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Discovery of 1,7-cyclized indoles as a new class of potent and highly selective human β_3-adrenergic receptor agonists with high cell permeability

Kazuhiro Mizuno,[a,*] Masaaki Sawa,[a] Hiroshi Harada,[a] Ikuko Taoka,[a] Haruhisa Yamashita,[a] Mayumi Oue,[b] Hiroshi Tsujiuchi,[b] Yukiyo Arai,[c] Shinya Suzuki,[c] Yasuji Furutani[b] and Shiro Kato[a]

[a]*Chemistry Research Laboratories, Dainippon Pharmaceutical Co., Ltd, 33-94, Enoki, Suita 564-0053, Japan*
[b]*Pharmacology and Microbiology Research Laboratories, Dainippon Pharmaceutical Co., Ltd, 33-94,Enoki, Suita 564-0053, Japan*
[c]*Pharmacokinetics and Physico-Chemical Property Research Loboratories, Dainippon Pharmaceutical Co., Ltd, Enoki 33-94, Suita 564-0053, Japan*

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Abstract—The synthesis and evaluation of a novel series of 1,7-cyclized indole-based human adrenergic receptor (β_3-AR) agonists are reported. The synthesis of a variety of 1,7-cyclized indole part was accomplished by the Mitsunobu reaction or a ring closing metathesis (RCM) reaction. SAR studies revealed that expansion of the ring size resulted in considerable selectivity against the β_1- and β_2-ARs. Compound **26**, an eight-membered ring analogue with a double bond on its 1,7-linker portion, was found to be a potent β_3-AR agonist (EC_{50} = 0.75 nM, IA = 90%) with extremely high selectivity for the β_3-AR over the β_1- and β_2-ARs.

1. Introduction

The β_3-adrenergic receptor (β_3-AR) has been shown to mediate various pharmacological and physiological effects such as lipolysis, thermogenesis, and relaxation of the urinary bladder. Activation of the β_3-AR is thought to be a possible approach for the treatment of obesity, noninsulin dependent diabetes mellitus (NIDDM),[1] and frequent urination.[2] Therefore, the β_3-AR is recognized as an attractive target for drug discovery. On the other hand, activation of the β_1- or β_2-AR would cause undesirable side effects such as increased heart rate or muscle tremors. Consequently, a number of recent efforts in this field have been directed toward the design of selective agonists for the β_3-AR.[3] During the past decade, several groups have reported the importance of an acidic group on the right-hand side (RHS) of the ethanolamine pharmacophore on potency and selectivity of the β_3-AR agonists (Fig. 1). For example, Sher et al.

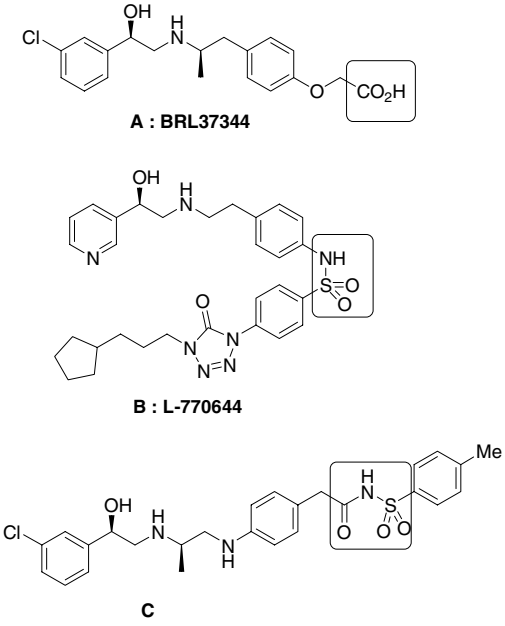

Figure 1.

Keywords: β_3-Adrenergic receptor; Agonist; 1,7-Cyclized indole.
* Corresponding author. Tel.: +81 663375904; fax: +81 663376010; e-mail: kazuhiro-mizuno@dainippon-pharm.co.jp

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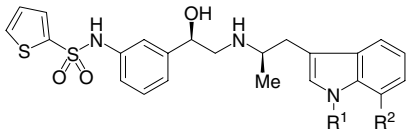
showed that a negatively charged carboxylate group on the RHS in compound **A** played a role in enhancing selectivity for the β_3-AR.[4] Researchers at Merck disclosed a novel series of β_3-AR agonists having an arylsulfonamide on the RHS, represented by compound **B**.[5] In these compounds, the NH group of the sulfonamide function seems to act as an acidic group instead of the traditional carboxyl group, and contributes to the selectivity for the β_3-AR. Workers at GlaxoSmithKline also reported β_3-AR agonists having an acylsulfonamide on the RHS as a carboxylic acid isosteres (compound **C**).[6] In addition, they suggested that the steric bulk of the RHS substituent also contributed to the potency and selectivity of the β_3-AR agonists. However, it is thought that introduction of such hydrophilic groups may generally cause low oral bioavailability due in part to poor absorption.[7]

Previous work in our laboratory has demonstrated that a series of tryptamine derivatives with a 2-thiophenesulfonamide group, represented by **1**, showed strong agonistic activity for human β_3-AR.[8] The structure–activity relationship (SAR) studies on the 7-position of the indole ring indicated that introduction of an aliphatic substituent such as a methoxy group in **2** increased agonistic activity, but modestly decreased subtype selectivity for the β_3-AR. Further SAR studies on the indole ring revealed that substitution of the hydrogen at the 1-position in **1** with an alkyl group significantly altered subtype selectivity (**3**). Based on these results, we assumed that substitutions at both the 1- and 7-positons of the indole ring might induce subtype selectivity without loss of potency for the β_3-AR. Furthermore, these 1,7-disubstitutions seem to offer a potential advantage in the drug's properties, since these compounds possess no acidic group on the RHS, which would make oral bioavailability of the compounds worse. In this study, we report the synthesis and pharmacological evaluation of a novel series of 1,7-disubstituted indole-based β_3-AR agonists with a 2-thiophenesulfonamide group on their left-hand side (LHS).



	EC_{50} (nM)		
	β_3	β_1	β_2
1 : R^1 = H, R^2 = H	0.88	66	21
2 : R^1 = H, R^2 = OMe	0.55	29	6.6
3 : R^1 = Et, R^2 = H	0.61	>100	42

Figure 2.

2. Chemistry

The general strategy adopted for the preparation of the target compounds listed in Figure 2 and Tables 1 and 2 was a convergent route in which various tryptamine derivatives were coupled with the common intermediate **4** as shown in Figure 3. Synthesis of the key intermediate **4** was carried out as depicted in Scheme 1. Asymmetric reduction of the commercially available 3-nitrophenacyl bromide **5** was accomplished by treatment with a BH_3–THF complex in the presence of Corey's CBS-borane reagent.[9] After recrystallization from hexane/*i*-Pr_2O, the desired (*R*)-alcohol **6**[10] was obtained in 78% yield with an excellent enantiomeric purity (>99% ee). Protection of the secondary hydroxyl group in **6** was accomplished with *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of an excess amount of KBr to avoid the undesirable formation of the chloride **7b**, which was derived by a halogen exchange reaction of **7a**. The nitro compound **7a** was then subjected to catalytic hydrogenation in the presence of Pt on charcoal, followed by treatment with 2-thiophenesulfonyl chloride to provide the desired sulfonamide **4** in 91% yield from the (*R*)-alcohol **6**.

The preparation of **1** and its *N*-ethyl derivative **3** is shown in Scheme 2. (*R*)-α-Methyltryptamine **8**[11] was coupled with the bromide **4** in the presence of *i*-Pr_2NEt and KI, and subsequent deprotection of the TBDMS group supplied **1** in 48% yield. Alternatively, the amino group in **8** was protected with a Boc group, and subsequent *N*-ethylation of the indole ring with EtI in the presence of NaH gave *N*-ethylated compound **10** in 76% yield from **8**. The Boc group in **10** was then deprotected, and the resulting amine was coupled with the key intermediate **4** in the presence of *i*-Pr_2NEt and KI. Finally, removal of the TBDMS group afforded the desired compound **3** in 29% yield from compound **10**.

The synthesis of 7-methoxy derivatives **2** and **16a**,**b** was carried out as shown in Scheme 3. The amino group in **11**[12] was protected with a Boc group, and the benzyl group in **12** was then removed by hydrogenation to give 7-hydroxyindole derivative **a** in 96% yield from **11**. *O*-Alkylation of **13** was performed with MeI and K_2CO_3 to provide *O*-methylated compound **14** in 99% yield. Deprotection of the Boc group in **14**, coupling of the resulting amine with the key intermediate **4**, and subsequent removal of the TBDMS group afforded the desired compound **2** in 50% yield from **14**. Alternatively, further alkylation of **14** with MeI or BnBr in the presence of NaH gave *N*,*O*-dialkylated indoles **15a**,**b** in good yield. Subsequent conversion of the tryptamines **15a**,**b**

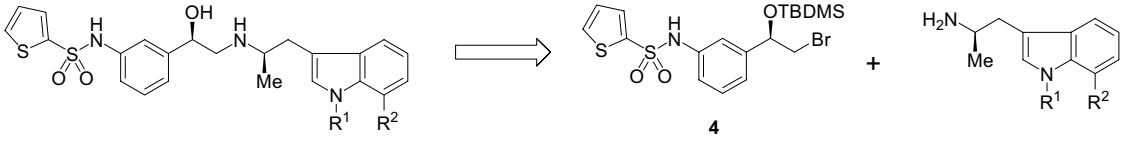
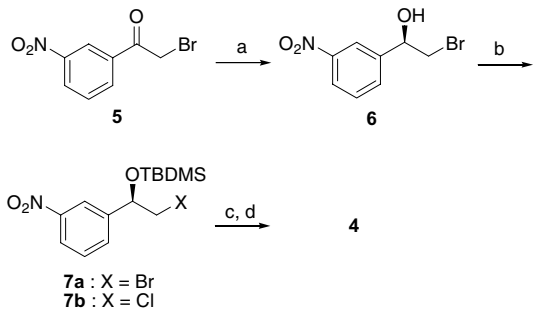


Figure 3.



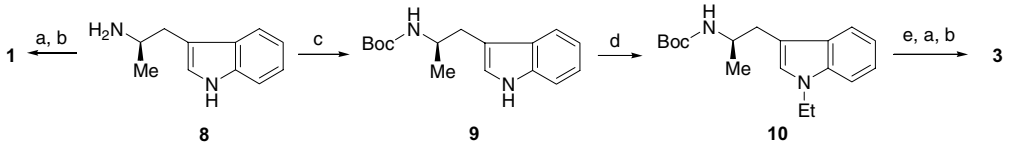
Scheme 1. Reagents: (a) (*R*)-2-methyl-CBS-oxazaborolidine, BH_3–THF complex, THF; (b) TBDMSCl, KBr, imidazole, DMF; (c) 5% Pt/C, H_2, EtOAc; (d) 2-thiophenesulfonyl chloride, pyridine, CH_2Cl_2.

into the corresponding final products **16a**,**b** was accomplished using the method already described.

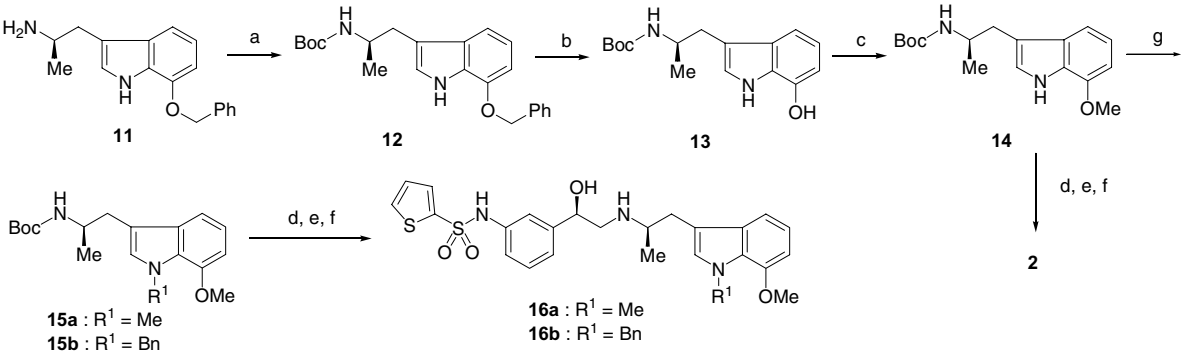
Preparation of 1,7-cyclized indole derivatives **19a**–**f** was conducted starting from 7-hydroxyindole derivative **13** and commercially available chlorohydrins in five steps as shown in Scheme 4. *O*-Alkylation of **13** was conducted using appropriate chlorohydrins under the general conditions of the Mitsunobu reaction (diethyl azodicarboxylate (DEAD), Ph_3P, room temperature), except dimethyl derivative **17e**, which required reflux temperature. The resulting chloroalkyl compounds **17a**–**f** were treated with strong bases such as NaH and KOH to afford cyclized compounds **18a**–**f**. Cyclization of **17a**–**d** proceeded easily by treating with NaH at room temperature, yielding six- to nine-membered simple rings in a good to moderate yield, although the yield of **17d** was not satisfactory (18%). Cyclization of **17e** required heating due to the steric bulk of the dimethyl group. Several attempts to obtain compound **18f**, containing two ether oxygen atoms in the linker part, were somehow unsuccessful. Compound **18f** was finally obtained in 32% yield, according to the procedures Hearny and Ley outlined for the *N*-alkylation of an indole ring (KOH in DMSO).[13] The obtained tryptamines **18a**–**f** were converted into the desired products **19a**–**f** by removal of the Boc group, coupling with the sulfonamide **4**, and deprotection of the TBDMS group.

