties than **8** in rats. We also conducted a docking study at the β_3-AR binding site, which identified the structural features required for high affinity for the β_3-AR as well as high selectivity for the β_3-AR over α_{1A}-, β_1-, and β_2-ARs. Thus, we have successfully identified compound **15** as a highly potent,

selective, and orally available β_3-AR agonist lacking cardiovascular side effects.



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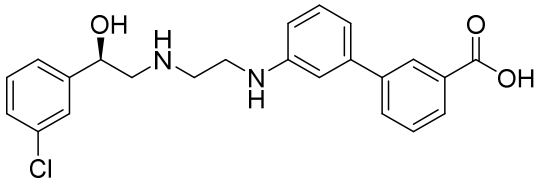

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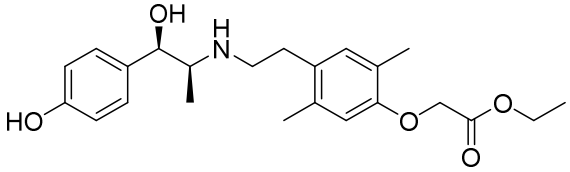

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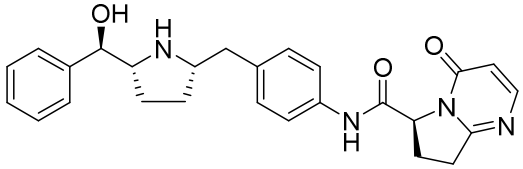

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Figure 1. Chemical structures of **1**, **2**, **3**, and **4**.

Results and Discussion

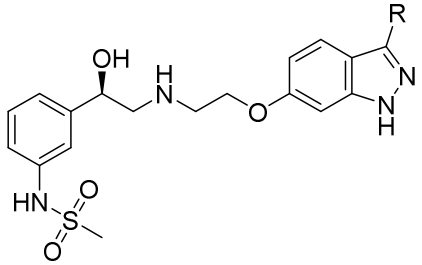
Characteristics of compound 8. We evaluated the *in vitro* ADME and PK profiles of four 3-substituted indazole β_3-AR agonists **5**-**8** previously reported by us.[10c] As shown in Table 1, the human unbound microsomal intrinsic clearance ($hCL_{int,u}$) and the rat unbound microsomal intrinsic clearance ($rCL_{int,u}$) for **7** and **8** were determined to be unacceptably higher (thereby indicating greater metabolic instability) than **5**, even though **7** and **8** possessed excellent

β_3-AR selectivity while **5** was poorly selective for the β_3-AR over α_{1A}-AR. Furthermore, **8** showed an undesirable PK profile (C_{max} and AUC) in rats compared with **5** (Table 2). On the other hand, **8** had moderate MDCK cell monolayer permeability and good solubility in aqueous solutions at a pH 1.2 and pH 6.8 (Table 2). The low C_{max} and AUC of **8** may be due to its metabolic instability.

These previous results indicated that the selectivity of our indazole compounds for the β_3-AR and α_{1A}-AR changed depending on the substituent at the 3-positon. Therefore, when ordered in terms of their selectivity for the β_3-AR over α_{1A}-AR, the indazole compounds ranked as follows: *iso*-propyl (**8**) > cyclobutyl (**7**) > ethyl (**6**) > methyl (**5**). In contrast, the rank order of these compounds in terms of their metabolic stability ($hCL_{int,u}$) was as follows: methyl (**5**) > ethyl (**6**) > *iso*-propyl (**8**) > cyclobutyl (**7**). Thus, highly selective compounds (**7** and **8**) showed poor metabolic stability.

Our aim was to improve the metabolic stability of these compounds while maintaining highly selective β_3-AR agonist activity. In sulfonamide-containing AR agonists, the α_{1A}- or β_3-AR agonistic activity can be manipulated by changing the sulfonamide structure.[26] Therefore, we decided to optimize the metabolically stable 3-methyl indazole derivative **5** by modifying its sulfonamide moiety in order to improve its β_3-AR agonistic selectivity over α_{1A}-AR.

Table 1. AR Agonist Activity and *In Vitro* Metabolic Stability

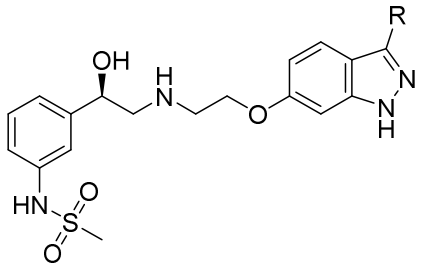


		$h\beta_3$[a]		$h\alpha_{1A}$[a]		Selectivity	Metabolic Stability[a]	
Compound	R	EC_{50}	IA[b]	EC_{50}	IA[c]	α_{1A} / β_3	$hCL_{int,u}$	$rCL_{int,u}$

		(nM)	(%)	(nM)	(%)		(mL/min/kg)	(mL/min/kg)
5	-Me	21 ± 0.67	82	219 ± 6.9	71	10	<10	103
6	-Et	14 ± 0.58	78	857 ± 164	31	61	42	113
7		66	68	>10000	4	>151	463	641
8	-*i*-Pr	13 ± 1.5	69	>10000	9.1	>769	193	211

[a]The results are shown as the mean ± SEM (n = 3) or are presented as the average of two experiments. [b]IA (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [c]IA: maximum response induced by norepinephrine was defined as 100%.

Table 2. *In vitro* ADME and *In Vivo* Pharmacokinetics in Rats



Compound	**5**	**8**
R	-Me	-*i*-Pr
Physicochemical Properties and *in vitro* ADME Profiles		
Metabolic Stability[a]		
$hCL_{int,u}$ (mL/min/kg)	<10	193
$rCL_{int,u}$ (mL/min/kg)	103	211

Permeability[a]		
MDCK ($x10^{-6}$ cm/s)	2.4	4.1
Solubility[a]		
pH 1.2 solution (μM)	285	295
pH 6.8 solution (μM)	273	267
Pharmacokinetics Parameters in Rats[a]		
5 mg/kg[b]		
C_{max} (μg/mL)	0.107 ± 0.034	0.0452
AUC (μg·hr/mL)	0.542 ± 0.065	0.200

[a]The results are shown as the mean ± SD (n = 3) or are presented as the average of two experiments. [b]Compounds were administered orally to rats in solution with saline or water.

Altenbach *et al.* reported a SAR study on potent sulfonamide-containing α_{1A}-AR agonist **9** (Figure 2),[26a] for which increases in the size of the aliphatic group (R^1) of the sulfonamide resulted in decreased α_{1A}-AR potency, while aromatic substitution of the sulfonamide made the compounds inactive to α_{1A}-AR.[26a] On the other hand, when Sawa *et al.* compared aliphatic sulfonamide **10a** with aromatic sulfonamide **10b**, the β_3-AR agonistic potency of **10b** was higher than that of **10a**.[26b] Taking these findings into account, we hypothesized that changing the size of the substitution on the sulfonamide of compound **5** would improve selectivity for β_3-AR agonistic activity over α_{1A}-AR without sacrificing its desirable human microsome, rat microsome, and rat PK profile (Figure 2).

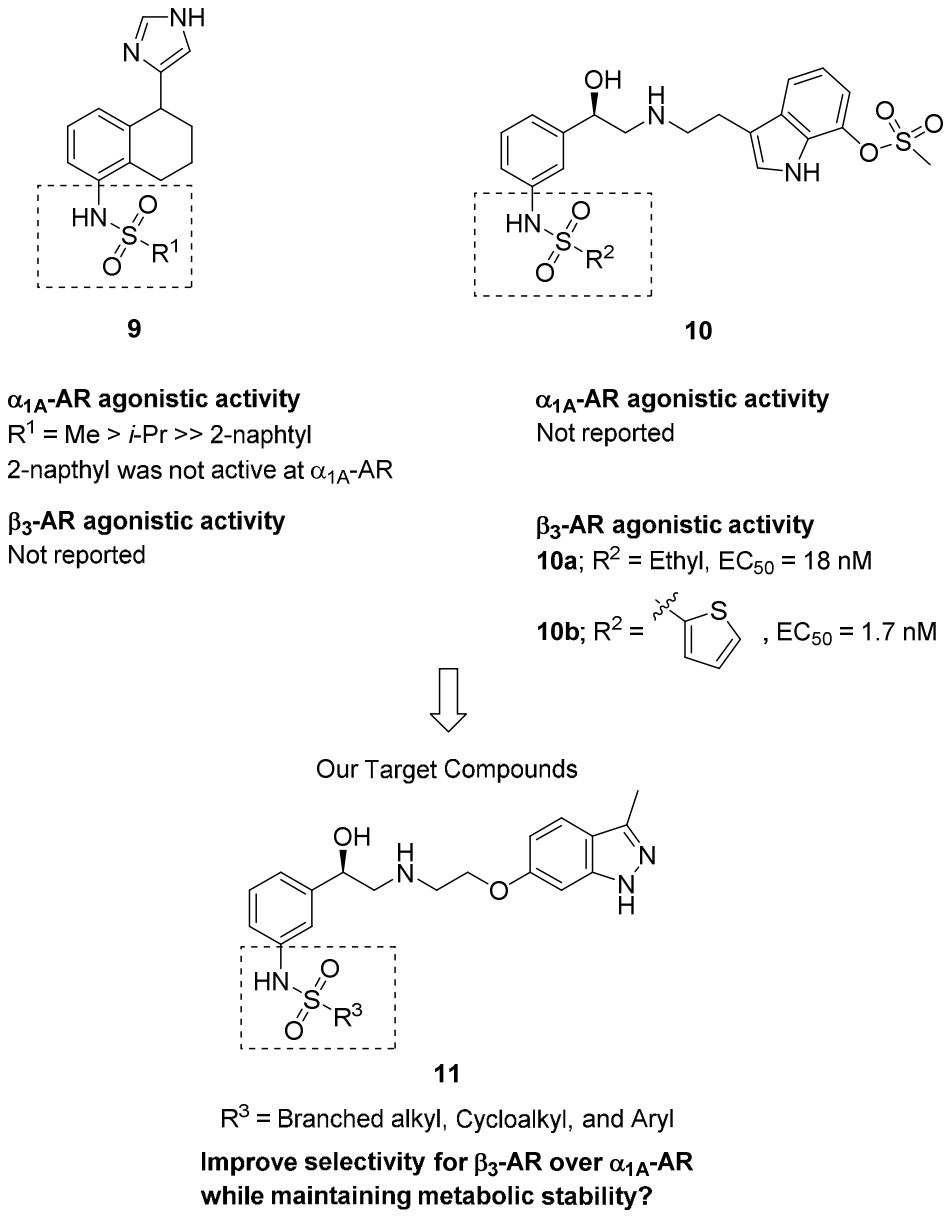


Figure 2. Design strategy of sulfonamide moiety.

