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Synthesis and pharmacological characterization of 1-benzyl-4-aminoindole-based thyroid hormone receptor β agonists

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ABSTRACT

A series of 1-benzylindole-based TRβ agonists were prepared and evaluated. Compounds **11b′** and **11c′** were found to have cholesterol-lowering in a rat model with marginal effects on cardiac function and HPT axis. The present work illustrates the potential use of indoles as inner ring isosteres.

1. Introduction

Management of dyslipidemia is established as reliable clinical intervention to reduce the morbidity and mortality associated with atherosclerotic cardiovascular diseases (CVD). Among a number of therapeutic approaches, statins are the mainstay of treatment for dyslipidemia in CVD patients.[1] Statins, however, are not equally effective in all patients, and are less effective in lowering triglycerides than low-density lipoprotein (LDL) cholesterol.[2] In addition, the withdrawal of cerivastatin in 2001 has led to recommendations for the appropriate use of statins, including cautions, contraindications, and safety monitoring for statin therapy.[3] Therefore, the development of new lipid-lowering drugs with novel mechanisms of action and better safety profiles is still desirable.[4]

Thyroid hormones (TH) are involved in growth, development, and metabolism via their two homologous receptors, TRα and TRβ, which belong to the nuclear receptor superfamily of ligand-dependent transcription factors.[5] TH synthesis and secretion are strictly regulated by the hypothalamic/pituitary/thyroid (HPT) axis, and their receptors are ubiquitously expressed in human and rodents. Plasma levels of circulating TH thereby link to various pathological states. For example, hyperthyroidism is characterized by decreased serum cholesterol and triglycerides levels, body weight loss, tachycardia, arrhythmia, muscle wasting, and other complaints.[5a]

The efficacy and safety of the thyromimetic dextrothyroxine sodium (D-T_4) were investigated in a large clinical trial using coronary heart disease (CHD) patients with hyperlipidemia.[6] Treatment with D-T_4 decreased cholesterol and triglyceride levels by 12% and 15–20%, respectively, compared to a concomitant increase for the placebo group. Although these beneficial effects lasted over three years, treatment with D-T_4 resulted in a significantly higher overall mortality than that with the placebo. In addition, the risk of death increased in patients with a history of myocardial infarction or angina pectoris. The clinical trial was eventually discontinued, however, intensive efforts for the development of safer thyromimetics are continuing (Fig. 1).[7] Recently,

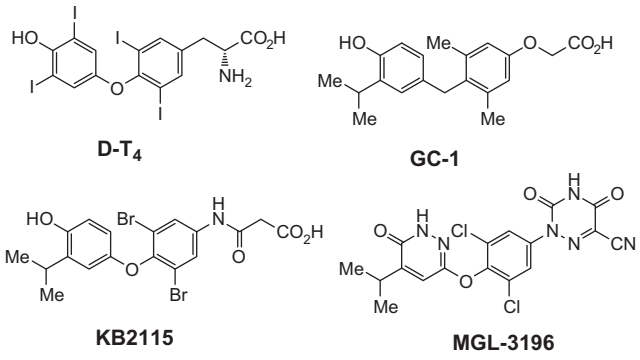

Figure 1. Structures of representative TR agonists.

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a new clue was provided from studies on phenotypes of TR-deficient mice.[8] While TRα1-deficient mice had low heart rate and low body temperature, TRα-deficient (e.g., deletion of TRα1 and TRα2) mice displayed severe hypothyroidism, intestinal malformation, and growth-retardation. As for mice with TRβ-deficiency, they exhibited a modest increase in cholesterol level and had impaired T_3-dependent regulation of cholesterol metabolism. The mice also had goiter, overproduced thyroxine (T_4) and thyroid stimulating hormone (TSH), and thus developed resistance to TH (RTH). It should be noted here that while TRα1 and TRβ double mutation results in exacerbated hypothyroidism, TRα2 and TRβ double mutation can be lethal after weaning. These findings indicate that the non-binding TRα2 receptor is crucial to survival.