Next, we designed compound **22**, containing a difluoromethylene unit on its 1,7-linker portion. Introduction of the difluoropropyl unit in **20** was first attempted by the Mitsunobu reaction (DEAD, PPh_3) with 3-chloro-2,2-difluoro-1-propanol.[14] However, the reaction was considerably slow even at reflux temperature, and prolonged reaction time led to the formation of by-products. This result implied that the reactivity of the difluoroalcohol toward the Mitsunobu reagent would be low compared to the alcohols used in Scheme 4. Tsunoda et al. reported that cyanomethylenetributylphosphorane (CMBP), an ylide-type Mitsunobu reagent,[15] is very effective in the reactions of secondary alcohols, which often show low reactivity. We therefore adopted Tsunoda's procedures for the preparation of **20**. As expected, with the use of CMBP, the reaction of **13** with the difluoroalcohol proceeded smoothly to provide the desired compound in quantitative yield. Cyclization of compound **20** was accomplished with KOH in DMSO, affording 1,7-cyclized indole derivative **21** in 52% yield. Tryptamine **21** also afforded the final compound **22** in 20% yield using the general three steps outlined in Schemes 2–4.

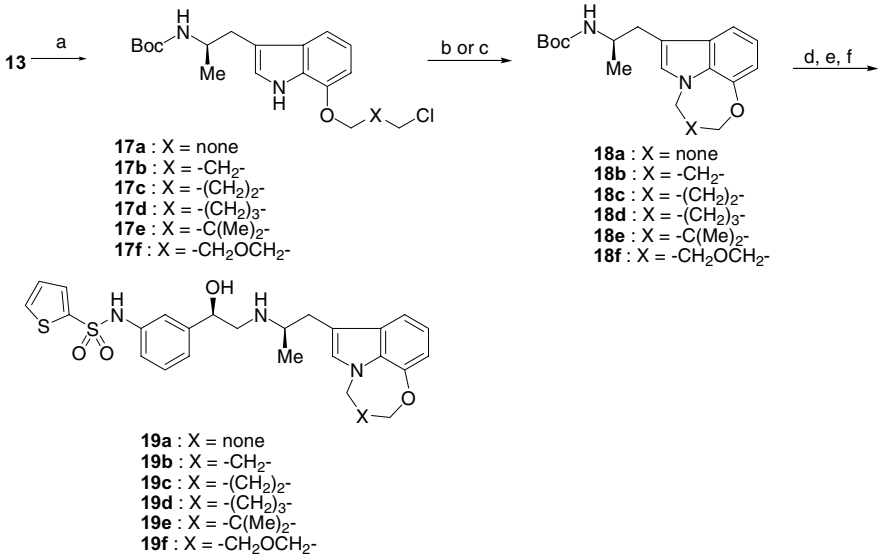
Synthesis of compound **26**, where a double bond was incorporated into the 1,7-linker portion, is depicted in Scheme 6. For the construction of an unsaturated ring system, we employed the ring closing metathesis (RCM) reaction. Thus, *O*-allylation of **13** with allyl



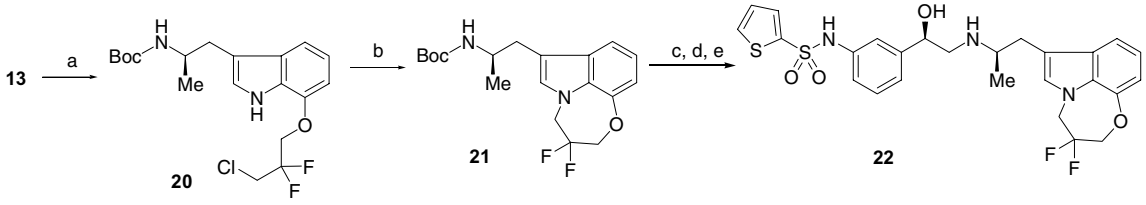
Scheme 2. Reagents: (a) **4**, KI, *i*-Pr_2NEt, MeCN; (b) 4 M HCl–EtOAc, EtOH; (c) $(Boc)_2O$, $CHCl_3$; (d) EtI, NaH, THF; (e) 4 M HCl–EtOAc.



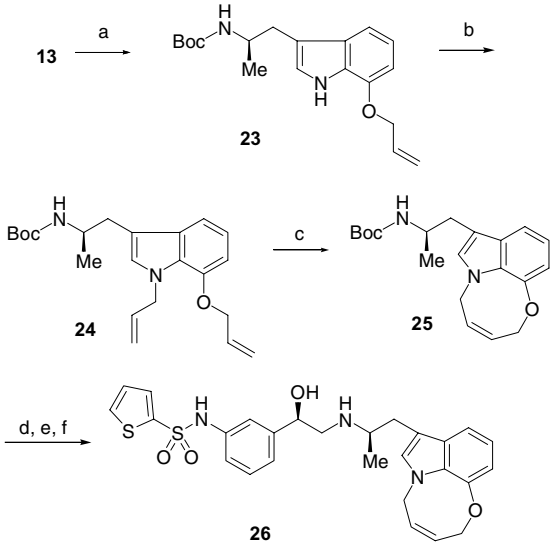
Scheme 3. Reagents: (a) $(Boc)_2O$, EtOAc; (b) 5% Pd–C, MeOH; (c) MeI, K_2CO_3, acetone; (d) 4 M HCl–EtOAc; (e) **4**, KI, *i*-Pr_2NEt, MeCN; (f) 4 M HCl–EtOAc, EtOH; (g) MeI or BnBr, NaH, DMF.



Scheme 4. Reagents: (a) $HOCH_2$–X–CH_2Cl, DEAD, Ph_3P, THF; (b) NaH, DMF; (c) KOH, DMSO; (d) 4 M HCl–EtOAc; (e) **4**, KI, *i*-Pr_2NEt, MeCN; (f) 4 M HCl–EtOAc, EtOH.



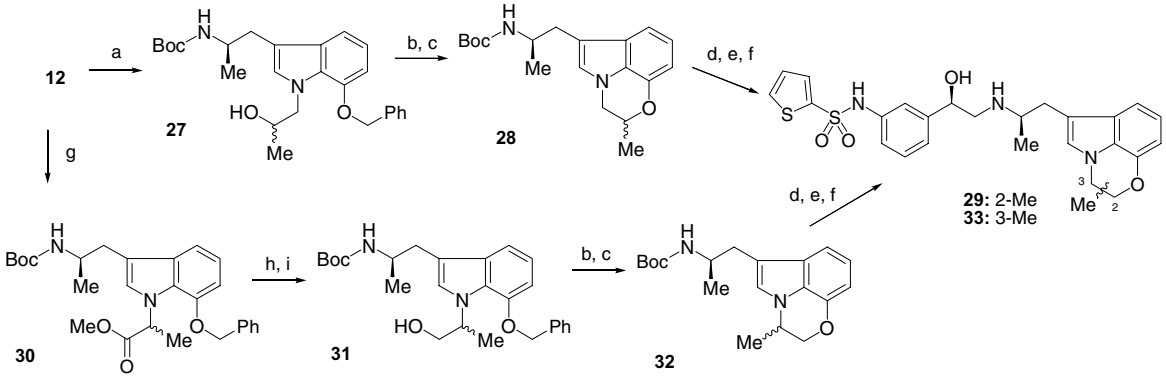
Scheme 5. Reagents: (a) $HOCH_2CF_2CH_2Cl$, $Bu_3P{=}CHCN$, toluene; (b) KOH, DMSO; (c) 4 M HCl–EtOAc; (d) **4**, KI, *i*-Pr_2NEt, MeCN; (e) 4 M HCl–EtOAc, EtOH.



Scheme 6. Reagents: (a) allyl iodide, K_2CO_3, acetone; (b) allyl bromide, NaH, DMF; (c) $Cl_2(PCy_3)_2Ru{=}CHPh$, CH_2Cl_2; (d) 4 M HCl–EtOAc; (e) **4**, KI, *i*-Pr_2NEt, MeCN; (f) 4 M HCl–EtOAc, EtOH.

iodide and K_2CO_3 gave *O*-allyl derivative **23** in 82% yield, and subsequent *N*-allylation with the use of allyl bromide and NaH gave *N*,*O*-diallyl compound **24** in 97% yield, which was subjected to the RCM reaction. The RCM reaction of **24** was successfully achieved using $Cl_2(PCy_3)_2Ru{=}CHPh$[16] as a catalyst to give **25** in 64% yield. Tryptamine derivative **25** was subjected to the general three steps as outlined in Schemes 2–5 to afford the final compound **26** in 23% yield.

An alternative synthetic method shown in Scheme 7 was developed to prepare **29** and **33**, which have a methyl group in the 1,7-linker unit. In this route, the order of cyclization step was reversed. Namely, initial alkylation at the nitrogen atom and the subsequent intramolecular Mitsunobu reaction provided the tryptamine derivatives **28** and **32**. For the synthesis of **28**, the nitrogen of the indole ring in compound **12** was alkylated with an excess amount of propylene oxide in the presence of *t*-BuOK to give **27** in 44% yield. The benzyl group in **27** was removed by Pd catalyzed hydrogenation, and the resulting 7-hydroxyindole derivative was subjected to the intramolecular Mitsunobu reaction. As expected, cyclization proceeded successfully and the desired compound **28** was obtained in 30% overall yield from compound **12**. Similarly, compound **32** was prepared as follows. *N*-Alkylation of **12** with methyl 2-bromopropionate in the presence of NaH gave **30** in 90% yield, and then, alkaline hydrolysis of the ester, followed by reduction of the resulting carboxylic acid with a BH_3–THF complex gave primary alcohol **31** in 47% yield. Removal of the benzyl group and subsequent intramolecular



Scheme 7. Reagents: (a) propylene oxide, *t*-BuOK, THF; (b) 10% Pd–C, H_2, EtOH; (c) DEAD, Ph_3P, THF; (d) 4 M HCl–EtOAc; (e) **4**, KI, *i*-Pr_2NEt, MeCN; (f) 4 M HCl–EtOAc, EtOH; (g) methyl 2-bromopropionate, NaH, DMF; (h) 1 M NaOHaq, MeOH; (i) BH_3–THF complex, THF.

Mitsunobu reaction afforded desired compound **32** in 75% yield. As with the case described above, tryptamines **28** and **32** were coupled with the sulfonamide part **4**, giving the desired compounds **29** and **33**.

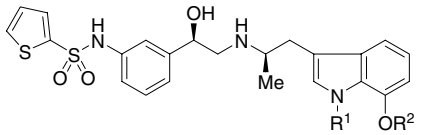
The assigned structures of the final compounds were fully confirmed by MS, ^1H NMR, and elemental analysis.

3. Results and discussion

All compounds listed in Tables 1 and 2 were evaluated in vitro for their ability to stimulate cAMP accumulation in CHO cells expressing the cloned human β_1-, β_2-, and β_3-ARs.[17] The binding affinity of the compounds listed in Table 2 was also determined for human β_1-, β_2-, and β_3-ARs in competition with the radioligand (−)-[^{125}I]-iodocyanopindolol. Compounds with a high potency for the β_3-AR were further evaluated for their ability to cross Caco-2 cell monolayers to predict their oral absorption potential.