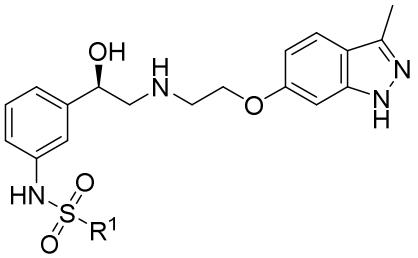
SAR of the sulfonamide moiety. Agonistic activities for the β_3-AR of analogues of **5**, which were modified at the sulfonamide moiety, are shown in Table 3. Replacement of the methyl substituent of **5** with an *n*-Pr (**12**) slightly increased β_3-AR agonistic potency (**5**, EC_{50} = 21 nM vs. **12**, EC_{50} = 6.5 nM). However, by replacing the methyl substituent with *i*-Pr (**13**), the β_3-AR agonistic activity was decreased (**5**, EC_{50} = 21 nM vs. **13**, EC_{50} = 70 nM). We next

synthesized and evaluated compounds with a cycloalkyl substitution; replacement with a cyclopropyl or cyclobutyl group increased β_3-AR agonistic potency (**13**, EC_{50} = 70 nM vs. **14**, EC_{50} = 43 nM; **15**, EC_{50} = 18 nM). However, replacement with a cyclopentyl group (**16**) decreased potency (**13**, EC_{50} = 70 nM vs. **16**, EC_{50} = 309 nM). On the other hand, the phenyl sulfonamide derivative **17** (EC_{50} = 3.9 nM) showed higher potency than the alkyl congeners. Thus, in order of their β_3-AR agonist activity, the compounds were ranked as follows: **17** > **12** > **15**, **5** > **14** > **13** > **16**.

The agonistic potency of these compounds for the α_{1A}-AR is also summarized in Table 3. Although replacement of the methyl substituent with an *n*-Pr did not change activity (**5**, EC_{50} = 219 nM vs. **12**, EC_{50} = 163 nM), replacement with *i*-Pr (**13**) and cyclopropyl (**14**) showed remarkably weakened agonistic activity for the α_{1A}-AR (**13**, EC_{50} = 1757 nM; **14**, EC_{50} = 1250 nM). Finally, the change to a cyclobutyl (**15**), cyclopentyl (**16**), or phenyl (**17**) group made these compounds inactive to α_{1A}-AR (all EC_{50} >10000 nM). Thus, in order of α_{1A}-AR agonist activity, the compounds were ranked as **5**, **12** > **13**, **14** >> **15**, **16**, **17**.

Based on their agonistic potencies for the β_3- and α_{1A}-ARs, these compounds were ranked in terms of their selectivity for β_3-AR over α_{1A}-AR as follows: **17** > **15** >> **12**, **13**, **14**, **16** > **5**. The most selective, **15** and **17**, were next evaluated for β_1-AR and β_2-AR agonistic activities, and the results indicated that both compounds were inactive to these two AR subtypes (Table 4). Thus, **15** and **17** were identified as highly selective β_3-AR agonists, and were selected for the next pharmacological and ADME evaluation.

Table 3. Human β_3- and α_{1A}-AR Agonistic Activities[a]



Compound	R^1	β_3[b]		α_{1A}[b]		Selectivity
		EC_{50} (nM)	IA (%)[c]	EC_{50} (nM)	IA (%)[d]	α_{1A} / β_3
5	-Me	21 ± 0.67	82	219 ± 6.9	71	10
12	-*n*-Pr	6.5 ± 0.94	79	163	84	25
13	-*i*-Pr	70 ± 6.4	90	1757	27	25
14		43 ± 4.3	72	1250	26	29
15		18 ± 2.6	105	>10000	0	>556
16		309 ± 69	58	>10000	0	>32
17	-Ph	3.9 ± 0.067	85	>10000	0	>2564
isoproterenol		86 ± 3.7	100	NT		
norepinephrine		NT		9.1 ± 0.52	100	

[a]The results are shown as the mean ± SEM (n = 3) or are presented as the average of two experiments. [b]Human β_3- or α_{1A}-AR agonist assay, reference 10c. [c]IA (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [d]IA: maximum response induced by norepinephrine was defined as 100%. NT: not tested.

Table 4. Summary of Human β- and α_1-ARs Agonistic Activities[a]

Compound	β_3[b]	β_1[b]	β_2[b]	α_{1A}[b]

	EC_{50} (nM)	IA (%)[c]	EC_{50} (nM)	IA (%)[c]	EC_{50} (nM)	IA (%)[c]	EC_{50} (nM)	IA (%)[d]
8	13 ± 1.5	69	>10000	11.9	>10000	5.6	>10000	9.1
15[e]	18 ± 2.6	105	>10000	4	>10000	4	>10000	0
17	3.9 ± 0.067	85	>10000	10	>10000	0	>10000	0
isoproterenol	86 ± 3.7	100	3.2 ± 0.29	100	13 ± 3.0	100	NT	
norepinephrine	NT		NT		NT		9.1 ± 0.52	100

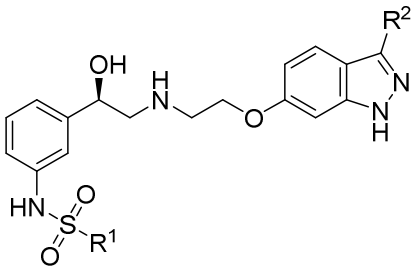
[a]The results are shown as the mean ± SEM (n = 3) or are presented as the average of two experiments. [b]Human β_1-, β_2-, β_3- or α_{1A}-AR agonist assay, reference 10c. [c]IA (intrinsic activity): maximum response induced by isoproterenol was defined as 100%. [d]IA: maximum response induced by norepinephrine was defined as 100%. [e]Compound **15** exhibited insignificant agonistic activity for α_{1B}-AR and α_{1D}-AR (EC_{50} >10000 nM). NT: not tested.

ADME profiles. ADME profiles of **15** and **17** as well as **8** *in vitro* are summarized in Table 5. Although human microsomal clearance of **15** was excellent ($hCL_{int,u}$ = 57 mL/min/kg), that of **17** was not acceptable ($hCL_{int,u}$ = 464 mL/min/kg, eight-fold worse than **15**). Rat microsomal clearance of **15** was also acceptable ($rCL_{int,u}$ = 52 mL/min/kg, four-fold better than **8**). Both **15** and **17** had moderate MDCK cell monolayer permeability and good solubility in pH 1.2 and pH 6.8 solutions. Compound **15** also had improved C_{max}, AUC and half-life values in comparison with **8**.

Compound **15** was next evaluated with a panel of cytochrome P450 enzymes, where it showed only insignificant inhibition of the CYPs (< 34% inhibition at 10 μM against CYP1A2, 2B6, 2C19, 2C8, 2C9, 2D6, and 3A4). In the same evaluation, **1** showed a significant inhibition of 87% at 10 μM against CYP2D6. The low inhibitory capacity of

15 against CYPs suggests that **15** has a much lower risk of drug–drug interactions than **1** when coadministered with CYP-metabolized drugs.

Table 5. *In Vitro* ADME and *In Vivo* Pharmacokinetics of 8, 15, and 17 in Rats



Compound	**8**	**15**	**17**
R^1	-Me		-Ph
R^2	-*i*-Pr	-Me	-Me
Physicochemical and *in vitro* ADMET profiles			
Metabolic Stability[a]			
$hCL_{int,u}$ (mL/min/kg)	193	57	464
$rCL_{int,u}$ (mL/min/kg)	211	52	NT
Permeability[a]			
MDCK ($x10^{-6}$ cm/s)	4.1	5.0	5.1
Solubility[a]			
pH 1.2 solution (μM)	295	299	300
pH 6.8 solution (μM)	267	278	229

Pharmacokinetics parameters in rat[a]

5 mg/kg[b]			
C_{max} (μg/mL)	0.0452	0.119 ± 0.077	NT
AUC (μg·hr/mL)	0.200	0.312 ± 0.100	NT
Half-life (hr)	1.62	3.01 ± 1.51	NT

[a]The results are shown as the mean ± SD (n = 3) or are presented as the average of two experiments. [b]Compounds were administered orally to rats in solution with saline or water. NT: not tested.

Cardiovascular safety profiles and pharmacology. We compared the cardiovascular safety profile of **15**, which has highly selective β_3-AR agonistic activity and an excellent ADME profile, with the clinical drug **1**. As shown in Table 6, **1** (i.v., 3 mg/kg) clearly increased HR and decreased mean blood pressure (MBP) in anesthetized rats. In the same evaluation, compound **15** (i.v., 3 mg/kg) tended to increase HR and decrease MBP less than **1** as a preliminary experiment.[27] These data indicate that **15** possesses a desirable cardiovascular safety profile likely due to its minimal off-target activities; it is highly active to the β_3-AR, but completely inactive to all other α_1- and β-ARs.