Given that TRβ selective modulation may produce beneficial effects on lipid metabolism without affecting cardiac functions, several TRβ-selective agonists were developed.[9] For instance, GC-1, which has a 10-fold selectivity for TRβ over TRα, dose-dependently lowered plasma cholesterol in cholesterol-fed rats without significant tachycardia.[10] In the clinical trials with healthy subjects, GC-1 was well tolerated and decreased LDL cholesterol level by up to 41% at the dose of 100 μg, QD over 2 weeks. KB2115, another TRβ-selective hepato-specific agonist,[11] decreased total and LDL cholesterol levels without affecting heart rate in obese subjects with 255 mg/dl of mean total plasma cholesterol. In particular, KB2115 decreased LDL cholesterol level by as much as 40% when given at the dose of 100 μg daily for 1 week. In patients with hypercholesterolemia, co-treatment with statin and KB2115 (100 μg daily for 12 weeks) further decreased serum LDL cholesterol level by 32%. This co-treatment also decreased triglycerides and Lp(a) lipoprotein without significant heart, bone, or pituitary adverse effects. Encouraged by these clinical data, current medicinal chemistry for thyromimetics is focusing on the design of more TRβ selective hepato-specific modulators that can activate only the target gene responsible for the beneficial effects in the liver.[12] Very recently, MGL-3196 has emerged as a new TRβ modulator with promising phase 1 trial results that support the high potential of this research area.[13]

Herein, we report the synthesis and pharmacological profile of 1-benzyl-4-aminoindoles as new TRβ selective agonists.

2. Results and discussion

Until comprehensive studies with TR-deficient mice and X-ray structural analysis of TR-ligand complexes,[8,14] it was believed that thyromimetics selective delivery to the liver was a key factor for dissociation of these agents beneficial lipid-lowering effects from the broad hormonal effects, and that this delivery was partly attributed to thyromimetics constrained conformations with both a perpendicular arrangement of the two aromatic rings and a distal position of the 3′-substituent to the inner aromatic ring (Fig. 2).[15] Early medicinal chemical efforts for thyromimetic were thereby centered on the design of isosteres of the 3′-iodo substituent in the outer ring, and successfully identified optimal groups, such as the pyridazinonyl group in SK&F L-94901,[16] that efficiently maintain the 'active' conformation. On the other hand, the explorations of these optimal groups resulted in a considerable reduction in intellectual property space around the outer ring, making modification around the inner ring an attractive option for further improvement.[17] From this point of view, the structure GC-1 is quite fascinating because it is rationally simplified and amenable to modification. A crystal structure of GC-1 in complex with the TRβ-ligand binding domain reveals that the pockets for the 3,5-dimethylphenyl outer ring are not fully filled.[14b] We therefore envisaged that when the outer ring is connected at the 1-position, a larger planar 2-methylindole might not only restrict free rotation of the outer ring, but also make better fit into the pockets.

To begin with, we prepared a series of compounds with various linkage lengths between the indole core and the terminal carboxylic acid (Scheme 1). The outer ring moiety of the benzyl chlorides **1**, was prepared from 2-isopropylphenol by four steps and immediately used without purification due to their instability. N-alkylation of the indoles **2** with **1** proceeded smoothly by use of sodium hydride as base. Alkaline hydrolysis of **3** gave the desired acids **4** in excellent yields. Elongation of **4a**,**b** with glycine ethyl ester was carried out under standard coupling conditions, followed by hydrolysis to give the desired acids **5a**,**b**. The binding affinity and transcriptional activity of **4** and **5** are shown in Table 1. The binding assay was carried out by radio-active ligand displacement in a range test compound concentrations at radiolabelled endogenous ligand thyronine (T_3) concentration of 0.16 nM, using recombinant hTRα1 and hTRβ1 ligand binding domains. Transcriptional activity was measured using monkey kidney CV-1 cells transiently transfected with hTRα1 or hTRβ1 and an alkaline phosphatase reporter gene. In this assay, T_3 showed strong binding affinity for TRα1 and TRβ1 with IC_{50} values of 3.2 and 2.9 nM, respectively and consistent transcriptional activity for both receptors. On the whole, the 2-methylindoles **4** and **5** preferred TRβ1 to TRα1, although they were at least 100-fold less potent in binding to both TRs than T_3. The length of the linkage seems to have an impact on the binding affinity to TRα1 rather than TRβ1, wherein **5b** showed the highest TRβ selectivity with a ratio of 19. The reporter assay results of **4c**–**e** also indicated a similar preference of all compounds for TRβ1, although the observed agonist activity of **4c**–**e** was higher than expected from their binding affinity. Malm and co-workers have also reported a similar discrepancy, albeit in the opposite sense, between the binding affinity and the functional activity and suggested that this discrepancy might be due to the lack of active transporters in the cell-based reporter assay.[14d,17a] In our case, there may be a favorable access of **4c**–**e** to target organelles, such as the nucleus.