To assess the possibility of the 1,7-disubstituted indole derivatives as potent and selective β_3-AR agonists, we initially synthesized 7-methoxy derivative **2** and its 1-methylated analogue **16a**. As expected, **16a** showed remarkable improvement in subtype selectivity against both the β_1- and β_2-ARs (>160-fold and 57-fold, respectively) compared to that of **2** (53-fold and 12-fold, respectively) while maintaining good agonistic activity for the β_3-AR (EC_{50} = 0.61 nM). However, the presence of an aromatic group at the 1-positon of the indole ring, such as a benzyl group in **16b**, was not tolerated by the β_3-AR (EC_{50} = 4.4 nM). Next, 1,7-cyclized indole-based compound **19a** was designed and synthesized. Surprisingly, inclusion of a ring at this position did not result in a dramatic change in either potency or selectivity. Namely, the tricyclic compound **19a** exhibited an almost identical in vitro profile with the noncyclized parent compound **16a**. These results encouraged us to further investigate the possibility of the other 1,7-cyclized indole derivatives as β_3-AR agonists.

Table 1. Agonistic activity of substituted indole derivatives for human β-ARs



Compd	R^1	R^2	EC_{50}, nM[a] (IA, %)[b]		
			β_3	β_1	β_2
2	H	Me	0.55 (101)	29 (36)	6.6 (67)
16a	Me	Me	0.61 (86)	>100 (57[c])	35 (57)
16b	Bn	Me	4.4 (80)	32 (65)	22 (64)
19a	–$(CH_2)_2$–		0.92 (85)	>88 (79[c])	36 (42)

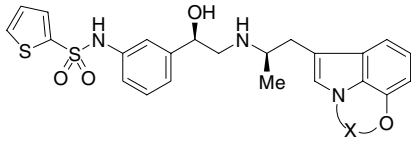
[a] Agonistic activity was assessed by measuring cAMP accumulation in CHO cells expressing human β-ARs.

[b] Values in parentheses represent the intrinsic activity (IA) given as percentage of maximal stimulation with isoproterenol.

[c] % Activity at 1000 nM.

To examine the available space in the binding sites, we initially synthesized seven- to nine-membered simple ring analogues **19b–d**, and the results are shown in Table 2. Despite the expansion of the ring size, all compounds, **19b–d**, showed potent agonistic activity for the β_3-AR (EC_{50} = 1.0, 1.4, and 1.4 nM, respectively). However, the selectivity against the β_1- and β_2-ARs was significantly affected by the ring size. Namely, the agonistic activity of the eight- and nine-membered ring analogues, **19c** and **19d**, for the β_1- and β_2-ARs was dramatically decreased, whereas the seven-membered ring analogue **19b** was still active for these receptors. While the origin of the high agonistic activity of 1,7-cyclized indoles for the β_3-AR is unknown, it is speculated to be the result of a positive hydrophobic interaction with the binding site and a more favorable conformational change of the receptor induced by the rigid ring structure. These tricyclic compounds, **19a–d**, also showed similar tendency in the binding assay with regard to subtype selectivity. The binding affinity for the β_3-AR was quite insensitive to the changes in the ring size (K_i = 3.2–7.7 nM), however the expansion of the ring size significantly attenuated the binding affinity for the β_1- and β_2-ARs. These data strongly suggested that the selectivity concerning the agonistic activity was elicited by steric hindrance of the ring moiety, and not caused by its antagonistic property.

Table 2. In vitro data for 1,7-linked tricyclic indole derivatives: agonistic activity and binding affinity for human β-ARs, and Caco-2 permeability



Compd	X	EC_{50}, nM[a] (IA, %)[b]			Binding K_i, nM[e]			Caco-2 Permeability[f]
		β_3	β_1	β_2	β_3	β_1	β_2	Class
19a	$-(CH_2)_2-$	0.92 (85)	>88 (79[c])	36 (42)	7.7	190	71	high
19b	$-(CH_2)_3-$	1.0 (81)	53 (70)	44 (40)	4. 8	120	59	high
19c	$-(CH_2)_4-$	1.4 (98)	>100 (50[c])	nd[d] (23[c])	3.2	540	170	high
19d	$-(CH_2)_5-$	1.4 (79)	>100 (49[c])	nd[d] (22[c])	5.5	560	280	high
26	$-CH_2CH{=}CHCH_2-$	0.75 (90)	>100 (72[c])	nd[d] (27[c])	3.1	550	150	high
19f	$-CH_2CH_2OCH_2CH_2-$	3.3 (77)	>100 (55[c])	>100 (48[c])	3.9	560	340	nt[g]
29	$-CH_2CH(Me)-$	1.2 (95)	>100 (63[c])	36 (51)	9.0	230	89	high
33	$-CH(Me)CH_2-$	0.66 (94)	>100 (56[c])	19 (42)	8.9	270	55	high
19e	$-CH_2C(Me)_2CH_2-$	11 (84)	>100 (48[c])	nd[d] (8[c])	23	160	140	nt[g]
22	$-CH_2CF_2CH_2-$	3.4 (92)	>100 (52[c])	nd[d] (15[c])	10	370	96	nt[g]

[a] Agonistic activity was assessed by measuring cAMP accumulation in CHO cells expressing human β-ARs.
[b] Values in parentheses represent the intrinsic activity (IA) given as percentage of maximal stimulation with isoproterenol.
[c] % Activity at 1000 nM.
[d] nd = not determined.
[e] Binding affinity is reported as K_i, the binding inhibition constant, determined by inhibition of ^{125}I-iodocyanopindolol binding.
[f] According to the degree of permeability across Caco-2 monolayers, the compounds were classified for their absorption potential as follows: high, $P_{app} \geq 1.0 \times 10^{-6}$ cm/s; moderate, 0.5×10^{-6} cm/s < P_{app} < 1.0×10^{-6} cm/s; low, $P_{app} \leq 0.5 \times 10^{-6}$ cm/s.
[g] nt = not tested.

Based on the good potency of the 1,7-cyclized indoles, additional eight- and nine-membered ring analogues were synthesized as shown by **26** and **19f**. Incorporation of a double bond into the eight-membered ring (**26**) resulted in a 1.9-fold improvement in potency for the β_3-AR (EC_{50} = 0.75 nM) compared to the saturated eight-membered ring analogue **19c**. This preference for the alkenyl moiety might be due to either its electron-donating property, which would strengthen the binding ability to the receptor, or a more favorable conformational bias imparted by the double bond. In contrast, the insertion of one ether oxygen, as in **19f**, resulted in a moderate decrease in potency for the β_3-AR. As can be seen from Table 2, these compounds exhibited excellent selectivity against the β_1- and β_2-ARs both in an agonistic assay and in the binding test as expected.

Next, we examined the effect of substituents on the linker portion of 1,7-cyclized indoles. The ability to introduce this substitution at the linker portion not only provides an increase in potency and/or selectivity but also affords the possibility of increasing the metabolic stability against metabolic enzymes such as Cytochrome P450 proteins.[18] Therefore, we synthesized six-membered ring analogues with a methyl group (**29** and **33**) and a seven-membered ring analogue with a dimethyl group (**19e**), and the results are shown in Table 2. The methyl group at either carbon atom in the linker was acceptable to β_3-AR (**29** and **33**), while geminal substitution, as in the dimethyl analog **19e**, attenuated potency for β_3-AR, possibly due to steric effects. However, these mono-methyl substitutions did not result in enhancement of selectivity, especially against the β_2-AR. Replacement of the dimethyl group with a smaller difluoro group (**22**) resulted in improving the potency for the β_3-AR (EC_{50} = 3.4 nM) without affecting potency against the β_1- and β_2-ARs.

As a first step toward understanding the overall pharmacokinetic profile of the 1,7-cyclized indole-based derivatives, an attempt was made to determine the permeability of compounds with high potency for the β_3-AR (EC_{50} < 3.0 nM). As shown in Table 2, all the compounds examined showed high cellular permeability ($P_{app} \geq 1.0 \times 10^{-6}$ cm/s) as expected from their structure. These data indicate that the 1,7-cyclized-based indole series of compounds represent a leading group of β_3-agonists with markedly improved bioavailability.

4. Conclusions

In this study, we have disclosed the synthesis and biological evaluation of a new series of 1,7-linked tricyclic indole derivatives as potent and highly selective human β_3-AR agonists. The synthesis of a variety of tricyclic indole parts was accomplished by the construction of the 1,7-linker portion, through either the Mitsunobu reaction or a ring closing metathesis (RCM) reaction. All the 1,7-linked tricyclic indole analogues synthesized showed potent agonistic activity for the β_3-AR with excellent subtype selectivity. SAR studies for the linker portion revealed that expansion of the ring size resulted in considerable selectivity against the β_1- and β_2-ARs (**19a** vs **19d**). These observations are explained in terms of poor binding affinity for the β_1- and β_2-ARs due to steric effects. Introduction of a double bond into the linker portion resulted in further improvement in the agonistic activity for the β_3-AR. These 1,7-cyclized

indole-based β_3-agonists showed high cellular permeability ($P_{app} \geq 1.0 \times 10^{-6}$ cm/s) in the Caco-2 cell membrane assays. These results revealed the potential of 1,7-linked tricyclic indole-based derivatives as a new and leading set of candidates for human β_3-AR agonists with good oral bioavailability.

5. Experimental

5.1. Chemistry

Melting points were determined using a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-LA300 (300 MHz) spectrometer. Chemical shifts were expressed as δ (ppm) value from tetramethylsilane as an internal standard. Elemental analyses were performed on an Elementar Vario EL analyzer. Mass spectra were recorded on a Shimadzu LCMS-QP8000A spectrometer (APCI).

5.1.1. (*R*)-2-Bromo-1-(3-nitrophenyl)ethanol (6).[10] To a stirred solution of 3-nitrophenacyl bromide (**5**) (100 g, 410 mmol) and (*R*)-2-methyl-CBS-oxazaborolidine (1 M in toluene, 50 mL, 50 mmol) in THF (500 mL), was added dropwise BH_3–THF complex (1 M in THF, 800 mL, 800 mmol) over a period of 30 min at room temperature. After the addition was completed, stirring was continued for 2 h at ambient temperature. Then the reaction mixture was cooled in an ice-bath, saturated NH_4Cl solution (400 mL) was slowly added. After evaporation of THF under reduced pressure, the residual solution was partitioned between *i*-Pr_2O (500 mL) and H_2O (500 mL). The mixture was stirred for 30 min, and the insoluble was filtered off. The organic layer was separated from the filtrate, and the remaining aqueous layer was extracted twice with *i*-Pr_2O. Combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 4/1 to 2/1 to give crude **6** as a solid. This solid was dissolved in *i*-Pr_2O (160 mL), and to the solution, hexane (240 mL) was added dropwise with stirring. The formed crystals were collected by filtration and dried under vacuum to afford **6** (78 g, 77%). Optical purity of **6** was determined to be >99% ee by a chiral HPLC (Daicel Chiralpack AD column, 0.5 mL/min, hexane/ethanol 1/1, 25 °C). The retention times were: **6** (*R*)-isomer, (9.38 min); corresponding (*S*)-isomer, (8.34 min). Mp: 65–66 °C; ^{1}H NMR ($CDCl_3$): 2.81 (d, J = 3.7 Hz, 1H), 3.56 (dd, J = 10.6, 8.3 Hz, 1H), 3.70 (dd, J = 10.6, 3.5 Hz, 1H), 5.06 (m, 1H), 7.57 (m, 1H), 7.75 (m, 1H), 8.20 (m, 1H), 8.29 (m, 1H); Anal. Calcd for $C_8H_8BrNO_3$, 1/20*i*-Pr_2O: C, 39.69; H, 3.49; N, 5.58. Found: C, 39.58; H, 3.32; N, 5.70.

5.1.2. (*R*)-2-Bromo-1-(*tert*-butyldimethylsilyloxy)-1-(3-nitrophenyl)ethane (7a). To a stirred suspension of **6** (49 g, 199 mmol) and KBr (119 g, 1.0 mol) in DMF (100 mL), was added a solution of TBDMSCl (45 g, 300 mmol) and imidazole (41 g, 603 mmol) in DMF (600 mL). After 18 h of stirring, the reaction mixture was poured into 1 L of water and extracted with *i*-Pr_2O (500 mL × 2). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 50/1 to 20/1 to give **7a** (69 g, 95%) as a light yellow solid. Mp: 43 °C; ^{1}H NMR ($CDCl_3$): −0.06 (s, 3H), 0.14 (s, 3H), 0.91 (s, 9H), 3.44 (dd, J = 10.4, 5.3 Hz, 1H), 3.50 (dd, J = 10.4, 6.8 Hz, 1H), 4.96 (dd, J = 6.8, 5.3 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.71 (d, J = 7.7 Hz, 1H), 8.17 (m, 1H), 8.25 (t, J = 2.0 Hz, 1H).