Next, we investigated the effect of **15** on the relaxation of urinary bladder smooth muscle strips from marmosets.[10c] As shown in Figure 3, **15** fully relaxed isolated marmoset urinary bladder smooth muscle that was pre-contracted with KCl, and its efficacy was similar to that of the non-selective β-AR agonist isoproterenol.

Based on its excellent β_3-AR selectivity, oral availability, low risk of drug–drug interactions, and favorable relaxation effect on marmoset urinary bladder smooth muscle, the optimized compound **15** was identified as a candidate drug for the treatment of OAB without cardiovascular side effects.

Table 6. Effects of Intravenous Administration of 15 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
1	3	10.0 ± 2.5	ND	3.5 ± 3.5	38.3 ± 3.4
15	3	5.8 ± 0.4	0.9 ± 0.4	2.0 ± 0.9	12.9 ± 0.9

[a]Compounds were administered intravenously to rats (3 mg/kg) and HR and MBP recorded for 30 min. Maximum increase or decrease of HR or MBP is shown as the percentage change relative to baseline. The results are shown as the mean ± SEM. See reference 10c for further details of the experimental method. ND: Not detected.

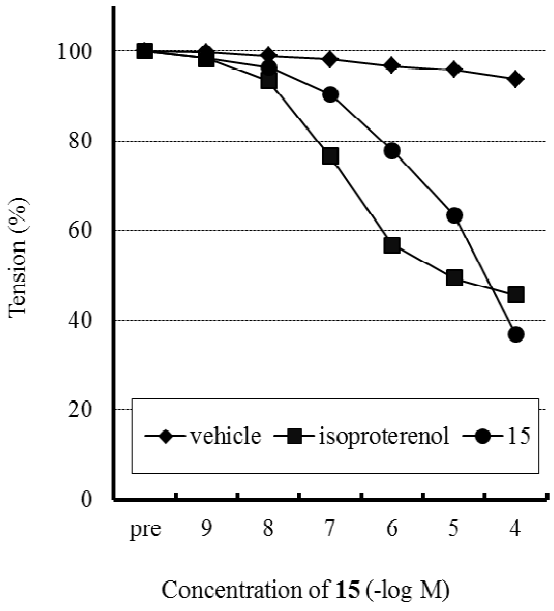


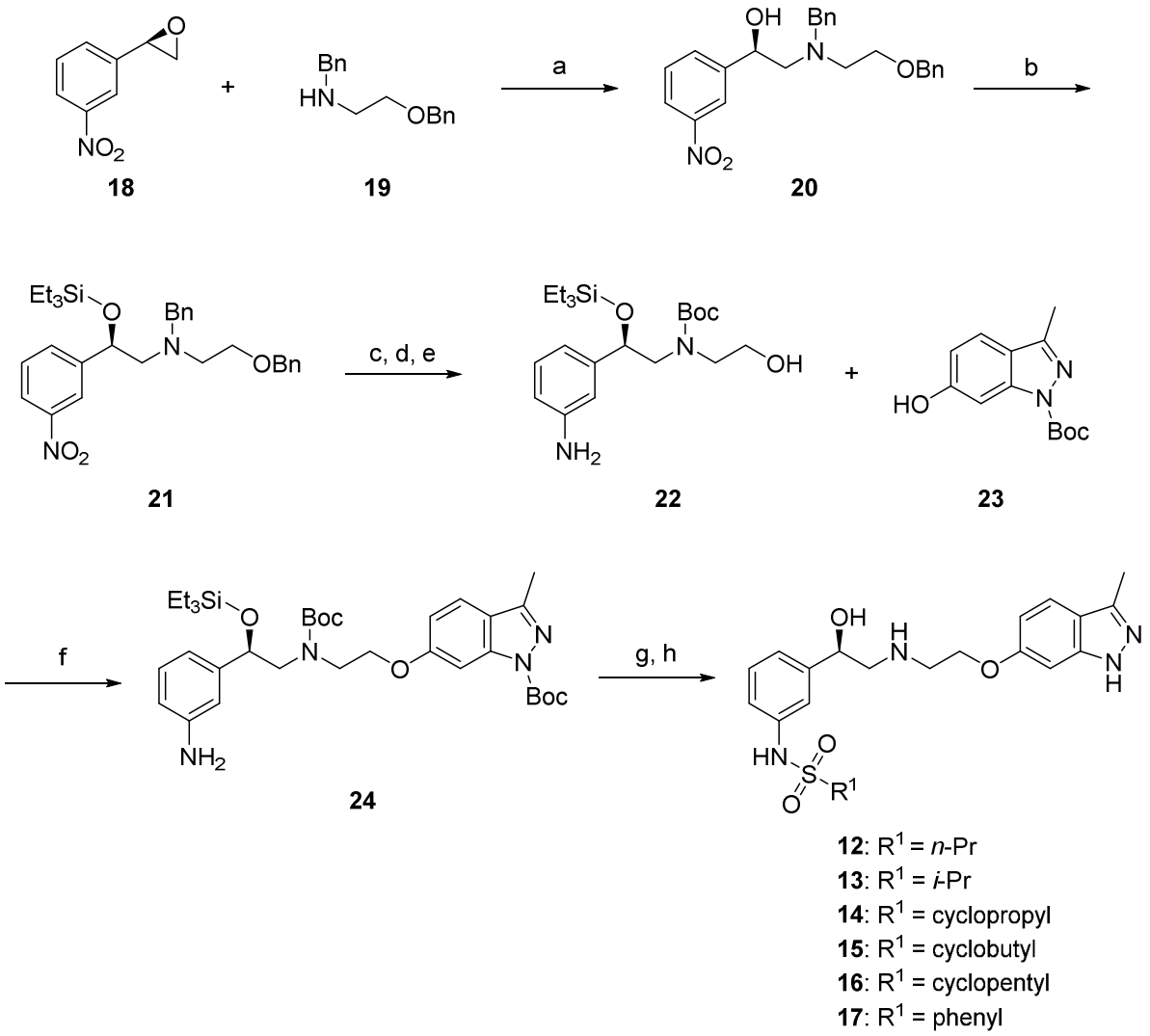
Figure 3. Relaxation of marmoset urinary bladder smooth muscle by **15**. Each point represents the mean of two

experiments. See reference 10c for further details of the experimental method.

Chemistry

The synthesis of compounds **12-17** is shown in Scheme 1. Aminoalcohol **20** was prepared by coupling (*R*)-2-(3-nitrophenyl)oxirane **18**[28] with benzylamine **19** in 2-PrOH. Protection of the hydroxyl of **20** with triethylsilylchloride (TESCl) and imidazole afforded **21**. Hydrogenolysis of **21** reduced the nitro group and deprotected *N*-Bn, and the alkyl amine was protected by using Boc_2O, and then the *O*-Bn group was deprotected under hydrogenolysis conditions to afford alcohol **22**. Mitsunobu reaction of **22** and **23**[10c] using DMEAD[29] and triphenylphosphine proceeded to give the aniline **24**. Coupling of a sulfonyl chloride with aniline **24** and subsequent deprotection of Boc and TES groups afforded the corresponding target compounds **12-17**.

Scheme 1. Synthesis of Target Compounds[a]



[a]Reagents and conditions: (a) 2-PrOH, reflux; (b) TESCl, imidazole, DMF, rt; (c) H_2, 10% Pd/C, EtOH, 50 °C; (d) Boc_2O, THF, rt; (e) H_2, 20% $Pd(OH)_2$/C, THF, MeOH, 50 °C; (f) DMEAD, PPh_3, toluene, rt; (g) R^1-SO_2Cl (R^1 = *n*-Pr, cyclopropyl, cyclobutyl, or phenyl), pyridine, CH_2Cl_2 or R^1-SO_2Cl (R^1 = *i*-Pr or cyclopentyl), DBU, CH_2Cl_2, rt; (h) for **12**-**14**, **16**, and **17**: 4 M HCl in dioxane, rt, for **15**[30]: 1 M TBAF in THF, THF, rt then 4 M HCl in dioxane, rt.

Docking Study to Characterize β_3-AR Agonistic Activity and Selectivity over α_{1A}-, β_1-, and β_2-ARs.

A docking study of the indazole series of the agonists was undertaken in order to understand the SAR underlying the β_3- and α_{1A}-AR agonistic activities. No crystal structure of the β_3-AR is known; therefore, we constructed a homology

model of the β_3-AR. Previous studies showed that β_3-AR homology models, which were constructed using the crystal structure of the β_2-AR, were useful for providing structural insight into ligand binding conformation, performing QSAR analyses, and conducting in silico drug discovery.[10a, 10d, 31] Recent resolutions of the β_2-AR active conformation[32] allowed us to generate more accurate models of the β_3-AR with β_3-AR agonist using homology modeling and docking simulation. We constructed an in silico homology model of the β_3-AR based on the β_2-AR active conformation (PDB entry 3P0G). The β_3-AR sequence was aligned with the β_1-, β_2-, and α_{1A}-ARs using MOE 2015 software (Chemical Computing Group, Montreal, Canada), and the alignment was refined manually to better match the position of amino acids with the corresponding β_2-AR structure (Figure S1, Supporting Information). The sequence identity between β_3- and β_2-ARs is 55.7% based on sequence alignment.[31b]

First, we constructed a homology model of the β_3-AR with compound **17**, which is the most potent β_3-AR agonist in the indazole series. Using this model, we performed a docking simulation with a structurally-diverse indazole series of eight compounds, which included the previously reported compounds,[10c] in addition to another series that consisted of KUC-7322 (active form of compound **3**),[22] two analogues of **10**,[26b] and isoproterenol. Previously, our group used a similar strategy for a docking simulation to characterize the histamine H_3 receptor binding mode.[33] The correlation between the calculated docking score and the -pEC_{50} was determined. As shown in Figure 4, a strong correlation was observed between docking score and -pEC_{50} (R = 0.67 for all 12 compounds; R = 0.90 for the eight indazole compounds). These results show that the homology model and docking pose of the ligand are in agreement with the SAR. To further validate the docking pose of compound **15**, which offers the best profile for potency, selectivity, and ADME within our indazole series, a molecular dynamics (MD) simulation was performed. An enormous difference between the pose suggested by docking and final ligand pose after a MD simulation has been reported in spite of a high

docking score.[34] To examine temporal changes in compound **15** with the β_3-AR during 20 ns of the MD simulation, the root-mean-square deviation (RMSD) was calculated for compound **15** and the β_3-AR throughout the simulation time (Figure S2 in Supporting Information). The time series of the RMSD of compound **15** show that the binding mode is overall stable. Accordingly, compound **15** with the β_3-AR after the MD refinement was further evaluated for its interaction with and selectivity for the β_3-AR over α_{1A}-, β_1-, and β_2-ARs.