Crystallographic studies have demonstrated that the two subtype receptors differ in a single amino acid residue, that is, Asn for TRβ and Ser for TRα, in the ligand-binding pocket. Furthermore, these two residues form a hydrogen bond network with two

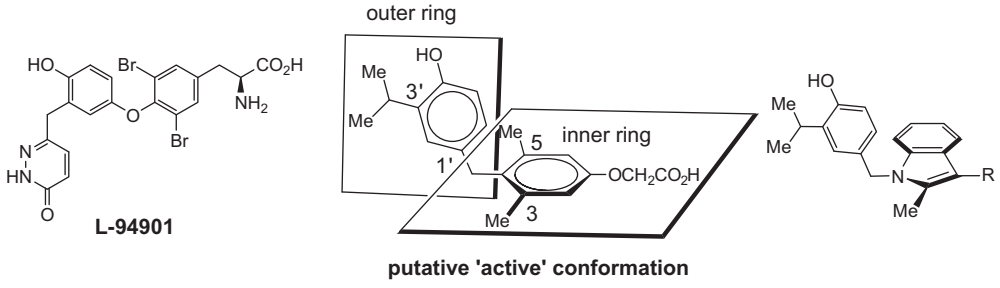
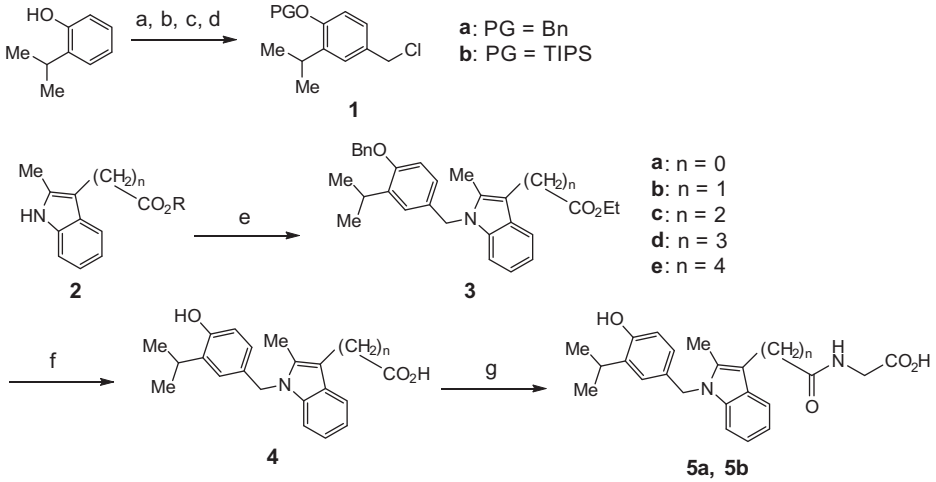
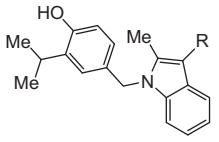


Figure 2. Structure of L-94901 and representation of a putative 'active' conformation of TR agonists.



Scheme 1. Synthesis of **5**. Reagents and conditions: (a) NBS, MeCN; (b) PG = Bn: BnCl, $NaHCO_3$, NaI, MeCN, 60 °C, PG = TIPS: TIPSCl, imidazole, DMF; (c) (i) ^{t}BuLi, THF, −78 °C, (ii) NaOH, MeOH, 60 °C; (d) $SOCl_2$, CH_2Cl_2; (e) (i) NaH, DMF, 0 °C, (ii) compound **1a**, 0 °C; (f) (i) H_2/Pd-C, EtOH/EtOAc, (ii) NaOH, MeOH, 60 °C; (g) (i) glycine ethyl ester HCl, WSC, HOBt, Et_3N, DMF, (ii) NaOH, MeOH, 60 °C.