5.1.3. (*R*)-*N*-{3-[2-Bromo-1-(*tert*-butyldimethylsilyloxy)-ethyl]phenyl}(2-thiophene)sulfonamide (4). A mixture of **7a** (131 g, 364 mmol), 5% Pt/C (4 g), and EtOAc (1.3 L) was hydrogenated under H_2 for 6 h. The catalyst was then removed by filtration, and the filtrate was concentrated under reduced pressure. After the oily residue and pyridine (35 mL, 433 mmol) were then dissolved in CH_2Cl_2 (1.5 L), 2-thiophenesulfonyl chloride (66 g, 363 mmol) was added portionwise at 0 °C. The mixture was stirred overnight at room temperature, 1 M aqueous HCl was added, and the organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 10/1 to give **4** (166 g, 96%). ^{1}H NMR ($CDCl_3$): −0.13 (s, 3H), 0.07 (s, 3H), 0.87 (s, 9H), 3.34 (dd, J = 10.3, 5.0 Hz, 1H), 3.39 (dd, J = 10.3, 7.0 Hz, 1H), 4.78 (dd, J = 7.0, 5.0 Hz, 1H), 6.68 (s, 1H), 6.99 (dd, J = 5.0, 3.8 Hz, 1H), 7.07–7.16 (m, 3H), 7.27 (m, 1H), 7.47 (dd, J = 3.8, 1.3 Hz, 1H), 7.52 (dd, J = 5.0, 1.3 Hz, 1H).

5.1.4. 3-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-1*H*-indole (1). A mixture of **8**[11] (2.6 g, 15 mmol), **4** (4.76 g, 10 mmol), *i*-Pr_2NEt (2.2 mL, 13 mmol), KI (1.7 g, 10 mmol), and MeCN (100 mL) was heated under reflux for 48 h. After the reaction mixture was concentrated to dryness under reduced pressure, the residue was partitioned between EtOAc and H_2O. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH = 100/1 to give desired coupling product (3.4 g, 60% from **4**).

The compound obtained above was dissolved in EtOH (10 mL), treated with 4 M HCl/EtOAc (30 mL) at room temperature for 2 h. After the reaction mixture was concentrated in vacuo, the residue was partitioned between EtOAc and aqueous K_2CO_3. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH = 10/1 to give the title compound **1** (2.2 g, 82%) as an amorphous solid. ^{1}H NMR (DMSO-d_6): 0.97 (d, J = 6.1 Hz, 3H), 2.55–2.74 (m, 2H), 2.87 (dd, J = 3.9, 4.6 Hz, 1H), 2.98 (m, 1H), 3.44 (m, 1H), 4.55 (dd, J = 7.9, 4.0 Hz, 1H), 6.93–7.20 (m, 8H), 7.33 (d, J = 8.0 Hz, 1H), 7.47–7.51 (m, 2H), 7.81 (d, J = 5.0 Hz, 1H), 10.81 (s, 1H); MS *m*/*z*: 456 (MH^+); Anal. Calcd for $C_{23}H_{25}N_3O_3S_2$: C, 60.63; H, 5.53; N, 9.22. Found: C, 60.65; H, 5.70; N, 8.82.

5.1.5. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-1*H*-indole (9). To a stirred solution of **8** (1.74 g, 10 mmol) in $CHCl_3$ (20 mL), was added dropwise a solution di-*tert*-butyl dicarbonate (2.6 g, 12 mmol) in $CHCl_3$ (20 mL) at room temperature. After stirring overnight, the reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 3/1 to give **9** (2.8 g, quant.) as a colorless solid. ^{1}H NMR ($CDCl_3$): 1.13 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.85 (dd, J = 14.3, 6.9 Hz, 1H), 2.97 (dd, J = 14.3, 5.1 Hz, 1H), 4.03 (br, 1H), 4.44 (br, 1H), 7.02 (d, J = 2.2 Hz, 1H), 7.09–7.22 (m, 2H), 7.36 (d, J = 7.9 Hz, 1H), 7.64 (d, J = 7.7 Hz, 1H), 8.07 (s, 1H).

5.1.6. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-1-ethyl-1*H*-indole (10). To a stirred suspension of NaH (60% dispersion in mineral oil, 48 mg, 1.2 mmol) in THF (5 mL), was added a solution of **9** (0.27 g, 1.0 mmol) in THF (5 mL). After stirring at room temperature for 10 min, ethyl iodide (0.16 mL, 2.0 mmol) was added, and stirring was continued for 2 h. H_2O was then added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 10/1 to give **10** (0.23 g, 76%) as an amorphous solid. ^{1}H NMR ($CDCl_3$): 1.13 (d, J = 6.6 Hz, 3H), 1.44 (s, 9H), 1.44 (t, J = 7.3 Hz, 3H), 2.84 (dd, J = 14.3, 6.8 Hz, 1H), 2.95 (dd, J = 14.3, 5.1 Hz, 1H), 4.01 (br, 1H), 4.13 (q, J = 7.3 Hz, 2H), 4.43 (br, 1H), 6.94 (s, 1H), 7.09 (m, 1H), 7.20 (m, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 8.1 Hz, 1H).

5.1.7. 1-Ethyl-3-{(2*R*)-2-{{(2*R*)-2-hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-1*H*-indole (3). Compound **10** (0.35 g, 1.16 mmol) was treated with 4 M HCl in EtOAc (10 mL) at room temperature for 2 h, and the resulting mixture was concentrated under vacuum. The residue was partitioned between EtOAc and aqueous K_2CO_3, and the organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated under vacuum to give (*R*)-3-(2-aminopropyl)-1-ethyl-1*H*-indole (0.15 g, 64%), which was used in the next step without further purification.

A mixture of the product obtained above, **4** (0.33 g, 0.69 mmol), *i*-Pr_2NEt (0.18 mL, 1.0 mmol), KI (0.11 g, 0.69 mmol), and MeCN (8 mL) was heated under reflux for 48 h. After the reaction mixture was concentrated under reduced pressure, the residue was partitioned between EtOAc and H_2O. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH = 100/1 to give desired coupling product (0.19 g, 46% from **4**).

The compound obtained above was dissolved in EtOH (4 mL), treated with 4 M HCl in EtOAc (10 mL) at room temperature for 2 h. After the solvent was evaporated to dryness, the residue was partitioned between EtOAc and aqueous K_2CO_3. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with $CHCl_3$/MeOH = 10/1 to give the title compound **3** (0.15 g, quant.) as an amorphous solid. ^{1}H NMR (DMSO-d_6): 0.98 (d, J = 6.2 Hz, 3H), 1.31 (t, J = 7.2 Hz, 3H), 2.60 (dd, J = 13.9, 7.9 Hz, 1H), 2.67–2.76 (m, 2H), 2.87 (dd, J = 13.9, 5.1 Hz, 1H), 2.97 (m, 1H), 4.12 (q, J = 7.2 Hz, 2H), 4.58 (dd, J = 7.7, 4.6 Hz, 1H), 6.96–7.20 (m, 8H), 7.40 (d, J = 8.3 Hz, 1H), 7.48 (dd, J = 3.8, 1.3 Hz, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.80 (dd, J = 5.1, 1.5 Hz, 1H); MS *m*/*z*: 484 (MH^+); Anal. Calcd for $C_{25}H_{29}N_3O_3S_2$: C, 62.08; H, 6.04; N, 8.69. Found: C, 61.84; H, 6.13; N, 8.45.

5.1.8. (*R*)-7-Benzyloxy-3-[2-(*tert*-butoxycarbonylamino)-propyl]-1*H*-indole (12). To a stirred solution of **11**[12] (112 g, 399 mmol) in EtOAc (800 mL), was added dropwise a solution di-*tert*-butyl dicarbonate (105 g, 481 mmol) in EtOAc (200 mL) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure. To the resulting syrupy oil, hexane (400 mL) was added, and the solid formed was collected by filtration, dried under vacuum to give **12** (146 g, 96%). Mp: 94–95 °C; ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.83 (dd, J = 14.5, 6.7 Hz, 1H), 2.94 (dd, J = 14.5, 5.1 Hz, 1H), 4.00 (m, 1H), 4.44 (m, 1H), 5.18 (s, 2H), 6.71 (d, J = 7.5 Hz, 1H), 6.97 (d, J = 2.2 Hz, 1H), 7.02 (dd, J = 7.9, 7.5 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H), 7.24–7.51 (m, 5H), 8.30 (s, 1H).

5.1.9. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-7-hydroxy-1*H*-indole (13). A mixture of **12** (60 g, 158 mmol), 5% Pd/C (3 g), and MeOH (500 mL) was hydrogenated under H_2 at room temperature for 3 h. The catalyst was then removed by filtration, and the filtrate was concentrated under reduced pressure. Hexane (250 mL) was added to the resulting syrupy oil, and the solid formed was collected by filtration, dried under vacuum to give **13** (46 g, quant.). Mp: 164–166 °C; ^{1}H NMR (CD_3OD): 1.08 (d, J = 6.6 Hz, 3H), 1.40 (s, 9H), 2.73 (dd, J = 13.9, 7.5 Hz, 1H), 2.88 (m, 1H), 3.89 (m, 1H), 6.49 (d, J = 7.5 Hz, 1H), 6.80 (t, J = 7.7 Hz, 1H), 6.98 (s, 1H), 7.08 (d, J = 7.9 Hz, 1H).

5.1.10. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-7-methoxy-1*H*-indole (14). A mixture of **13** (1.1 g, 3.5 mmol), methyl iodide (1.1 mL, 17.7 mmol), K_2CO_3 (0.59 g, 4.3 mmol), and acetone (40 mL) was refluxed overnight. The solvent was then removed under vacuum, and the residue was treated with EtOAc, washed successively with H_2O and brine. The organic layer was dried over $MgSO_4$ and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 4/1 to give **14** (1.1 g, 99%) as a syrupy oil. ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.83 (dd, J = 14.3, 7.0 Hz, 1H), 2.94 (dd, J = 14.3, 5.1 Hz, 1H), 3.95 (s, 3H), 3.98 (m, 1H), 4.45 (br, 1H), 6.64 (d, J = 7.7 Hz, 1H), 6.98 (d, J = 2.0 Hz, 1H), 7.03 (t, J = 7.7 Hz, 1H), 7.24 (d, J = 7.7Hz, 1H), 8.29 (s, 1H).

5.1.11. 3-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-7-methoxy-1*H*-indole (2). This compound was prepared from **14** (5.36 g, 17.6 mmol) using the procedure described for

the preparation of **3**. Yield 2.9 g (50% from **4**). ^{1}H NMR (DMSO-d_6): 0.95 (d, J = 6.0 Hz, 3H), 2.52–2.74 (m, 3H), 2.83 (dd, J = 13.9, 5.0 Hz, 1H), 2.94 (m, 1H), 3.89 (s, 3H), 4.54 (dd, J = 8.2, 4.0 Hz, 1H), 6.62 (d, J = 7.5 Hz, 1H), 6.89 (t, J = 7.8 Hz, 1H), 6.96–7.19 (m, 8H), 7.47 (dd, J = 3.7, 1.3 Hz, 1H), 7.80 (dd, J = 5.0, 1.3 Hz, 1H), 10.89 (s, 1H); MS *m/z*: 486 (MH^+); Anal. Calcd for $C_{24}H_{27}N_3O_4S_2$: C, 59.36; H, 5.60; N, 8.65. Found: C, 59.07; H, 5.68; N, 8.28.

5.1.12. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-7-methoxy-1-methyl-1*H*-indole (15a). To a stirred suspension of NaH (60% dispersion in mineral oil, 88 mg, 2.2 mmol) in DMF (5 mL) was added a solution of **14** (0.56 g, 1.84 mmol) in DMF (5 mL) at room temperature. After stirring for 10 min, methyl iodide (0.137 mL, 2.2 mmol) was added, and stirring was continued for 2 h. The reaction was then quenched with water, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 10/1 to give **15a** (0.52 g, 89%) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.6 Hz, 3H), 1.44 (s, 9H), 2.78 (dd, J = 14.3, 6.8 Hz, 1H), 2.90 (dd, J = 14.3, 5.3 Hz, 1H), 3.91 (s, 3H), 3.97 (br, 1H), 4.00 (s, 3H), 4.42 (br, 1H), 6.60 (d, J = 7.7 Hz, 1H), 6.74 (s, 1H), 6.97 (m, 1H), 7.18 (dd, J = 8.0, 0.8 Hz, 1H).