The proposed binding mode of compound **15** with the β_3-AR after the MD refinement is shown in Figure 5A. The obtained mode suggested that compound **15** interacts with the three key conserved residues Asp117 (transmembrane [TM] 3), Ser208 (TM5), and Asn332 (TM7), as observed in the crystal structure of the BI-167107-β_2-AR complex (PDB entry 3P0G). An additional hydrogen bonding was predicted between the indazole NH of compound **15** and backbone of Cys196 (extracellular loop [ECL] 2); the indazole moiety participates in hydrophobic interactions with residues Leu97 (TM2), Leu329 (TM7), and Trp333 (TM7) (Figure 5A and B). Moreover, hydrogen bonding also occurred between the sulfonamide carbonyl oxygen and Asn312 (TM6); a T-shaped π-π interaction was predicted between the phenyl ring and Phe309 (TM6); and the cyclobutane moiety occupied a hydrophobic pocket formed by residues Val118 (TM3), Ile173 (TM4), and Tyr204 (TM5) (Figure 5A and B).

We next examined selectivity for the β_3-AR over α_{1A}-AR based on the proposed β_3-AR binding model of compound **15** and the sequence alignment with the α_{1A}-AR. Previously, we reported that the selectivity of different indazole 3-moieties for the β_3-AR over α_{1A}-AR was ranked as follows: *i*-Pr (**8**) > cyclobutyl (**7**) > ethyl (**6**) > -Me (**5**) > -CH_2OH.[10c] These results suggest that a hydrophobic substituent effectively induces improved selectivity for the β_3-AR over α_{1A}-AR. The indazole moiety participates in hydrophobic interactions with residues Leu97 (TM2), Leu329 (TM7), and Trp333 (TM7) of the β_3-AR in the binding model. The residue Leu329 is not conserved in the α_{1A}-AR; in α_{1A}-AR,

these residues are mutated to the hydrophilic residue Lys309. Therefore, the environment of this pocket in the α_{1A}-AR might be more hydrophilic than that in the β_3-AR. Thus, the SAR results on selectivity for the β_3-AR over α_{1A}-AR are supported by the results obtained from our docking model of the β_3-AR. On the other hand, when compared by their selectivity for the β_3-AR over α_{1A}-AR, the substituents at the sulfonamide moiety were ordered as follows: phenyl (**17**) > cyclobutyl (**15**) >> n-propyl (**12**) > methyl (**5**) (Table 2). This ordering suggests that a more bulky substituent improves selectivity for the β_3-AR over α_{1A}-AR. The cyclobutyl substituent displayed hydrophobic interactions with Val118 (TM3), Ile173 (TM4), and Tyr204 (TM5) in β_3-AR homology modeling. The residue Ile173 is not conserved, and is replaced with Leu162 in the α_{1A}-AR. Moreover, Phe198, Ala199, and Ser200 of ECL2, which is located proximal to the sulfone group of the sulfonamide in the β_3-AR, are not conserved in the α_{1A}-AR; in the α_{1A}-AR, these residues correspond to Ile178, Asn179, and Glu180, respectively. Interactions of α_{1A}-AR's residues with the cyclobutyl substituent and sulfone group may thus differ significantly from those of β_3-AR's residues, based on differences in their overall size, protonation potential, charge density, and overall hydrogen bond-forming ability with neighboring residues or ligands. Consequently, these differences may account for the selectivity of β_3-AR over α_{1A}-AR agonistic activity in our indazole analogues.

We also considered the selectivity for the β_3-AR over β_1- and β_2-ARs based on the proposed β_3-AR binding model of compound **15**. As previously observed by Edmondon *et al.*,[10a] the Ala197 (ECL2) residue of β_3-AR is a critical component for selectivity for activation of β_3-AR over binding to β_1- and β_2-ARs. The indazole moiety of compound **15** formed a hydrogen-bonding interaction with the backbone of Cys196, and the Ala197 residue is located proximal to the indazole NH group (Figure 5A). The Ala197 replaces an Asp residue in β_1- and β_2-ARs (Asp217 in β_1-AR, Asp192 in β_2-ARs). The Asp residue in β_1- and β_2-ARs may lead to unfavorable interactions with this hydrogen-bonding with

respect to both electrostatic interactions and size. Therefore compound **15** is likely to show high selectivity for the β_3-AR over β_1- and β_2-ARs.

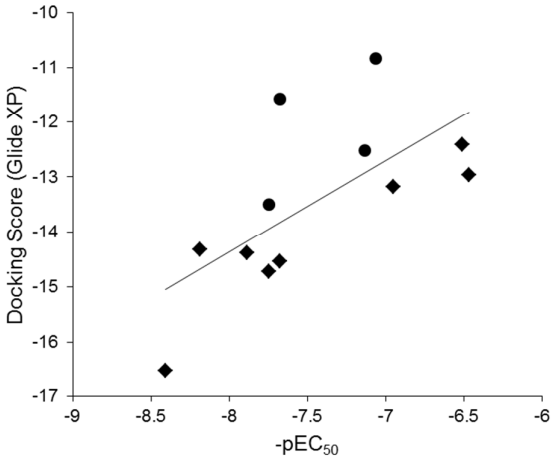


Figure 4. Plot of docking score calculated by Glide extra precision (XP) based on the compound **17** β_3-AR model versus experimental agonistic activity ($-pEC_{50}$) for eight indazole analogues (diamonds) and four other compounds (circles). The coefficient of determination, R^2, was 0.45 for the docking scores and $-pEC_{50}$ values of all 12 compounds. See Supporting Information (Table S1) for individual $-pEC_{50}$ values and docking scores.

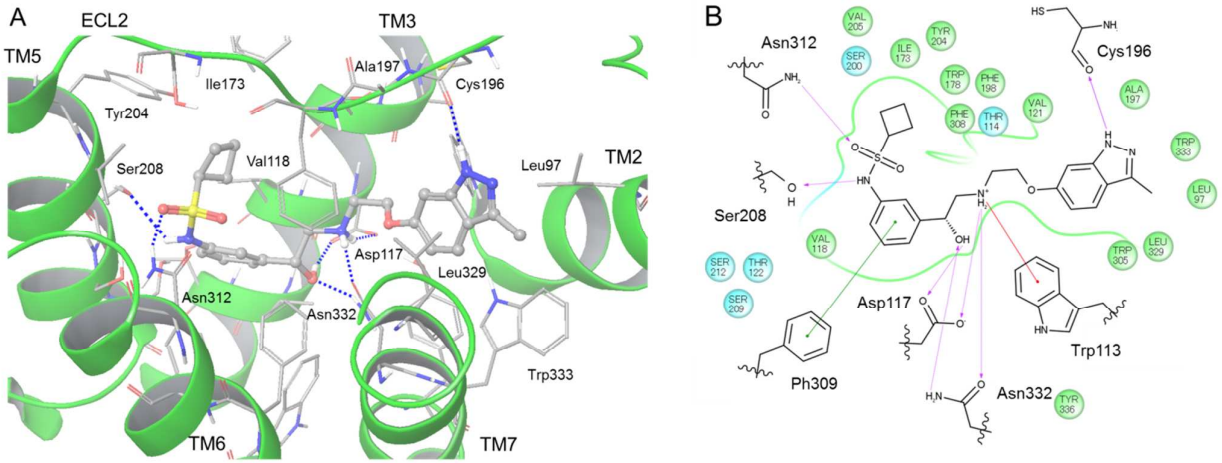


Figure 5. (A) Proposed model of compound **15** binding to the homology model of β_3-AR after MD refinement.

Receptor residues within 4.0 Å of compound **15** are represented by lines. Compound **15** is shown as a ball and stick model. All nonpolar hydrogen atoms of the receptor residues are omitted for clarity. Hydrogen bonding and salt bridges to side chains of Asn117, Ser208, Asn312, and Asn332 are depicted by blue dots. Hydrogen bonding to the backbone of Cys196 is also depicted by blue dots. (B) Schematic representation of the interactions between compound **15** and β_3-AR residues using the Maestro Ligand Interaction Diagram module (Schrödinger, LLC, Portland, OR, USA). Hydrogen bonding is indicated with dashed arrows, salt bridge with purple lines, cation-π interactions with red lines, and π-π interactions with green lines.