Table 1
Potency of compounds **4** and **5** for TRs

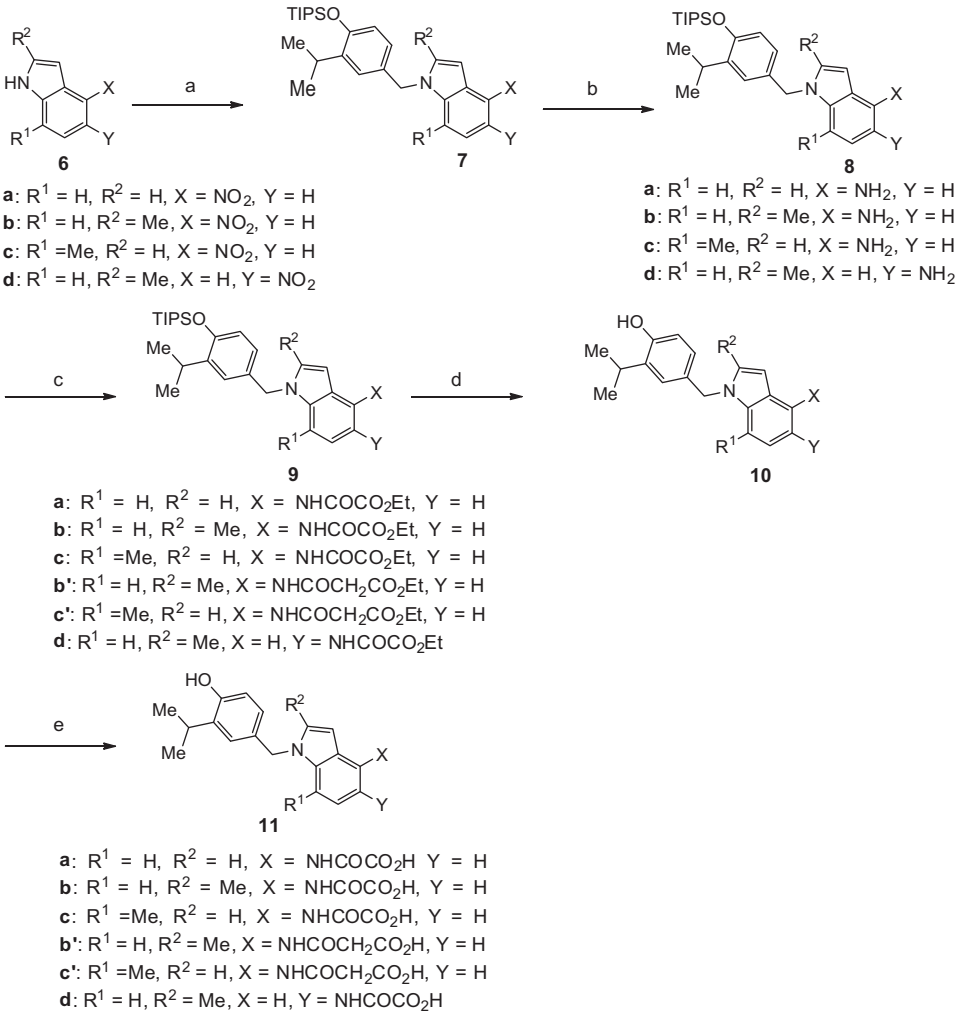


Compd	R	Binding assay[a] (IC_{50}, μM)			Reporter assay (EC_{50}, μM)		
		TRα	TRβ	α/β	TRα	TRβ	α/β
T_3		0.0032	0.0029	1.1	0.0021	0.002	1.0
4b	CH_2CO_2H	—	—	—	0.45	0.15	3.0
4c	$CH_2CH_2CO_2H$	1.3	0.76	2.6	0.039	0.037	1.0
4d	$CH_2CH_2CH_2CH_2CO_2H$	3.4	0.29	>2.1	0.18	0.037	4.8
4e	$CH_2CH_2CH_2CH_2CH_2CO_2H$	>10	1.4	>7.1	0.10	0.062	1.6
5b	$CH_2CONHCH_2CO_2H$	8.2	0.43	19	—	—	—
5a	$CONHCH_2CO_2H$	>10	1.1	>9.0	—	—	—

[a] Values are the means of quadruplicate experiments.

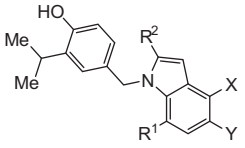
neighboring Arg residues, that is, the carboxylate of a ligand and water molecules in the individual binding pockets. Therefore, it is proposed that this subtle difference renders the selectivity in binding largely dependent on the position of the ligand carboxylate. The proposed relationship fairly rationalizes our results with the 2-methylindoles **4** and **5**,[18] and thereby necessitates further optimization around the carboxylic acid side chain. To this end, we chose the nitroindoles **6** as a starting precursor with a nitro group that can be easily connected to a carboxylic acid side chain after conversion into an amino group (Scheme 2). Thus, **6** was obtained according to a procedure described in the literature (see, Section 3), and introduction of the outer ring moiety was as described above. Reduction of **7** smoothly proceeded by catalytic hydrogenation. Condensation of **7** with diethyl oxalate or ethyl malonyl chloride and subsequent removal of the silyl protecting group followed by hydrolysis gave the desired acids **11**. Similarly, the 5-subsutituted congener **11d** was prepared from the commercially available 2-methyl-5-nitroindole. At this stage, to see whether the observed discrepancy of the results with **4** was a case only with the monkey cell line, the transcriptional activity of **11** was evaluated using HepG2 cells as source of a human cell line. Unexpectedly, only a small gap between the binding and reporter assays was observed regardless of the cell line used, although HepG2-based assay provided better agreement with the binding affinity (Table 2). A short explanation is worthwhile here. First, a marked difference can be seen between **11b** and **11d**. Compound **11b** showed the highest TRβ1 binding affinity with an IC_{50} value of 40 nM, whereas **11d** affinity for this receptor was the worst. Apparently, the chain extension at the 4-position is far more favorable for better binding affinity than that at the 5-position. Second, absence of the 2-methyl substituent (**11a**) resulted in a more than fourfold decrease in binding affinity, which actually supports our working hypothesis for restricted rotation of the outer ring. Interestingly, a positive substituent effect of a methyl group was also observed with 7-methyl analogue **11c**. In this line, extension with one methylene spacer unit was evaluated in both the 2- and 7-methyl analogues **11b′**, **c′**. Gratifyingly, both analogues produced significant improvement in TRβ1 selectivity. Although the HepG2-based assay showed an apparent difference in TRβ selectivity between **11b′** and **11c′**, we considered these two analogs to be virtually the same in terms of potency and selectivity. In contrast, the two analogues showed a considerable decrease in TRβ1 binding affinity compared to **11b**,**c**, and therefore no further extension was attempted.