5.1.13. (*R*)-1-Benzyl-3-[2-(*tert*-butoxycarbonylamino)propyl]-7-methoxy-1*H*-indole (15b). This compound was prepared from **14** (0.51 g, 1.68 mmol) and benzyl bromide (0.22 mL, 1.85 mmol) in a similar manner to that described for **15a**. Yield 0.68 g (quant.). ^{1}H NMR ($CDCl_3$): 1.09 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.79 (dd, J = 14.3, 7.0 Hz, 1H), 2.92 (dd, J = 14.3, 5.3 Hz, 1H), 3.82 (s, 3H), 3.97 (m, 1H), 4.42 (br, 1H), 5.58 (s, 2H), 6.61(d, J = 7.5 Hz, 1H), 6.84 (s, 1H), 7.00 (t, J = 7.9 Hz, 1H), 7.07–7.09 (m, 2H), 7.20–7.29 (m, 4H).

5.1.14. 3-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-7-methoxy-1-methyl-1*H*-indole (16a). This compound was prepared from **15a** (0.83 g, 2.6 mmol) using the procedure described for the preparation of **3**. Yield 0.27 g (49% from **4**). ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.2 Hz, 3H), 2.57 (dd, J = 12.1, 8.6 Hz, 1H), 2.67–2.84 (m, 3H), 3.00 (m, 1H), 3.93 (s, 3H), 4.00 (s, 3H), 4.50 (dd, J = 8.6, 3.7 Hz, 1H), 6.63 (d, J = 7.3 Hz, 1H), 6.72 (s, 1H), 6.93–7.25 (m, 7H), 7.43–7.47 (m, 2H); MS *m/z*: 500 (MH^+); Anal. Calcd for $C_{25}H_{29}N_3O_4S_2$: C, 60.10; H, 5.85; N, 8.41. Found: C, 59.91; H, 5.91; N, 8.23.

5.1.15. 1-Benzyl-3-{(2*R*)-2-{{(2*R*)-2-hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-7-methoxy-1*H*-indole (16b). This compound was prepared from **15b** (0.91 g, 2.3 mmol) using the procedure described for the preparation of **3**. Yield 0.27 g (46% from **4**). ^{1}H NMR ($CDCl_3$): 1.10 (d, J = 6.2 Hz, 3H), 2.55 (dd, J = 12.1, 8.8 Hz, 1H), 2.74–2.83 (m, 3H), 3.00 (m, 1H), 3.85 (s, 3H), 4.47 (dd, J = 8.3, 4.0 Hz, 1H), 5.57 (d, J = 15.7 Hz, 1H), 5.61 (d, J = 15.7 Hz, 1H), 6.65 (d, J = 7.7 Hz, 1H), 6.83–7.30 (m, 13H), 7.41–7.47 (m, 2H); MS *m/z*: 576 (MH^+); Anal. Calcd for $C_{31}H_{33}N_3O_4S_2$: C, 64.67; H, 5.78; N, 7.30. Found: C, 64.36; H, 5.78; N, 7.23.

5.1.16. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-7-(2-chloroethoxy)-1*H*-indole (17a). To a stirred solution of **13** (0.58 g, 2.0 mmol), 2-chloroethanol (0.27 mL, 4.0 mmol), and triphenylphosphine (1.05 g, 4.0 mmol) in THF (15 mL), was added dropwise DEAD (0.63 mL, 4.0 mmol) at room temperature. After stirring overnight, the reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 4/1 to give **17a** (0.59 g, 84%) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.83 (dd, J = 14.3, 7.0 Hz, 1H), 2.95 (dd, J = 14.3, 5.3 Hz, 1H), 3.88 (t, J = 5.7 Hz, 2H), 4.00 (br, 1H), 4.40 (t, J = 5.7 Hz, 2H), 4.43 (br, 1H), 6.63 (d, J = 7.7 Hz, 1H), 6.99–7.04 (m, 2H), 7.28 (d, J = 7.9 Hz, 1H), 8.33 (s, 1H).

5.1.17. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-7-(3-chloropropoxy)-1*H*-indole (17b). This compound was prepared from **13** (0.58 g, 2.0 mmol) and 3-chloro-1-propanol (0.33 mL, 4.0 mmol) in a similar manner to that described for **17a**. Yield 0.60 g (82%). ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.31 (m, 2H), 2.83 (dd, J = 14.1, 6.9 Hz, 1H), 2.95 (dd, J = 14.1, 5.1 Hz, 1H), 3.78 (t, J = 6.4 Hz, 2H), 4.00 (m, 1H), 4.29 (t, J = 5.9 Hz, 2H), 4.44 (br, 1H), 6.66 (d, J = 7.7 Hz, 1H), 7.00 (s, 1H), 7.02 (t, J = 7.7 Hz, 1H), 7.25 (d, J = 7.7 Hz, 1H), 8.24 (s, 1H).

5.1.18. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-7-(4-chlorobutoxy)-1*H*-indole (17c). This compound was prepared from **13** (2.9 g, 10 mmol) and 4-chloro-1-butanol (1.5 mL, 15 mmol) in a similar manner to that described for **17a**. Yield 1.45 g (38%). ^{1}H NMR ($CDCl_3$): 1.12 (d, J = 6.8 Hz, 3H), 1.44 (s, 9H), 2.00–2.04 (m, 4H), 2.83 (dd, J = 14.2, 6.6 Hz, 1H), 2.95 (dd, J = 14.2, 5.1 Hz, 1H), 3.65 (m, 2H), 4.01 (m, 1H), 4.17 (m, 2H), 4.43 (br, 1H), 6.62 (d, J = 7.7 Hz, 1H), 6.98–7.01 (m, 2H), 7.25 (m, 1H), 8.24 (s, 1H).

5.1.19. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-7-(5-chloropentyloxy)-1*H*-indole (17d). This compound was prepared from **13** (1.74 g, 6.0 mmol) and 5-chloro-1-pentanol (1.05 mL, 9.0 mmol) in a similar manner to that described for **17a**. Yield 2.0 g (85%). ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.6 Hz, 3H), 1.44 (s, 9H), 1.68 (m, 2H), 1.81–1.93 (m, 4H), 2.83 (dd, J = 14.3, 7.0 Hz, 1H), 2.95 (dd, J = 14.3, 5.1 Hz, 1H), 3.58 (t, J = 6.6 Hz, 2H), 4.00 (br, 1H), 4.14 (t, J = 6.2 Hz, 2H), 4.44 (br, 1H), 6.62 (d, J = 7.5 Hz, 1H), 6.98–7.03 (m, 2H), 7.23 (d, J = 8.0 Hz, 1H), 8.27 (s, 1H).

5.1.20. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-7-(3-chloro-2,2-dimethylpropoxy)-1*H*-indole (17e). This compound was prepared from **13** (1.16 g, 4.0 mmol) and 3-chloro-2,2-dimethyl-1-propanol (0.74 g, 6.0 mmol) by the method described for **17a** except that the reaction was conducted under reflux temperature. Yield 1.26 g (80%). ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.6 Hz, 3H), 1.17 (s, 6H), 1.43 (s, 9H), 2.83 (dd, J = 14.3, 7.0 Hz,

1H), 2.95 (dd, J = 14.3, 5.1 Hz, 1H), 3.62 (s, 2H), 3.93 (s, 2H), 4.00 (br, 1H), 4.45 (br, 1H), 6.66 (d, J = 7.7 Hz, 1H), 6.99–7.04 (m, 2H), 7.25 (d, J = 8.0 Hz, 1H), 8.26 (s, 1H).

5.1.21. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-7-[2-(2-chloroethoxy)ethoxy]-1*H*-indole (17f). This compound was prepared from **13** (1.74 g, 6.0 mmol) and 2-(2-chloroethoxy)ethanol (0.95 mL, 9.0 mmol) in a similar manner to that described for **17a**. Yield 1.5 g (62%). ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.6 Hz, 3H), 1.44 (s, 9H), 2.83 (dd, J = 14.3, 7.0 Hz, 1H), 2.95 (dd, J = 14.3, 5.3 Hz, 1H), 3.69 (t, J = 5.7 Hz, 2H), 3.85 (t, J = 5.7 Hz, 2H), 3.92 (m, 2H), 4.00 (m, 1H), 4.31 (m, 2H), 4.44 (br, 1H), 6.67 (d, J = 7.5 Hz, 1H), 6.98–7.03 (m, 2H), 7.27 (m, 1H), 8.58 (s, 1H).

5.1.22. (*R*)-6-[2-(*tert*-Butoxycarbonylamino)propyl]-2,3-dihydropyrrolo[1,2,3-*de*]-1,4-benzoxazine (18a). To a stirred solution of **17a** (0.59 g, 1.67 mmol) in DMF (8 mL), was added NaH (60% dispersion in mineral oil, 0.13 g, 3.36 mmol) at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was quenched with 1 M aqueous HCl, extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 4/1 to give **18a** (0.43 g, 81%) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.13 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.84 (dd, J = 14.3, 6.8 Hz, 1H), 2.92 (dd, J = 14.3, 5.1 Hz, 1H), 4.00 (m, 1H), 4.22 (t, J = 4.7 Hz, 2H), 4.46 (br, 1H), 4.51 (t, J = 4.7 Hz, 2H), 6.64 (d, J = 7.5 Hz, 1H), 6.89 (s, 1H), 6.97 (t, J = 7.8 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H).

5.1.23. (*R*)-7-[2-(*tert*-Butoxycarbonylamino)propyl]-3,4-dihydro-2*H*-pyrrolo[1,2,3-*ef*]-1,5-benzoxazepine (18b). This compound was prepared from **17b** (0.6 g, 1.64 mmol) using the procedure described for the preparation of **18a**. Yield 0.31 g (57%). ^{1}H NMR ($CDCl_3$): 1.14 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.35 (m, 2H), 2.81 (dd, J = 14.3, 6.8 Hz, 1H), 2.92 (dd, J = 14.3, 5.7 Hz, 1H), 4.00 (m, 1H), 4.12 (m, 2H), 4.29 (t, J = 4.7 Hz, 2H), 4.43 (br, 1H), 6.81 (d, J = 7.7 Hz, 1H), 6.90 (s, 1H), 7.00 (t, J = 7.7 Hz, 1H), 7.25 (d, J = 7.7 Hz, 1H).

5.1.24. (*R*)-8-[2-(*tert*-Butoxycarbonylamino)propyl]-2,3,4,5-tetrahydropyrrolo[1,2,3-*fg*]-1,6-benzoxazocine (18c). This compound was prepared from **17c** (1.45 g, 3.8 mmol) using the procedure described for the preparation of **18a**. Yield 1.28 g (99%). ^{1}H NMR ($CDCl_3$): 1.12 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 1.60 (m, 2H), 2.01 (m, 2H), 2.81 (dd, J = 14.3, 6.8 Hz, 1H), 2.92 (dd, J = 14.3, 5.5 Hz, 1H), 3.99 (m, 1H), 4.24 (t, J = 5.4 Hz, 2H), 4.43 (br, 1H), 4.47 (t, J = 6.2 Hz, 2H), 6.77 (s, 1H), 6.81 (d, J = 7.1 Hz, 1H), 6.98 (t, J = 7.7 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H).

5.1.25. (*R*)-9-[2-(*tert*-Butoxycarbonylamino)propyl]-3,4,5,6-tetrahydro-2*H*-pyrrolo[1,2,3-*gh*]-1,7-benzoxazonine (18d). This compound was prepared from **17d** (1.09 g, 2.9 mmol) using the procedure described for the preparation of **18a**. Yield 0.19 g (18%). ^{1}H NMR ($CDCl_3$): 1.12 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 1.49 (m, 2H), 1.74–1.83 (m, 4H), 2.80 (dd, J = 14.2, 6.6 Hz, 1H), 2.91 (dd, J = 14.2, 5.1 Hz, 1H), 3.99 (m, 1H), 4.24 (t, J = 5.1 Hz, 2H), 4.43 (br, 1H), 4.56 (m, 2H), 6.79–6.82 (m, 2H), 6.97 (t, J = 7.8 Hz, 1H), 7.29 (dd, J = 7.8, 0.9 Hz, 1H).