Conclusion

We successfully developed the highly potent and selective β_3-AR agonist **15**, which possesses a desirable metabolic stability and PK profile. Compound **15** showed dose-dependent β_3-AR-mediated responses in marmoset urinary bladder smooth muscle and exhibited an excellent cardiovascular safety profile. These promising characteristics are likely due to its minimal off-target effects: **15** is highly active towards only the β_3-AR, and completely inactive towards all other α- and β-ARs. Furthermore, **15** is likely to have a lower risk of drug–drug interactions due to its low inhibition of CYPs. Thus, compound **15** could be a drug candidate for the treatment of OAB without cardiovascular side effects. Detailed pharmacology of **15** will be reported in a future pharmacology paper. We also developed a docking model of the β_3-AR with **15** and related β_3-AR agonists, which identified the structural features required for highly selective binding to the β_3-AR over α_{1A}-, β_1-, and β_2-ARs, and which could therefore be useful for the design of other potent β_3-AR agonists.

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 F_{254} (Darmstadt, Germany). Chromatography on silica gel was carried out using prepacked silica gel cartridges (Yamazen Hi-Flash Column Silica gel, Purifpack-Si series or Biotage KP-Sil series) and the indicated solvent system (Yamazen Multi Prep YFLC, a Moritex Purif-α2 (50F) or Quad1 preparative isolation system). ^{1}H NMR spectra were recorded on either a JEOL AL-300 (300 MHz) or a Bruker BIOPIN AVANCE III HD (400 MHz), with chemical shifts reported in δ values (ppm) relative to trimethylsilane. Electrospray ionization (ESI) high-resolution mass spectra (HRMS) were recorded using a Thermo Scientific Orbitrap Velos Pro (Waltham, MA, USA). Liquid chromatography-mass spectrometry (LC/MS) data were recorded using a Waters single quadrupole type mass spectrometer UPLC/SQD system (Milford, MA, USA) with an ESI method. All the final compounds were purified to ≥ 95% purity, as determined by high-performance liquid chromatography (HPLC). The HPLC method conditions were as follows: YMC Meteoric Core C18 column (Kyoto, Japan), 2.7 μm, 3.0 × 50 mm, 40 °C; solvent A consisting of water (10 mM ammonium acetate); solvent B consisting of acetonitrile; 1.0 mL/min flow rate; linear mobile phase gradient of 10-95% B (2 min), 95% B (2 min), 10% B (2 min); photodiode array detection (254 nm). None of the final compounds were recognized as PAINS compounds.[35] All animal experiments were approved by the Committee on Ethics in Animal Experiments of Asahi Kasei Pharma Corporation.

(*R*)-N-(3-(1-Hydroxy-2-((2-((3-methyl-1H-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)propane-1-sulfonamide (12). To a solution of **24** (95 mg, 0.15 mmol) and pyridine (75 μL, 0.9 mmol) in anhydrous CH_2Cl_2 (1 mL) was added

propane-1-sulfonyl chloride (86 mg, 0.6 mmol) at room temperature, and the solution was shaken (600 min^{-1}) overnight. The mixture was purified by flash column chromatography (4:3 n-hexane/ethyl acetate). The sulfonamide product was diluted with MTBE (100 μL) and 4 M HCl in 1,4-dioxane (1.5 mL, 6.0 mmol) was added at room temperature. The mixture was shaken (600 min^{-1}) overnight and nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was dried to give compound **12** (40.9 mg, 0.095 mmol, 63% yield) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.93 (3H, t, J = 7.6), 1.68 (2H, quin, J = 7.6), 2.44 (3H, s), 3.03-3.09 (1H, m), 3.07 (2H, t, J = 7.6), 3.23-3.27 (1H, m), 3.46 (2H, t, J = 5.1), 4.33-4.37 (2H, m), 4.99 (1H, dd, J = 2.2, 10.2), 6.21 (1H, brs), 6.77 (1H, dd, J = 1.8, 8.7), 6.90 (1H, d, J = 1.8), 7.11 (1H, d, J = 7.8), 7.15 (1H, dd, J = 1.3, 7.8), 7.30 (1H, s), 7.33 (1H, t, J = 7.8), 7.60 (1H, d, J = 8.7), 8.97 (1H, brs), 9.22 (1H, brs), 9.88 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 11.4, 12.5, 16.7, 45.9, 52.2, 53.6, 63.4, 67.9, 92.1, 111.6, 116.6, 117.1, 118.6, 120.9, 121.0, 129.3, 138.5, 140.7, 141.7, 143.0, 157.2; HRMS calculated for $C_{21}H_{28}N_4O_4S + H^+$, 433.1904, found ESI: $[M+H]^+$, 443.1897; LC/MS-ESI (*m/z*): $[M+H]^+$, 433; HPLC: purity 100%, R_T 2.0 min.

(*R*)-*N*-(3-(1-Hydroxy-2-((2-((3-methyl-1H-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)propane-2-sulfonamide (13).

Step 1. To a solution of **24** (96 mg, 0.15 mmol) and pyridine (18 μL, 0.23 mmol) in anhydrous CH_2Cl_2 (2 mL) was added propane-2-sulfonyl chloride (26 mg, 0.18 mmol) at room temperature, and the solution was stirred overnight. To the mixture was added DBU (134 μL, 0.9 mmol) and propane-2-sulfonyl chloride (20 μL, 0.18 mmol) at room temperature. The mixture was stirred overnight and DBU (33.5 μL, 0.23 mmol) and propane-2-sulfonyl chloride (20 μL, 0.18 mmol) were added at room temperature. The mixture was stirred for 4 days and DBU (33.5 μL, 0.23 mmol) and propane-2-sulfonyl chloride (30 μL, 0.26 mmol) were added at room temperature. The mixture was stirred overnight

and purified by flash column chromatography (81:19 to 60:40 n-hexane/ethyl acetate) to give a mixture of **24** and sulfonamide product (41.5 mg). The mixture was diluted with CH_2Cl_2 (1.5 mL) and 1.7 mmol/g MP-Isocyanate (118 mg, 0.2 mmol) was added at room temperature. The mixture was stirred overnight, filtered, and concentrated to give *tert*-butyl (*R*)-6-(2-((tert-butoxycarbonyl)(2-(3-((1-methylethyl)sulfonamido)phenyl)-2-((triethylsilyl)oxy)ethyl)amino)ethoxy)-3-methyl-1*H*-indazole-1-carboxylate (40 mg). LC/MS-ESI (*m/z*): $[M+H]^+$, 747.

Step 2. To *tert*-butyl (*R*)-6-(2-((tert-butoxycarbonyl)(2-(3-((1-methylethyl)sulfonamido)phenyl)-2-((triethylsilyl)oxy)ethyl)amino)ethoxy)-3-methyl-1*H*-indazole-1-carboxylate (40 mg) was added 4 M HCl in 1,4-dioxane (1.5 mL, 6.0 mmol) at room temperature. The mixture was shaken (600 min^{-1}) overnight and nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was dried to give compound **13** (28 mg, 0.055 mmol, 36% yield) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.24 (6H, d, J = 6.8), 2.44 (3H, s), 3.02-3.09 (1H, m), 3.23 (1H, septet, J = 6.8), 3.24-3.26 (1H, m), 3.44-3.47 (2H, m), 4.31-4.40 (2H, m), 4.98 (1H, dd, J = 2.2, 10.3), 6.22 (1H, brs), 6.77 (1H, dd, J = 2.1, 8.7), 6.90 (1H, d, J = 2.1), 7.09 (1H, d, J = 7.7), 7.17 (1H, dd, J = 1.3, 8.1), 7.30-7.34 (2H, m), 7.60 (1H, d, J = 8.7), 8.95 (1H, brs), 9.20 (1H, brs), 9.85 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 11.4, 16.0, 45.9, 51.2, 53.6, 63.4, 68.0, 92.1, 111.6, 116.5, 117.1, 118.5, 120.8, 120.9, 129.3, 138.8, 140.8, 141.6, 143.0, 157.0; HRMS calculated for $C_{21}H_{28}N_4O_4S + H^+$, 433.1904, found ESI: $[M+H]^+$, 433.1895; LC/MS-ESI (*m/z*): $[M+H]^+$, 433; HPLC: purity 100%, R_T 1.9 min.

(*R*)-*N*-(3-(1-Hydroxy-2-((2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)cyclopropanesulfonamide

(14). To a solution of **24** (96 mg, 0.15 mmol) and pyridine (18 μL, 0.23 mmol) in anhydrous CH_2Cl_2 (2 mL) was added cyclopropanesulfonyl chloride (25 mg, 0.18 mmol) at room temperature, and the solution was stirred overnight. To the mixture was added pyridine (24 μL, 0.3 mmol) and cyclopropanesulfonyl chloride (42 mg, 0.3 mmol) at room temperature. The mixture was stirred overnight and purified by flash column chromatography (4/3 n-hexane/ethyl acetate). The sulfonamide product was diluted with 1,4-dioxane (0.2 mL) to which was added 4 M HCl in 1,4-dioxane (1.6 mL, 6.4 mmol) at room temperature. The mixture was shaken (600 min^{-1}) overnight and nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was dried to give compound **14** (63 mg, 0.12 mmol, 83% yield) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.90-0.97 (4H, m), 2.44 (3H, s), 2.58-2.64 (1H, m), 3.03-3.10 (1H, m), 3.23-3.28 (1H, m), 3.46 (2H, t, J = 5.0), 4.32-4.44 (2H, m), 5.01 (1H, dd, J = 2.2, 10.3), 6.25 (1H, brs), 6.77 (1H, dd, J = 2.1, 8.8), 6.91 (1H, d, J = 2.1), 7.12 (1H, d, J = 7.8), 7.18 (1H, dd, J = 1.3, 7.8), 7.32-7.36 (2H, m), 7.60 (1H, d, J = 8.8), 8.99 (1H, brs), 9.26 (1H, brs), 9.82 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 4.9, 11.5, 29.5, 45.9, 53.6, 63.4, 67.9, 92.1, 111.5, 117.2, 117.4, 119.4, 120.8, 121.1, 129.1, 138.5, 140.8, 141.7, 142.9, 157.1; HRMS calculated for $C_{21}H_{26}N_4O_4S + H^+$, 431.1748, found ESI: $[M+H]^+$, 431.1754; LC/MS-ESI (*m/z*): $[M+H]^+$, 431; HPLC: purity 100%, R_T 1.9 min.