5.1.26. (*R*)-7-[2-(*tert*-Butoxycarbonylamino)propyl]-3,4-dihydro-3,3-dimethyl-2*H*-pyrrolo[1,2,3-*ef*]-1,5-benzoxazepine (18e). This compound was prepared from **17e** (1.26 g, 3.2 mmol) by the method described for **18a** except that the reaction was conducted at 70 °C. Yield 0.52 g (45%). ^{1}H NMR ($CDCl_3$): 1.14 (s, 6H), 1.14 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.79 (dd, J = 14.5, 7.0 Hz, 1H), 2.90 (dd, J = 14.5, 5.5 Hz, 1H), 3.78 (s, 2H), 3.97 (s, 2H), 4.00 (m, 1H), 4.45 (br, 1H), 6.77 (d, J = 7.5 Hz, 1H), 6.82 (s, 1H), 6.99 (t, J = 7.7 Hz, 1H), 7.24 (d, J = 7.9 Hz, 1H).

5.1.27. (*R*)-9-[2-(*tert*-Butoxycarbonylamino)propyl]-2,3,5,6-tetrahydropyrrolo[1,2,3-*gh*]-1,4,7-benzodioxazonine (18f). A mixture of **17f** (0.97 g, 2.4 mmol), KOH (0.26 g, 4.0 mmol), and DMSO (8 mL) was heated at 80 °C. After stirring for 66 h, the reaction mixture was poured into H_2O, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 2/1 to give **18f** (0.28 g, 32%) as an amorphous solid. ^{1}H NMR (CD_3OD): 1.08 (d, J = 6.6 Hz, 3H), 1.39 (s, 9H), 2.72 (dd, J = 14.3, 7.3 Hz, 1H), 2.87 (m, 1H), 3.75 (m, 2H), 3.82 (m, 2H), 3.80-4.50 (m, 5H), 6.75 (dd, J = 7.7, 0.9 Hz, 1H), 6.89 (s, 1H), 6.91 (t, J = 7.7 Hz, 1H), 7.30 (d, J = 7.7 Hz, 1H).

5.1.28. 6-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-2,3-dihydropyrrolo[1,2,3-*de*]-1,4-benzoxazine (19a). This compound was prepared from **18a** (0.8 g, 2.5 mmol) using the procedure described for the preparation of **3**. Yield 0.30 g (50% from **4**). ^{1}H NMR (DMSO-d_6): 0.98 (d, J = 6.2 Hz, 3H), 2.59 (dd, J = 13.7, 7.5 Hz, 1H), 2.68 (d, J = 6.2 Hz, 2H), 2.82 (dd, J = 13.7, 5.2 Hz, 1H), 2.94 (m, 1H), 4.20 (t, J = 4.6 Hz, 2H), 4.44 (t, J = 4.6 Hz, 2H), 4.54 (t, J = 6.2 Hz, 1H), 6.50 (d, J = 7.5 Hz, 1H), 6.84 (t, J = 7.8 Hz, 1H), 6.98–7.08 (m, 5H), 7.13–7.21 (m, 2H), 7.47 (dd, J = 3.7, 1.3 Hz, 1H), 7.80 (dd, J = 4.9, 1.3 Hz, 1H); MS *m*/*z*: 498 (MH^+); Anal. Calcd for $C_{25}H_{27}N_3O_4S_2$: C, 60.34; H, 5.47; N, 8.44. Found: C, 59.97; H, 5.60; N, 8.16.

5.1.29. 7-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-3,4-dihydro-2*H*-pyrrolo[1,2,3-*ef*]-1,5-benzoxazepine (19b). This compound was prepared from **18b** (0.73 g, 2.2 mmol) using the procedure described for the preparation of **3**. Yield 0.30 g (51% from **4**). ^{1}H NMR (DMSO-d_6): 0.97 (d, J = 6.2 Hz, 3H), 2.24 (m, 2H), 2.56 (dd, J = 14.0, 7.3 Hz, 1H), 2.67 (d, J = 6.2 Hz, 2H), 2.79 (dd, J = 14.0, 5.3 Hz, 1H), 2.91 (m, 1H), 4.08 (t, J = 5.6 Hz, 2H), 4.22 (dd, J = 5.7, 3.7 Hz, 2H), 4.53 (t, J = 6.2 Hz, 1H), 6.64 (d, J = 7.7 Hz, 1H), 6.89 (t, J = 7.7 Hz, 1H), 6.96–7.06 (m, 4H), 7.09–7.20 (m, 3H), 7.46 (dd, J = 3.7, 1.3 Hz, 1H), 7.79 (dd, J = 4.9, 1.3 Hz, 1H); MS *m*/*z*: 512 (MH^+); Anal. Calcd for $C_{26}H_{29}N_3O_4S_2$: C,

61.03; H, 5.71; N, 8.21. Found: C, 60.75; H, 5.89; N, 7.90.

5.1.30. 8-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-2,3,4,5-tetrahydropyrrolo[1,2,3-*fg*]-1,6-benzoxazocine (19c). This compound was prepared from **18c** (1.34 g, 3.9 mmol) using the procedure described for the preparation of **3**. Yield 0.33 g (59% from **4**). ^{1}H NMR (DMSO-d_6): 0.97 (d, J = 6.2 Hz, 3H), 1.43 (m, 2H), 1.99 (m, 2H), 2.56 (dd, J = 14.0, 7.5 Hz, 1H), 2.65–2.68 (m, 2H), 2.80 (dd, J = 14.0, 5.3 Hz, 1H), 2.93 (m, 1H), 4.15 (t, J = 5.2 Hz, 2H), 4.39 (t, J = 6.1 Hz, 2H), 4.53 (t, J = 6.2 Hz, 1H), 6.70 (d, J = 7.6 Hz, 1H), 6.89 (t, J = 7.7 Hz, 1H), 6.95–7.01 (m, 3H), 7.05 (dd, J = 4.9, 3.9 Hz, 1H), 7.13–7.20 (m, 2H), 7.26 (d, J = 7.9 Hz, 1H), 7.47 (dd, J = 3.9, 1.3 Hz, 1H), 7.80 (dd, J = 4.9, 1.3 Hz, 1H); MS *m*/*z*: 526 (MH^+); Anal. Calcd for $C_{27}H_{31}N_3O_4S_2$: C, 61.69; H, 5.94; N, 7.99. Found: C, 61.44; H, 6.07; N, 7.65.

5.1.31. 9-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-3,4,5,6-tetrahydro-2*H*-pyrrolo[1,2,3-*gh*]-1,7-benzoxazonine (19d). This compound was prepared from **18d** (0.6 g, 1.68 mmol) using the procedure described for the preparation of **3**. Yield 0.26 g (42% from **4**). ^{1}H NMR (DMSO-d_6): 0.98 (d, J = 6.2 Hz, 3H), 1.34 (m, 2H), 1.62–1.74 (m, 4H), 2.59 (dd, J = 13.8, 7.7 Hz, 1H), 2.63–2.78 (m, 2H), 2.85 (dd, J = 13.8, 5.1 Hz, 1H), 2.99 (m, 1H), 4.15 (m, 2H), 4.47 (m, 2H), 4.58 (m, 1H), 6.74 (d, J = 7.6 Hz, 1H), 6.90 (t, J = 7.7 Hz, 1H), 6.95–7.08 (m, 4H), 7.14–7.23 (m, 3H), 7.50 (dd, J = 3.9, 1.3 Hz, 1H), 7.82 (dd, J = 4.9, 1.3 Hz, 1H); MS *m*/*z*: 540 (MH^+); Anal. Calcd for $C_{28}H_{33}N_3O_4S_2$: C, 62.31; H, 6.16; N, 7.79. Found: C, 62.02; H, 6.27; N, 7.68.

5.1.32. 7-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-3,4-dihydro-3,3-dimethyl-2*H*-pyrrolo[1,2,3-*ef*]-1,5-benzoxazepine (19e). This compound was prepared from **18e** (0.52 g, 1.45 mmol) using the procedure described for the preparation of **3**. Yield 0.19 g (37% from **4**). ^{1}H NMR (DMSO-d_6): 0.99 (d, J = 6.0 Hz, 3H), 1.05 (s, 6H), 2.57 (dd, J = 14.2, 7.6 Hz, 1H), 2.70 (d, J = 6.2 Hz, 2H), 2.81 (dd, J = 14.2, 5.3 Hz, 1H), 2.98 (m, 1H), 3.80 (s, 2H), 3.94 (s, 2H), 4.57 (t, J = 6.2 Hz, 1H), 6.63 (d, J = 6.9 Hz, 1H), 6.88 (t, J = 7.8 Hz, 1H), 6.99–7.03 (m, 3H), 7.05 (dd, J = 4.9, 3.9 Hz, 1H), 7.12–7.21 (m, 3H), 7.48 (dd, J = 3.7, 1.4 Hz, 1H), 7.81 (dd, J = 5.0, 1.4 Hz, 1H); MS *m*/*z*: 540 (MH^+); Anal. Calcd for $C_{28}H_{33}N_3O_4S_2$: C, 62.31; H, 6.16; N, 7.79. Found: C, 62.01; H, 6.30; N, 7.49.

5.1.33. 9-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-2,3,5,6-tetrahydropyrrolo[1,2,3-*gh*]-1,4,7-benzodioxazonine (19f). This compound was prepared from **18f** (0.27 g, 0.75 mmol) using the procedure described for the preparation of **3**. Yield 0.08 g (25% from **4**). ^{1}H NMR (CD_3OD): 1.11 (d, J = 6.0 Hz, 3H), 2.68 (dd, J = 12.1, 4.9 Hz, 1H), 2.74–2.86 (m, 3H), 3.02 (m, 1H), 3.74 (m, 2H), 3.84 (m, 2H), 3.92–4.42 (m, 4H), 4.60 (dd, J = 7.9, 5.0 Hz, 1H), 6.77 (d, J = 7.0 Hz, 1H), 6.83 (s, 1H), 6.89–6.98 (m, 3H), 7.03 (m, 1H), 7.07–7.16 (m, 2H), 7.43 (dd, J = 3.8, 1.3 Hz, 1H), 7.57 (dd, J = 5.1, 1.3 Hz, 1H); MS *m*/*z*: 542 (MH^+); Anal. Calcd for $C_{27}H_{31}N_3O_5S_2$, 1/2H_2O: C, 58.89; H, 5.86; N, 7.63. Found: C, 58.81; H, 5.85; N, 7.26.

5.1.34. (*R*)-3-[2-(*tert*-Butoxycarbonylamino)propyl]-7-(3-chloro-2,2-difluoropropoxy)-1*H*-indole (20). A solution of **13** (1.16 g, 4.0 mmol), 3-chloro-2,2-difluoro-1-propanol (0.73 mL, 8.0 mmol), and CMBP (1.93 g, 8.0 mmol) in toluene (30 mL) was refluxed for 18 h under Ar atmosphere. The reaction mixture was concentrated under reduced pressure, and the residue was chromatographed on silica gel, eluting with hexane/EtOAc = 4/1 to give **20** (1.61 g, quant.) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.12 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.83 (dd, J = 14.3, 7.0 Hz, 1H), 2.95 (dd, J = 14.3, 5.1 Hz, 1H), 3.95 (t, J = 12.5 Hz, 2H), 4.00 (m, 1H), 4.45 (br, 1H), 4.47 (t, J = 11.3 Hz, 2H), 6.67 (d, J = 7.5 Hz, 1H), 7.01-7.06 (m, 2H), 7.33 (d, J = 8.0 Hz, 1H), 8.31 (s, 1H).

5.1.35. (*R*)-7-[2-(*tert*-Butoxycarbonylamino)propyl]-3,3-difluoro-3,4-dihydro-2*H*-pyrrolo[1,2,3-*ef*]-1,5-benzoxazepine (21). This compound was prepared from **20** (0.8 g, 2.0 mmol) using the procedure described for the preparation of **18f**. Yield 0.38 g (52%). ^{1}H NMR ($CDCl_3$): 1.13 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.78 (dd, J = 14.5, 6.8 Hz, 1H), 2.88 (m, 1H), 4.00 (m, 1H), 4.43 (br, 1H), 4.52 (t, J = 12.7 Hz, 2H), 4.53 (t, J = 12.6 Hz, 2H), 6.80–6.84 (m, 2H), 7.00 (t, J = 7.9 Hz, 1H), 7.33 (d, J = 7.9 Hz, 1H).