(*R*)-*N*-(3-(1-Hydroxy-2-((2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)cyclobutanesulfonamide

(15). *Step 1.* To a solution of **24** (14.95 g, 23.4 mmol) and pyridine (13.2 mL, 164 mmol) in anhydrous CH_2Cl_2 (100 mL) was added cyclobutanesulfonyl chloride (12.73 g, 81.9 mmol) at room temperature, and the solution was stirred overnight. The mixture was diluted with ethyl acetate, washed twice with 0.5 M HCl, washed with brine, dried over Mg_2SO_4, and concentrated in vacuo. The residue (28.72 g) was purified by flash column chromatography (73:27 to

52:48 n-hexane/ethyl acetate) to afford the sulfonamide product (11.14 g, 14.7 mmol, 63% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 rotamers) δ 0.54 (6H, q, *J* = 7.8), 0.88 (9H, t, *J* = 7.8), 1.23-1.31 (2H, m), 1.47 (9H, s), 1.71 (9H, s), 1.88-2.02 (2H, m), 2.19-2.23 (2H, m), 2.47-2.60 (5H, m), 3.21-4.16 (5H, m), 4.88-4.93 and 5.02-5.06 (1H, each m), 6.03 and 6.44 (1H, each s), 6.86 (1H, d, *J* = 8.7), 7.09-7.12 (1H, m), 7.15-7.30 (2H, m), 7.47 (1H, dd, *J* = 1.6, 8.7), 7.54 (1H, s); LC/MS-ESI (*m/z*): $[M+H]^+$, 759.

Step 2. To a solution of sulfonamide (11.14 g, 14.7 mmol) in anhydrous THF (100 mL) was added 1 M TBAF in THF (30 mL, 30 mmol) at room temperature, and the mixture was stirred for 2 h. The mixture was diluted with ethyl acetate and the organic layer was washed with brine, water, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (39:61 to 18:82 n-hexane/ethyl acetate) to give the alcohol product (8.99 g, 13.9 mmol, 95% yield). LC/MS-ESI (*m/z*): $[M+H]^+$, 645.

Step 3. To a solution of the alcohol (8.99 g, 13.9 mmol) in anhydrous 1,4-dioxane (13.9 mL) was added 4 M HCl in 1,4-dioxane (139 mL, 556 mmol) at room temperature, and the mixture was stirred for 2 h. The sticky precipitate was allowed to settle to the bottom of the flask, and the supernatant solution was decanted. The residue was diluted with ethanol (150 mL) and water (15 mL). The solution was concentrated under reduced pressure. To the residue was added ethanol, and the precipitate was collected by suction filtration to give compound **15** (5.15 g, 10 mmol, 72% yield) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.81-1.93 (2H, m), 2.11-2.19 (2H, m), 2.27-2.36 (2H, m), 2.48 (3H, s), 3.02-3.09 (1H, m), 3.22-3.25 (1H, m), 3.46 (2H, t, *J* = 5.0), 3.86-3.94 (1H, quintet, *J* = 8.2), 4.34-4.43 (2H, m), 5.81 (1H, brs), 5.02 (1H, dd, *J* = 1.9, 10.2), 6.82 (1H, dd, *J* = 2.0, 8.8), 6.93 (1H, dd, *J* = 2.0), 7.11 (1H, d, *J* = 7.8), 7.14 (1H, d, *J* = 7.8), 7.29 (1H, s), 7.31 (1H, t, *J* = 7.8), 7.65 (1H, d, *J* = 8.8), 9.09 (1H, brs), 9.47 (1H, brs), 9.80 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 11.2, 16.2, 23.2, 45.8, 53.1, 53.7, 63.4, 67.9, 92.1, 112.2, 116.8, 117.2, 119.1, 121.1,

121.2, 129.1, 138.5, 140.7, 141.5, 142.9, 157.6; HRMS calculated for $C_{22}H_{28}N_4O_4S + H^+$ 445.1904, found ESI: $[M+H]^+$, 445.1895; LC/MS-ESI (*m/z*): $[M+H]^+$, 445; HPLC: purity 100%, R_T 2.0 min.

(*R*)-*N*-(3-(1-Hydroxy-2-((2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)cyclopentanesulfonamide (16). *Step 1.* To a solution of **24** (95 mg, 0.15 mmol) and DBU (134 µL, 0.9 mmol) in anhydrous CH_2Cl_2 (1 mL) was added cyclopentanesulfonyl chloride (111 mg, 0.6 mmol) in CH_2Cl_2 (1 mL) at room temperature, and the mixture was stirred for 2 days. Nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was diluted with methanol (2 mL) and 5 M NaOH (200 µL, 1 mmol) was added at room temperature. The mixture was stirred for 3 days and poured into saturated aq NH_4Cl. The aqueous layer was extracted twice with ethyl acetate. The organic layers were washed twice with 2 M HCl, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (47:53 to 20:80 n-hexane/ethyl acetate) and preparative TLC (10:1 CH_3Cl/MeOH) to give *tert*-butyl (*R*)-(2-(3-(cyclopentanesulfonamido)phenyl)-2-hydroxyethyl)(2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)carbamate (35 mg, 0.063 mmol, 42% yield). LC/MS-ESI (*m/z*): $[M+H]^+$, 559.

Step 2. To the *tert*-butyl (*R*)-(2-(3-(cyclopentanesulfonamido)phenyl)-2-hydroxyethyl)(2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)carbamate (35 mg, 0.063 mmol) was added 4 M HCl in 1,4-dioxane (1.5 mL) at room temperature. The mixture was shaken (600 min^{-1}) overnight and nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was dried to provide the product (34 mg, 0.064 mmol, quant.) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.48-1.57 (2H, m), 1.61-1.70 (2H, m), 1.79-1.95 (4H, m), 2.43 (3H, s), 3.02-3.09 (1H, m), 3.22-3.28 (1H, m), 3.46 (2H,

t, J = 4.7), 3.50-3.56 (1H, m), 4.32-4.40 (2H, m), 4.98 (1H, dd, J = 2.0, 10.1), 6.23 (1H, brs), 6.76 (1H, dd, J = 2.1, 8.7), 6.90 (1H, d, J = 2.1), 7.10 (1H, d, J = 7.8), 7.17 (1H, dd, J = 1.4, 7.8), 7.31 (1H, s), 7.33 (1H, t, J = 7.8), 7.59 (1H, d, J = 8.7), 8.94 (1H, brs), 9.16 (1H, brs), 9.82 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 11.5, 25.3, 27.2, 45.9, 53.6, 59.6, 63.4, 67.9, 92.1, 111.5, 116.9, 117.2, 118.9, 120.9, 121.0, 129.2, 138.7, 140.1, 141.6, 143.0, 157.1; HRMS calculated for $C_{23}H_{30}N_4O_4S + H^+$, 459.2061, found ESI: $[M+H]^+$, 459.2068; LC/MS-ESI (*m/z*): $[M+H]^+$, 459; HPLC: purity 100%, R_T 2.0 min.

(*R*)-*N*-(3-(1-Hydroxy-2-((2-((3-methyl-1*H*-indazol-6-yl)oxy)ethyl)amino)ethyl)phenyl)benzenesulfonamide (17).

To a solution of **24** (96 mg, 0.15 mmol) and pyridine (18 μL, 0.23 mmol) in anhydrous CH_2Cl_2 (2 mL) was added benzenesulfonyl chloride (32 mg, 0.18 mmol) at room temperature, and the solution was stirred overnight. The mixture was purified by flash column chromatography (4:3 n-hexane/ethyl acetate). The sulfonamide product was diluted with 1,4-dioxane (0.2 mL) and 4 M HCl in 1,4-dioxane (1.6 mL, 6.4 mmol) was added at room temperature. The mixture was shaken (600 min^{-1}) overnight and nitrogen gas was blown into the reaction solution to evaporate the solvent. The residue was dried to provide the product (67 mg, 0.12 mmol, 83% yield) as dihydrochloride salt. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.44 (3H, s), 2.88-2.99 (1H, m), 3.14-3.17 (1H, m), 3.43 (2H, t, J = 4.9), 4.29-4.38 (2H, m), 4.92 (1H, dd, J = 2.0, 10.4), 6.20 (1H, brs), 6.76 (1H, dd, J = 2.1, 8.8), 6.90 (1H, d, J = 2.1), 7.02 (1H, d, J = 8.0), 7.05 (1H, d, J = 8.0), 7.22 (1H, s), 7.23 (1H, t, J = 8.0), 7.52-7.56 (2H, m), 7.58-7.62 (2H, m), 7.77-7.79 (2H, m), 8.93 (1H, brs), 9.17 (1H, brs), 10.41 (1H, s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 11.4, 45.9, 53.5, 63.4, 67.8, 92.1, 111.6, 117.1, 117.2, 119.0, 120.9, 121.4, 126.6, 129.1, 132.8, 137.8, 139.4, 140.8, 141.6, 142.9, 157.2; HRMS calculated for $C_{24}H_{26}N_4O_4S + H^+$, 467.1748, found ESI: $[M+H]^+$, 467.1735; LC/MS-ESI (*m/z*): $[M+H]^+$, 467; HPLC: purity 95% R_T 2.0 min.

(*R*)-2-(Benzyl(2-(benzyloxy)ethyl)amino)-1-(3-nitrophenyl)ethan-1-ol (20). *Step 1.* A mixture of 2-(benzyloxy)ethan-1-amine (12.31 g, 81 mmol), benzaldehyde (8.72 g, 82 mmol), and Na_2SO_4 (67.79 g, 477 mmol) in CH_2Cl_2 (150 mL) was stirred overnight at ambient temperature. The mixture was filtered and concentrated under reduced pressure. The residue was diluted with methanol (150 mL) and sodium borohydride (3.41 g, 90 mmol) was added at 0 °C. The mixture was allowed to warm to ambient temperature and was stirred for 2 h. The mixture was concentrated under reduced pressure, quenched with water, and diluted with ethyl acetate. The layers were separated, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude *N*-benzyl-2-(benzyloxy)ethan-1-amine **19** (20.19 g, >100% yield) was carried forward without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.72 (1H, brs), 2.84 (2H, t, *J* = 5.2), 3.62 (2H, t, *J* = 5.2), 3.80 (2H, s), 4.52 (2H, s), 7.20-7.37 (10H, m).

Step 2. A mixture of (*R*)-2-(3-nitrophenyl)oxirane[28] **18** (13.65 g, 83 mmol) and **19** (20.21 g, 84 mmol) in 2-propanol (205 mL) was stirred for 36 h at reflux. The mixture was concentrated under reduced pressure. The residue was diluted with toluene (100 mL) and concentrated under reduced pressure. The residue was purified by flash column chromatography (85:15 to 80:20 n-hexane/ethyl acetate) to give compound **20** (30.76 g, 76 mmol, 92% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.62 (1H, dd, *J* = 10.2, 13.1), 2.75-2.87 (2H, m), 2.92-3.01 (1H, m), 3.51-3.64 (2H, m), 3.78 (2H, dd, *J* = 13.6, 69.1), 4.54 (2H, s), 4.70 (1H, dd, *J* = 3.3, 10.2), 7.27-7.39 (10H, m), 7.45 (1H, t, *J* = 7.9), 7.59 (1H, d, *J* = 7.9), 8.09 (1H, dd, *J* = 1.1, 2.3, 7.9), 8.16 (1H, d, *J* = 2.3); LC/MS-ESI (*m/z*):$[M+H]^+$, 407.

(*R*)-*N*-Benzyl-*N*-(2-(benzyloxy)ethyl)-2-(3-nitrophenyl)-2-((triethylsilyl)oxy)ethan-1-amine (21). To a solution of **20** (30.37 g, 75 mmol) and imidazole (6.13 g, 90 mmol) in anhydrous DMF (150 mL) was added chlorotriethylsilane (15.1 mL, 90 mmol) at room temperature, and the solution was stirred overnight. The mixture was quenched with water and extracted twice with ethyl acetate. The combined organic layers were washed twice with water, washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography (100:0 to 87:13 n-hexane/ethyl acetate) to give compound **21** (36.66 g, 70 mmol, 93% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.42-0.56 (6H, m), 0.85 (9H, t, *J* = 7.9), 2.65-2.85 (4H, m), 3.37-3.47 (2H, m), 3.62 (2H, dd, *J* = 13.6, 42.9), 4.43 (2H, s), 4.68 (1H, dd, *J* = 5.4, 7.4), 7.06-7.11 (2H, m), 7.16-7.20 (3H, m), 7.26-7.37 (6H, m), 7.56 (1H, d, *J* = 7.7), 8.05 (1H, dd, *J* = 1.1, 2.3, 7.7), 8.12 (1H, dd, *J* = 2.3, 2.3); LC/MS-ESI (*m/z*): $[M+H]^+$, 521.

***tert*-Butyl (*R*)-(2-(3-aminophenyl)-2-((triethylsilyl)oxy)ethyl)(2-hydroxyethyl)carbamate (22).** *Step 1.* A mixture of **21** (36.46 g, 70 mmol) and 10% palladium on activated charcoal (15.12 g, PE-type, NE Chemcat) in ethanol (175 mL) was evacuated, placed under a hydrogen atmosphere, and stirred for 9 h at 50 °C. The mixture was evacuated, placed under a hydrogen atmosphere, and stirred for 4 h at 50 °C. The reaction mixture was cooled to room temperature, passed through a membrane filter, and the solvent was concentrated under reduced pressure to give (*R*)-3-(2-(2-benzyloxyethylamino)-1-triethylsilyloxy-ethyl)aniline (25.08 g). LC/MS-ESI (*m/z*): $[M+H]^+$, 401.

Step 2. To a solution of (*R*)-3-(2-(2-benzyloxyethylamino)-1-triethylsilyloxy-ethyl)aniline (25.08 g, 70 mmol) in THF (175 mL) was added Boc_2O (14.60 g, 67 mmol) at room temperature, and the solution was stirred for 1.5 h. The mixture was concentrated under reduced pressure. The residue was added to $Pd(OH)_2$ on activated charcoal (15.02 g, NE Chemcat), THF (80 mL), and MeOH (80 mL). The mixture was evacuated, placed under a hydrogen atmosphere,

and stirred for 8 h at 50 °C. The reaction mixture was cooled to room temperature, passed through a membrane filter, and the solvent was concentrated under reduced pressure. The residue was purified by flash column chromatography (75:25 to 54:46 n-hexane/ethyl acetate) to give compound **22** (17.94 g, 44 mmol, 62% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 3:2 rotamers) δ 0.44-0.63 (6H, m), 0.88 (9H, t, *J* = 7.9), 1.49 and 1.51 (9H, each s), 2.95-3.87 (8H, m), 4.90-4.95 and 5.17-5.21 (1H, each m), 6.57-6.78 (3H, m), 7.09 (1H, t, *J* = 7.7); LC/MS-ESI (*m/z*): $[M+H]^+$, 411.

***tert*-Butyl**

(*R*)-6-(2-((2-(3-Aminophenyl)-2-((triethylsilyl)oxy)ethyl)(tert-butoxycarbonyl)amino)ethoxy)-3-methyl-1*H*-indaz ole-1-carboxylate (24). To a solution of **22** (2.51 g, 6.1 mmol), **23**[10c] (1.99 g, 8.0 mmol), and triphenylphosphine (1.61 g, 6.1 mmol) in anhydrous toluene (25 mL) was added DMEAD[29] (1.43 g, 6.1 mmol) at room temperature, and the solution was stirred overnight at 50 °C under a nitrogen atmosphere. The reaction mixture was cooled to room temperature and washed three times with water. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography (74:26 to 53:47 n-hexane/ethyl acetate) to give a mixture of **24** and **23** (2.29 g). The mixture was diluted with CH_2Cl_2 (25 mL), to which 2.91 mmol/g MP-Carbonate (3.4 g, 9.9 mmol) was added at room temperature. The mixture was stirred overnight and 2.91 mmol/g MP-Carbonate (2.0 g, 5.8 mmol) was added at room temperature. The mixture was stirred for 5 h and filtered to give compound **24** (1.52 g, 2.4 mmol, 47% yield). ^{1}H-NMR (300 MHz, $CDCl_3$, 3:2 rotamers) δ 0.53 (6H, t, *J* = 7.9), 0.89 (9H, q, *J* = 7.9), 1.48 (9H, s), 1.70 (9H, s), 2.53 (3H, s), 3.16-3.78 (6H, m), 4.02-4.14 (2H, m), 4.80-4.84 and 4.96-5.00 (1H, each m), 6.57-6.60 (1H, m), 6.66 (1H, d, *J* = 2.3), 6.70 and 6.78 (1H, each d, *J* = 8.7 and 7.6), 6.87 (1H, dt, *J* = 2.3, 8.7), 7.09 (1H, t, *J* = 7.6), 7.46 (1H, dd, *J* = 1.8, 8.7), 7.55 (1H, s); LC/MS-ESI (*m/z*): $[M+H]^+$, 641.

Human and Rat Unbound Microsomal Intrinsic Clearance Determination.

Human Microsomal Stability Assay. The incubation mixtures were prepared in 96-well cluster tubes (1.4 mL PP; Micronic, Aston, PA, USA). The metabolic stability of the test compounds was determined with pooled, mixed-gender human microsomes (pool of n = 50; XenoTech, Kansas City, KS, USA). Each reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), liver microsomes (final concentration, 0.5 mg/mL), NADPH regenerating mix (final concentration, 1.0 mM NADP+, 4.0 mM glucose-6-phosphate, 3.0 mM $MgCl_2$, 0.4 U/mL of glucose-6-phosphate dehydrogenase, 5.0 mM UDP-GA, and 0.165 mM β-NAD), and the test compound (1 μM) in a total volume of 100 μL. The incubation mixture was prewarmed for 5 min. Liver microsomes were added to the mixture, and kept in a shaking water bath for 15 min at 37 °C. To the mixture was added chilled acetonitrile (200 μL) containing an internal standard to stop the reaction. The samples were centrifuged at 3500 rpm for 15 min at 4 °C. The supernatants were analyzed by LC-MS/MS. Controls were prepared by omitting NADPH regenerating mix from the reaction mixture, and adding microsomes after the reaction was terminated.

Human Liver Microsomal Binding Assay. Microsomal binding of the test compounds was determined by equilibrium dialysis. Dialysis mixtures contained the test compound (1 μM), microsomal solution (final concentration of microsomes, 0.5 mg/mL; ratio of components = 1 : 1 : 8 [microsomes at 5 mg/mL : PBS, pH 7.4 : 125 mM phosphate buffer, pH 7.4]), in a final volume of 150 μL. Duplicate mixtures were subjected to equilibrium dialysis against 150 μL of 100 mM potassium phosphate buffer (pH 7.4) using 96-well Micro-Equilibrium Dialysis Devices (HT Dialysis LLC, Gales Ferry, CT, USA). The dialyzing unit consisted of two chambers separated by an ultrathin membrane with a molecular weight cut-off of 12-14 kDa. The plate was rotated at 300 rpm for 6 h at 37 °C in the plate shaker (Taitec Inc.,

Japan). Upon completion of the dialysis, 50 μL samples were obtained from the microsome and buffer sides. For a matrix composition match, 50 μL of 100 mM phosphate buffer (pH 7.4) was added to the microsome side sample and the microsome mixture was added to the buffer side sample. To the mixture was added chilled acetonitrile (300 μL) containing an internal standard to stop the reaction; the samples were then analyzed by LC-MS/MS. Results are expressed as the area ratio of each sample versus control: human microsome fu = peak $area_{sample}$ / peak $area_{control}$.