5.1.36. 7-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-3,3-difluoro-3,4-dihydro-2*H*-pyrrolo[1,2,3-*ef*]-1,5-benzoxazepine (22). This compound was prepared from **21** (0.66 g, 1.8 mmol) using the procedure described for the preparation of **3**. Yield 0.11 g (20% from **4**). ^{1}H NMR (DMSO-d_6): 0.97 (d, J = 6.2 Hz, 3H), 2.55 (dd, J = 13.9, 7.7 Hz, 1H), 2.61–2.73 (m, 2H), 2.80 (dd, J = 13.9, 5.1 Hz, 1H), 2.94 (m, 1H), 4.54 (m, 1H), 4.58 (t, J = 12.6 Hz, 2H), 4.70 (t, J = 13.1 Hz, 2H), 6.76 (d, J = 7.6 Hz, 1H), 6.92–7.02 (m, 3H), 7.06 (dd, J = 5.0, 3.7 Hz, 1H), 7.11–7.21 (m, 3H), 7.26 (d, J = 7.7 Hz, 1H), 7.48 (dd, J = 3.9, 1.4 Hz, 1H), 7.82 (dd, J = 5.0, 1.3 Hz, 1H); MS *m*/*z*: 548 (MH^+); Anal. Calcd for $C_{26}H_{27}F_2N_3O_4S_2$: C, 57.02; H, 4.97; N, 7.67. Found: C, 57.20; H, 5.34; N, 7.37.

5.1.37. (*R*)-7-Allyloxy-3-[2-(*tert*-butoxycarbonylamino)-propyl-1*H*-]indole (23). This compound was prepared from **13** (1.45 g, 5.0 mmol) and allyl iodide (0.91 mL, 10 mmol) in a similar manner to that described for **14**. Yield 1.36 g (82%). ^{1}H NMR ($CDCl_3$): 1.12 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.83 (dd, J = 14.3, 6.9 Hz, 1H), 2.95 (dd, J = 14.3, 5.1 Hz, 1H), 4.01 (br, 1H), 4.44 (br, 1H), 4.69 (dt, J = 5.3, 1.5 Hz, 2H), 5.31 (m, 1H), 5.45 (m, 1H), 6.12 (m, 1H), 6.64 (d, J = 7.5 Hz, 1H), 6.98–7.03 (m, 2H), 7.24 (d, J = 8.2 Hz, 1H), 8.30 (s, 1H).

5.1.38. (*R*)-1-Allyl-7-allyoxy-3-[2-(*tert*-butoxycarbonylamino)propyl]indole (24). This compound was prepared from **23** (6.1 g, 18.4 mmol) and allyl bromide (1.88 mL, 22 mmol) in a similar manner to that described for **15a**. Yield 6.6 g (97%). ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.79 (dd, J = 14.5, 7.0 Hz, 1H), 2.91 (dd, J = 14.5, 5.3 Hz, 1H), 3.98 (m, 1H), 4.41 (m, 1H), 4.64 (dd, J = 3.8, 1.4 Hz, 2H), 4.91–5.09 (m, 4H), 5.29 (dd, J = 10.4, 1.5 Hz, 1H), 5.43 (dd, J = 17.2, 1.7 Hz, 1H), 5.97–6.17 (m, 2H), 6.62 (d, J = 7.7 Hz, 1H), 6.82 (s, 1H), 6.96 (t, J = 7.7 Hz, 1H), 7.21 (d, J = 7.9 Hz, 1H).

5.1.39. (*R*)-8-[2-(*tert*-Butoxycarbonylamino)propyl]-2,5-dihydropyrrolo[1,2,3-*fg*]-1,6-benzoxazocine (25). To a stirred solution of **24** (6.6 g, 17.8 mmol) in toluene (1 L), was added $Cl_2(PCy_3)_2Ru{=}CHPh$ (2.2 g, 2.67 mmol), and the mixture was heated under reflux for 4 h. The reaction mixture was concentrated under vacuum, and the residue was chromatographed on silica gel, eluting with hexane/EtOAc = 4/1 to give **25** (3.9 g, 64%) as an amorphous solid. ^{1}H NMR ($CDCl_3$): 1.12 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.80 (dd, J = 14.1, 6.6 Hz, 1H), 2.90 (dd, J = 14.1, 5.3 Hz, 1H), 3.97 (m, 1H), 4.42 (br, 1H), 4.85 (m, 2H), 4.90 (d, J = 8.3 Hz, 2H), 5.64 (m, 1H), 6.03 (m, 1H), 6.83 (s, 1H), 6.88 (dd, J = 7.5, 0.8 Hz, 1H), 7.00 (t, J = 7.8 Hz, 1H), 7.38 (dd, J = 8.0, 0.8 Hz, 1H).

5.1.40. 8-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-2,5-dihydropyrrolo[1,2,3-*fg*]-1,6-benzoxazocine (26). This compound was prepared from **25** (0.6 g, 1.75 mmol) using the procedure described for the preparation of **3**. Yield 0.16 g (23% from **4**). ^{1}H NMR (DMSO-d_6): 0.97 (d, J = 6.2 Hz, 3H), 2.56 (dd, J = 13.9, 7.8 Hz, 1H), 2.64–2.76 (m, 2H), 2.82 (dd, J = 13.9, 4.6 Hz, 1H), 2.96 (m, 1H), 4.57 (dd, J = 7.3, 5.0 Hz, 1H), 4.78 (s, 2H), 4.85 (d, J = 8.0 Hz, 2H), 5.67 (m, 1H), 5.99 (m, 1H), 6.80 (d, J = 7.3 Hz, 1H), 6.93 (t, J = 7.7 Hz, 1H), 6.99–7.07 (m, 4H), 7.14–7.21 (m, 2H), 7.29 (d, J = 7.7 Hz, 1H), 7.49 (dd, J = 3.7, 1.3 Hz, 1H), 7.82 (dd, J = 5.0, 1.3 Hz, 1H); MS *m*/*z*: 524 (MH^+); Anal. Calcd for $C_{27}H_{29}N_3O_4S_2$, 1/4H_2O: C, 61.40; H, 5.63; N, 7.96. Found: C, 61.39; H, 5.65; N, 7.73.

5.1.41. 7-Benzyloxy-3-[(2*R*)-2-(*tert*-butoxycarbonylamino)propyl]-1-[(2*RS*)-2-hydroxypropyl]-1*H*-indole (27). A mixture of **12** (1.52 g, 4.0 mmol), *t*-BuOK (0.54 g, 4.8 mmol), propylene oxide (10 mL), and THF (10 mL) was stirred at room temperature overnight. The reaction mixture was then concentrated under reduced pressure, and the residue was partitioned between EtOAc and aqueous 1 M HCl. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 2/1 to give **27** (0.77 g, 44%) as an amorphous solid. ^{1}H NMR ($CDCl_3$): 0.95 (d, J = 6.2 Hz, 3H), 1.10–1.16 (m, 3H), 1.33–1.44 (m, 9H), 2.76–2.84 (m, 2H), 3.85–4.12 (m, 3H), 4.38–4.48 (m, 2H), 5.09–5.17 (m, 2H), 6.72 (m, 1H), 6.86 (m, 1H), 7.00 (m, 1H), 7.23 (m, 1H), 7.35–7.47 (m, 5H).

5.1.42. 6-[(2*R*)-2-(*tert*-Butoxycarbonylamino)propyl]-2,3-dihydro-(2*RS*)-2-methylpyrrolo[1,2,3-*de*]-1,4-benzoxazine (28). A suspension of **27** (1.5 g, 3.4 mmol) and 20% $Pd(OH)_2$ (0.3 g) in EtOH (20 mL) was hydrogenated under H_2 for 2 h. The catalyst was then removed by filtration, and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 2/1 to give the corresponding 7-hydroxyindole derivative (0.99 g, 83%).

To a stirred solution of the 7-hydroxyindole derivative obtained above and triphenylphosphine (0.97 g, 3.7 mmol) in THF (20 mL), was added dropwise DEAD (0.58 mL, 3.7 mmol) at room temperature. After stirring overnight, the reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 3/1 to give the title compound **28** (0.77 g, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.13 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 1.55 (d, J = 6.4 Hz, 3H), 2.80–2.96 (m, 2H), 3.88 (dd, J = 12.1, 9.2 Hz, 1H), 4.00 (m, 1H), 4.18 (dd, J = 12.1, 3.0 Hz, 1H), 4.35–4.56 (m, 2H), 6.63 (d, J = 7.5 Hz, 1H), 6.86 (s, 1H), 6.96 (dd, J = 8.1, 7.5 Hz, 1H), 7.17 (d, J = 8.1 Hz, 1H).

5.1.43. 6-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}2,3-dihydro-(2*RS*)-2-methylpyrrolo[1,2,3-*de*]-1,4-benzoxazine (29). This compound was prepared from **28** (0.77 g, 2.3 mmol) using the procedure described for the preparation of **3**. Yield 0.29 g (55% from **4**). ^{1}H NMR (DMSO-d_6): 0.97 (d, J = 6.2 Hz, 3H), 1.45 (d, J = 6.2 Hz, 3H), 2.58 (m, 1H), 2.68 (d, J = 6.4 Hz, 2H), 2.82 (m, 1H), 2.95 (m, 1H), 3.80 (m, 1H), 4.32 (m, 1H), 4.43 (m, 1H), 4.54 (m, 1H), 6.50 (m, 1H), 6.84 (m, 1H), 6.97–7.07 (m, 5H), 7.13–7.20 (m, 2H), 7.47 (m, 1H), 7.81 (m, 1H); MS *m*/*z*: 512 (MH^+); Anal. Calcd for $C_{26}H_{29}N_3O_4S_2$: C, 61.03; H, 5.71; N, 8.21. Found: C, 60.70; H, 5.91; N, 7.87.

5.1.44. 7-Benzyloxy-3-[(2*R*)-2-(*tert*-butoxycarbonylamino)propyl]-1-[(2*RS*)-2-methoxycarbonylethyl]-1*H*-indole (30). To a stirred mixture of **12** (1.52 g, 4.0 mmol), NaH (60% dispersion in mineral oil, 0.19 g, 4.8 mmol), and DMF (10 mL), was added dropwise methyl 2-bromopropionate (0.54 mL, 4.8 mmol) at room temperature. After stirring for 30 min, the reaction mixture was quenched with aqueous 1 M HCl, extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 4/1 to give **30** (1.68 g, 90%) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.11 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 1.72 (d, J = 7.3 Hz, 3H), 2.76–2.96 (m, 2H), 3.57 (s, 3H), 4.00 (br, 1H), 4.49 (br, 1H), 5.17 (s, 2H), 5.93 (q, J = 7.3 Hz, 1H), 6.69 (d, J = 7.7 Hz, 1H), 6.95–7.01 (m, 2H), 7.23 (d, J = 8.1 Hz, 1H), 7.34–7.44 (m, 5H).

5.1.45. 7-Benzyloxy-3-[(2*R*)-2-(*tert*-butoxycarbonylamino)propyl]-1-{[(1*RS*)-1-hydroxymethyl]ethyl}-1*H*-indole (31). A mixture of **30** (1.68 g, 3.6 mmol), aqueous 1 M NaOH (15 mL), and MeOH (15 mL) was stirred at room

temperature for 18 h. After evaporation of MeOH under reduced pressure, the residue was partitioned between EtOAc and aqueous 1 M HCl. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 4/1 to 2/1 to give the corresponding carboxylic acid (1.5 g, 92%) as an amorphous solid.

To a stirred solution of the carboxylic acid obtained above in THF (10 mL), was added dropwise BH_3–THF complex (1 M in THF, 10 mL, 10 mmol) at room temperature. After 2 h stirring, the reaction mixture was quenched with saturated NH_4Cl solution, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 4/1 to give **31** (0.74 g, 51%) as a syrupy oil. ^{1}H NMR ($CDCl_3$): 1.11–1.17 (m, 3H), 1.28–1.47 (m, 9H), 1.46–1.49 (m, 3H), 2.78–2.90 (m, 2H), 3.74 (m, 2H), 3.97 (m, 1H), 4.37 (br, 1H), 5.19 (s, 2H), 5.41 (m, 1H), 6.69–6.74 (m, 1H), 6.95–7.08 (m, 2H), 7.21 (d, J = 8.0 Hz, 1H), 7.35–7.48 (m, 5H).

5.1.46. 6-[(2*R*)-2-(*tert*-Butoxycarbonylamino)propyl]-2,3-dihydro-(3*RS*)-3-methylpyrrolo[1,2,3-*de*]-1,4-benzoxazine (32). A suspension of **31** (0.74 g, 1.7 mmol) and 20% $Pd(OH)_2$ (0.2 g) in EtOH (15 mL) was hydrogenated under H_2 for 3 h. The catalyst was then removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 2/1 to give the corresponding 7-hydroxyindole derivative (0.53 g, 89%) as an amorphous solid.