Rat Microsomal Stability Assay. The incubation mixtures were prepared in Corning 96-well cluster plates. The metabolic stability of the test compounds was determined in pooled liver microsomes from male Sprague-Dawley rats (XenoTech). The reaction mixture consisted of 100 mM Tris-HCl buffer (pH 7.4), liver microsomes (5 mg/mL), NADPH regenerating mix (final concentrations of 0.165 mM NADP+, 4 mM glucose-6-phosphate, 3.0 mM $MgCl_2$, 0.1 U/mL of glucose-6-phosphate dehydrogenase, 5.0 mM UDP-GA, and 0.165 mM β-NAD), and the test compound (0.5 μM) in a total volume of 100 μL. Reactions were initiated by the addition of liver microsomes (final concentration, 0.5 mg/mL) and incubated in a shaking water bath for 20 min at 37 °C. To the mixture was added chilled acetonitrile (200 μL) containing an internal standard to stop the reaction. A control sample was prepared by adding acetonitrile to a reaction mixture lacking microsomes, and then adding microsomes after the reaction was terminated. The samples were centrifuged at 3500 rpm for 15 min at 4 °C. The supernatants were analyzed by LC-MS/MS.

Rat Liver Microsomal Binding Assay. Microsomal binding of the test compounds was determined by ultracentrifugation. Each ultracentrifugation mixture contained the test compound (0.5 μM), microsomal solution (final concentration, 0.5 mg/mL; ratio of components = 1 : 9 [microsomes at 5 mg/mL : 100 mM Tris-HCl buffer, pH 7.4]), in a final volume of 100 μL. Aliquots of 100 μL were placed in polyallomer ultracentrifuge tubes (8 mm × 34 mm; Beckman Coulter, Fullerton, CA, USA) and centrifuged at 160,000*g* for 16 h at 37 °C in an Optima TL ultracentrifuge (Beckman Coulter).

Upon completion of the ultracentrifugation, 50 μL of the supernatant was obtained. To the supernatant was added chilled acetonitrile (50 μL) containing an internal standard to stop the reaction; the samples were then analyzed by LC-MS/MS. Controls were prepared by adding the test compound to the ultracentrifugal supernatant of the microsomal solution. Results are expressed as the area ratio of sample versus control: rat microsome fu = peak $area_{sample}$ / peak $area_{control}$.

LC-MS/MS Analysis. The LC-MS/MS system consisted of an Agilent 1100 series gradient HPLC pump (Agilent Technologies, Palo Alto, CA, USA), a CTC HTS PAL Autosampler (AMR, Inc., Tokyo, Japan), and a Sciex API 3200 triple quadrupole mass spectrometer (Sciex, Foster City, CA, USA) equipped with a turbo ionspray interface. The samples were separated by reverse phase HPLC using an Inert Sustain RP C18 50 × 2.1 mm column (GL Science Inc., Tokyo, Japan) or Capcell Pak RP C18 50 × 2.1 mm column (Shiseido Inc., Tokyo, Japan). The compounds were eluted with an optimized gradient, which fell within the following method conditions: flow rate of 0.4 mL/min (range: 0-0.7 min); injection volume of 20 μL; solvent A consisting of 0.1% formic acid in water; solvent B consisting of acetonitrile or 0.1% formic acid in acetonitrile; the percentage of B was linearly increased from 5% to 90%, 90% B (1.3 min), returned to initial conditions, equilibrated (1.5 or 1.6 min). The area ratio of each test compound was calculated by comparing the peak area of the compound to the peak area of an internal standard.

Calculation of Human and Rat Unbound Microsomal Intrinsic Clearance ($hCL_{int,u}$ and $rCL_{int,u}$). Metabolic stability was determined by plotting the natural logarithm of the concentration of unchanged test compound as a function of time. The first-order rate constant was calculated using the equation $k = [Ln(C_0) – Ln(C)]/\text{incubation time}$, where C_0 was the initial concentration of the test compound, C was the concentration of the test compound remaining after incubation ($C = C_0 \times$ remaining ratio), and the incubation time was 15 min (human) or 20 min (rat). The half-life ($t_{1/2}$) was estimated

using the equation $t_{1/2} = 0.693/k$. The $hCL_{int,u}$ was estimated using the equation $hCL_{int,u} = k/$(microsomal protein concentration) × (microsomal protein per gram of liver) × (liver mass per kilogram of body mass) / (human microsome fu), where k was the first-order rate constant, the microsomal protein concentration was 0.5 mg/mL, the microsomal protein per gram of liver was 48.8 mg, the liver mass per kilogram of body mass was 25.7 g, and the human microsome fu was determined experimentally from the human liver microsomal binding assay.[36] The $rCL_{int,u}$ was estimated using the equation $rCL_{int,u} = k$ / (microsomal protein concentration) × (microsomal protein per gram of liver) × (liver mass per kilogram of body mass) / (rat microsome fu), where k was the first-order rate constant, the microsomal protein concentration was 0.5 mg/mL, the microsomal protein per gram of liver was 44.8 mg, liver mass per kilogram of body mass was 40.0 g, and the rat microsome fu was determined experimentally from the rat liver microsomal binding assay.[36]

PK Study. Compounds **5**, **8**, and **15** were subjected to PK studies in male Sprague-Dawley rats. Test compounds were administered orally to rats (n = 2 or 3) at a dose of 5 mg/kg in saline or water (dose volume 5 mL/kg). Blood samples were collected via the subclavian vein at 0.5, 1, 2, 4, 6, and 8 h post dose. Plasma was separated by centrifugation and stored frozen at -20 °C until analysis. Plasma compound concentrations were determined using LC-MS/MS. PK parameters were calculated using the non-compartmental analysis tool provided with WinNonlin® Enterprise software v6.4.0.768 (Certara, Princeton, NJ, USA).

MD simulation. The compound **15**-bound structure of human β_3-AR was subjected to 20 ns MD simulations using Desmond version 2.3[37] with OPLS3 force field.[38] The initial model structure was placed into a large

palmitoyloleoylphosphatidylcholine (POPC) bilayer at a depth consistent with the structure of human β_2-AR with BI-167107 (PDB entry 3P0G) from the Orientation of Proteins in Membranes (OPM) database[39] and TIP3P water molecules solvated with 0.15 M NaCl. After minimization and relaxation of the model, the production MD phase was performed for 20 ns in the isothermal-isobaric (NPT) ensemble at 300 K and 1 bar using Langevin dynamics. Long-range electrostatic setups were performed using Maestro (Schrödinger, LLC).

Supporting Information Available

Methods for the solubility assay, permeability assay, cytochrome P450 inhibition assay, human α_{1B} or α_{1D}-AR Agonist Assay, homology modeling of the β_3-AR, and docking; sequence alignment of human β_3-, α_{1A}-, β_1-, and β_2-AR; summary of individual -pEC_{50} and docking score values for β_3-AR; RMSD analysis of the compound **15** with β_3-AR within MD simulations; a CSV file containing molecular formula strings. This material is available free of charge via the Internet at http://pubs.acs.org.

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Acknowledgments

This research was partially supported by the Platform Project for Supporting in Drug Discovery and Life Science Research (Platform for Drug Discovery, Informatics, and Structural Life Science) from the Japan Agency for Medical Research and Development. We are grateful to Toshinori Sugahara, Masayo Koge and Eiichi Tanaka for *in vitro* assay support; Emiko Suzuki and Arata Saso for analysis of compounds; and Hisashi Aratake for project management. We thank Wataru Takahashi and Shiro Miyoshi for helpful discussions.

Abbreviations Used

ADME, absorption, distribution, metabolism, and excretion; DMF, N,N-dimethylformamide; DMEAD, di-2-methoxyethyl azodicarboxylate; MBP, mean blood pressure; MTBE, methyl *tert*-butyl ether; DBU, 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine; TBAF, tetra-*n*-butylammonium fluoride, THF, tetrahydrofuran; TM transmembrane; ECL, extracellular loop; PK, pharmacokinetic; MD, molecular dynamics; PAINS, pan assay

interference compounds.

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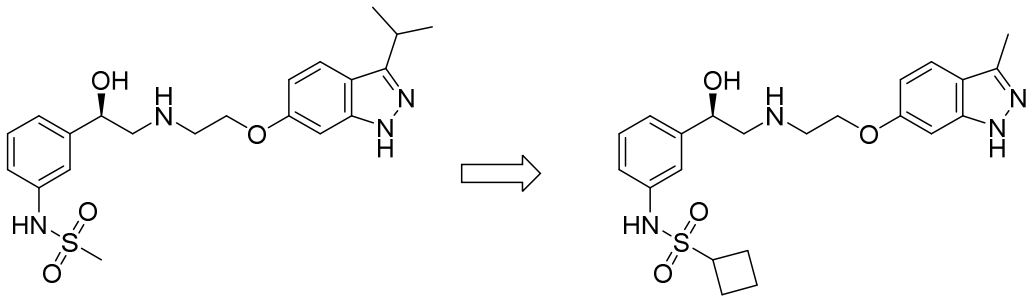
Table of Contents graphic.



8

β_3-AR EC_{50} = 13 nM
Poor metabolic stability
Poor pharmacokinetics
(C_{max} and AUC)

15

β_3-AR EC_{50} = 18 nM
Improved metabolic stability
Orally available
No cardiovascular side effects