To a stirred solution of the 7-hydroxyindole derivative obtained above and triphenylphosphine (0.52 g, 2.0 mmol) in THF (20 mL) was added dropwise DEAD (0.31 mL, 2.0 mmol) at room temperature. After stirring overnight, the reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with hexane/EtOAc = 3/1 to give the title compound **32** (0.42 g, 84%) as a colorless solid. ^{1}H NMR ($CDCl_3$): 1.13–1.15 (m, 3H), 1.43 (s, 9H), 1.51 (d, J = 6.4 Hz, 3H), 2.80–2.98 (m, 2H), 4.01 (m, 1H), 4.09 (dd, J = 11.0, 7.1 Hz, 1H), 4.30–4.49 (m, 3H), 6.64 (d, J = 7.3 Hz, 1H), 6.94–7.00 (m, 2H), 7.18 (d, J = 8.0 Hz, 1H).

5.1.47. 6-{(2*R*)-2-{{(2*R*)-2-Hydroxy-2-{3-[(2-thiophenesulfonyl)amino]phenyl}ethyl}amino}propyl}-2,3-dihydro-(3*RS*)-3-methylpyrrolo[1,2,3-*de*]-1,4-benzoxazine (33). This compound was prepared from **32** (0.61 g, 1.8 mmol) using the procedure described for the preparation of **3**. Yield 0.36 g (66% from **4**). ^{1}H NMR (DMSO-d_6): 0.98 (d, J = 5.9 Hz, 3H), 1.41 (d, J = 6.4 Hz, 3H), 2.59 (m, 1H), 2.68 (t, J = 7.1 Hz, 2H), 2.83 (m, 1H), 2.96 (m, 1H), 4.05 (m, 1H), 4.35 (m, 1H), 4.46 (dd, J = 11.2, 3.1 Hz, 1H), 4.54 (m, 1H), 6.51 (d, J = 7.5 Hz, 1H), 6.84 (t, J = 7.8 Hz, 1H), 6.98–7.08 (m, 4H), 7.14–7.20 (m, 3H), 7.47 (m, 1H), 7.81 (m, 1H); MS m/z: 512 (MH^+); Anal. Calcd for $C_{26}H_{29}N_3O_4S_2$: C, 61.03; H, 5.71; N, 8.21. Found: C, 60.66; H, 5.91; N, 7.89.

5.2. Pharmacological studies

5.2.1. cAMP accumulation studies. Each subtype of human β_1-, β_2-, and β_3-ARs was expressed on Chinese hamster ovary (CHO) cells. These cells were cultured with Dulbecco's MEM containing 10% fetal bovine serum, 100 μM nonessential amino acids, and 200 μg/mL G-418. On the day of assay, cells were harvested and resuspended in Hank's balanced salt solution containing 20 mM HEPES, 0.5 mM 3-isobutyl-methylxanthine, and 1 mM L(+)-ascorbic acid. The cell suspension was mixed with test compounds and allowed to stand at 37 °C. After 30 min of incubation, the reaction was terminated by boiling for 5 min. After centrifugation of the reaction mixture at 900*g* for 5 min at room temperature, cAMP concentrations in the supernatant were determined with a cAMP enzyme immunoassay (EIA) system (Amersham Bioscience).

5.2.2. Radioligand binding studies. Three CHO cell lines, expressing each subtype of human β_1-, β_2-, and β_3-ARs, were cultured with the above-mentioned medium. Preconfluent cells were washed with ice-cold PBS, and harvested with the ice-cold lysis buffer (10 mM Tris, 2 mM EDTA, 5 μg/mL leupeptin, 5 μg/mL benzamidine, and 10 μg/mL soybean trypsin inhibitor, pH 7.4 at 4 °C). Harvested membranes were washed twice in lysis buffer by centrifugation at 57,000*g* for 20 min and pellets were resuspended in the assay buffer (50 mM Tris, 2 mM EDTA, and 12.5 mM $MgCl_2$, pH 7.4 at 37 °C). Cell membranes were incubated for 30 min at 37 °C with various concentrations of test compounds in the presence of [^{125}I]-iodocyanopindolol (PerkinElmer) (1 nM for β_3-, 0.1 nM for β_1-, and 0.04 nM for β_2-ARs). Nonspecific binding was determined in the presence of 10 μM (−)-propranolol. The reaction was terminated by rapid filtration through GF/C filters (Whatman). The radioactivity of the ligand trapped on the filters was measured using a gamma counter (ALOKA). K_i values were calculated from IC_{50} values according to the method of Cheng and Prusoff.[19]

5.3. Caco-2 monolayer permeability studies

5.3.1. Materials. The Caco-2 cell line was obtained from the American Type Culture Collection at passage 38. Dulbecco's modified Eagle medium (DMEM) was purchased from Sigma. Nonessential amino acid (NEAA), fetal bovine serum (FBS), trypsin, EDTA, and penicillin–streptomycin (5000 IU/mL and 5000 μg/mL) were purchased from ICN Biomedical, Inc. Hank's balanced salt solution (HBSS) was purchased from Gibco Laboratories. HEPES was purchased from Nakarai Tesque. D(+)-glucose, bovine serum albumin (BSA), and 2-Morpholinoethanesrufonic acid monohydrate (MES) were purchased from Wako.

5.3.2. Preparation of Caco-2 monolayers. Caco-2 cells were grown in DMEM supplemented with 10% FBS and 1% NEAA at 37 °C in dishes (IWAKI, Japan) in a humidified air–5% CO_2 atmosphere. The cells were harvested with trypsin–EDTA, and suspended in culture medium containing 100 IU/mL penicillin and 100 μg/mL

streptomycin. This suspension was seeded onto polycarbonate filters (3 μm pores, 0.31 cm^2 growth area) inside HTS multiwell insert well plate (Nippon Becton Dikinson, Japan) at a density of $1*10^5$ cells/cm^2. The culture medium (0.3 mL in the insert and 40 mL in the feeder tray) was replaced every 48 h from 7 days to 12 days after seeding and every 24 h thereafter.

5.3.3. Drug transport study. Apical to Basal permeability of the drugs was measured using Caco-2 monolayers. The transport medium used for the drug transport study was HBSS supplemented with 25 mM glucose after adjusting the pH to 6.0 (Apical side) or 7.4 (Basal side) with 25 mM MES or 10 mM HEPES, respectively. After 20 min of incubation of both sides of the monolayers with the drug-free transport medium, each side of the medium was replaced with a drug-containing transport medium (apical side) or fresh transport medium with 4.5% BSA or without 4.5% BSA (basal side). Thereafter, the Caco-2 monolayers were incubated for 2 h at 37 °C. Drug concentration in the basal side medium was measured using a reversed-phase HPLC system (LC-2010C, Shimadzu, Japan). Permeability (apparent permeability coefficient, P_{app} (cm/s)) of each drug was calculated according to the following equation:

$$P_{app} = dM/dt * 1/AC_0$$

where dM/dt is the rate of the drug in amount transported to the basal side, C_0 is the initial drug concentration on the apical side, and A is the surface area of the monolayer.

References and notes

1. (a) de Souza, C. J.; Burkey, B. F. *Curr. Pharm. Des.* **2001**, *7*, 1433–1449; (b) Dow, R. L. *Exp. Opin. Invest. Drugs* **1997**, *6*, 1811–1825.
2. (a) Igawa, Y.; Yamazaki, Y.; Takeda, H.; Hayakawa, K.; Akahane, M.; Ajisawa, Y.; Yoneyama, T.; Nishizawa, O.; Andersson, K. E. *Br. J. Pharmacol.* **1999**, *126*, 819–825; (b) Igawa, Y.; Yamazaki, Y.; Takeda, H.; Akahane, M.; Ajisawa, Y.; Yoneyama, T.; Nishizawa, O. *Acta Physiol. Scand.* **1998**, *164*, 117–118.
3. (a) Washburn, W. N.; Sun, C. Q.; Bisacchi, G.; Wu, G.; Cheng, P. T.; Sher, P. M.; Ryono, D.; Gavai, A. V.; Poss, K.; Girotra, R. N.; McCann, P. J.; Mikkilineni, A. B.; Dejneka, T. C.; Wang, T. C.; Merchant, Z.; Morella, M.; Arbeeny, C. M.; Harper, T. W.; Slusarchyk, D. A.; Skwish, S.; Russell, A. D.; Allen, G. T.; Tesfamariam, B.; Frohlich, B. H.; Abboa-Offei, B. E.; Cap, M.; Waldron, T. L.; George, R. J.; Young, D.; Dickinson, K. E.; Seymour, A. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3525–3529; (b) Dow, R. L.; Paight, E. S.; Schneider, S. R.; Hadcock, J. R.; Hargrove, D. M.; Martin, K. A.; Maurer, T. S.; Nardone, N. A.; Tess, D. A.; DaSilva-Jardine, P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3235–3240.
4. Sher, P. M.; Mathur, A.; Fisher, L. G.; Wu, G.; Skwish, S.; Michel, I. M.; Seiler, S. M.; Dickinson, K. E. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1583–1588.
5. Shih, T. L.; Candelore, M. R.; Cascieri, M. A.; Chiu, S. H., Jr.; Colwell, L. F., Jr.; Deng, L.; Feeney, W. P.; Forrest, M. J.; Hom, G. J.; MacIntyre, D. E.; Miller, R. R.; Stearns, R. A.; Strader, C. D.; Tota, L.; Wyvratt, M. J.; Fisher, M. H.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1251–1254.
6. Uehling, D. E.; Donaldson, K. H.; Deaton, D. N.; Hyman, C. E.; Sugg, E. E.; Barrett, D. G.; Hughes, R. G.; Reitter, B.; Adkison, K. K.; Lancaster, M. E.; Lee, F.; Hart, R.; Paulik, M. A.; Sherman, B. W.; True, T.; Cowan, C. *J. Med. Chem.* **2002**, *45*, 567–583.
7. van de Waterbeemd, H.; Smith, D. A.; Beaumont, K.; Walker, D. K. *J. Med. Chem.* **2001**, *44*, 1313–1333.
8. Mizuno, K.; Sawa, M.; Harada, H.; Tateishi, H.; Oue, M.; Tsujiuchi, H.; Furutani, Y.; Kato, S. *Bioorg. Med. Chem. Lett.*, see doi:10.1016/j.bmc.2004.10.032.
9. Corey, E. J.; Helal, C. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1986–2012. A review.
10. Hu, B.; Ellingboea, J.; Gunawana, I.; Hanb, S.; Largisb, E.; Lia, Z.; Malamasa, M.; Mulveyb, R.; Oliphanta, A.; Suma, F. W.; Tillettb, J.; Wong, V. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 757–760.
11. Repk, D. B.; Ferguson, W. J. *J. Heterocycl. Chem.* **1976**, *13*, 775–778.
12. Fujii, A.; Fujima, Y.; Harada, H.; Ikunaka, M.; Inoue, T.; Kato, S.; Matsuyama, K. *Tetrahedron: Asymmetry* **2001**, *12*, 3235–3240.
13. Heaney, H.; Ley, S. V. *J. Chem. Soc., Perkin Trans. 1* **1973**, 499–500.
14. Guiot, J.; Ameduri, B.; Boutevin, B.; Fruchier, A. *New J. Chem.* **2002**, *26*, 1768–1773.
15. Tsunoda, T.; Ozaki, F.; Ito, S. *Tetrahedron Lett.* **1994**, *35*, 5081–5082.
16. Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100–110.
17. In a previous report,[20] we used CHO cells expressing a high level of the β_3-AR to measure compound activity, that is, the receptor densities were 150,000 receptors/cell (β_3-AR), 12,000 receptors/cell (β_1-AR), and 30,000 receptors/cell (β_2-AR). In this study, to better evaluate subtype selectivity, we used CHO cells expressing a low density of the β_3-AR (13,000 receptors/cell) and high densities of the β_1- and β_2-ARs (320,000 and 600,000 receptors/cell, respectively).
18. Mitsuya, M.; Kobayashi, K.; Kawakami, K.; Satoh, A.; Ogino, Y.; Kakikawa, T.; Ohtake, N.; Kimura, T.; Hirose, H.; Sato, N.; Numazawa, T.; Hasegawa, T.; Noguchi, K.; Mase, T. *J. Med. Chem.* **2000**, *43*, 5017–5029.
19. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
20. Harada, H.; Hirokawa, Y.; Suzuki, K.; Hiyama, Y.; Oue, M.; Kawashima, H.; Yoshida, N.; Furutani, Y.; Kato, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1301–1305.