To test in vivo potency of **11b′** and **11c′**, the two analogs were orally administered to cholesterol-fed rats (Table 3). Cholesterol feeding continued for 1 week before drug treatment with average



Scheme 2. Synthesis of **11**. Reagents and conditions: (a) (i) NaH, DMF, 0 °C, (ii) **1b**, 0 °C; (b) H_2/Pd-C, EtOH, (c) **9a**, **9b**, **9c**, **9d**: diethyl oxalate, 100 °C, **9b′** and **9d′**: ethyl malonyl chloride, Et_3N, CH_2Cl_2; (d) TBAF, THF; (e) NaOH, EtOH.

Table 2
Potency of compound **11** for TRs

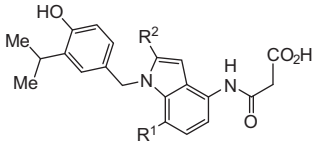


Substituents					Binding assay[a] (IC_{50}, μM)			Reporter assay[a] (EC_{50}, μM)			
								CV-1		HepG2	
Compd	X	Y	R^1	R^2	TRα	TRβ	α/β	TRβ	α/β	TRβ	α/β
11b	$NHCOCO_2H$	H	H	Me	0.23	0.04	5.7	0.59	2.2	0.11	1.5
11d	H	$NHCOCO_2H$	H	Me	0.23	1.5	0.15	–	–	–	–
11c	$NHCOCO_2H$	H	Me	H	0.19	0.054	3.5	0.50	1.5	0.19	2.5
11a	$NHCOCO_2H$	H	H	H	1.2	0.17	7.0	1.9	2.7	0.55	2.2
11b′	$NHCOCH_2CO_2H$	H	H	Me	5.2	0.23	21	1.7	4.5	0.22	16
11c′	$NHCOCH_2CO_2H$	H	Me	H	5.2	0.24	26	3.1	3.4	1.3	7.2

[a] Values are the means of quadruplicate experiments.

plasma cholesterol level reaching increased to 190 ± 49 mg/dl, compared to 66 ± 11 mg/dl in normal rats. We found that administration of **11b′** or **11c'** at the dose of 30 or 6 mg/kg QD, respectively, produced a significant reduction in total cholesterol level. Similar cholesterol-lowering effects were observed with T_3 at the dose of 50 μg/kg QD. Differences in in vivo potency between **11b′**, **c′** and

Table 3
Cholestrol-lowering effects of **11b′** and **11c′** in cholesterol-fed rats[a]



	R^1, R^2	Total Cho (mg/dl)	LDL-Cho (mg/dl)	Free Cho (mg/dl)	HDL-Cho (mg/dl)	TG (mg/dl)
Normal		66 ± 11	8.0 ± 2	39 ± 9	28 ± 3	100 ± 40
Control		190 ± 49	51 ± 15	36 ± 9	22 ± 6	62 ± 25
T_3		123 ± 15*	28 ± 5*	22 ± 2*	22 ± 2	61 ± 8
11c′	Me, H	94 ± 5*	20 ± 2*	15 ± 1*	20 ± 1	27 ± 7*
11b′	H, Me	111 ± 19*	25 ± 7*	19 ± 4*	23 ± 7	44 ± 9

[a] Cholesterol-fed rats were orally treated once daily for 1 week with **11b′** (30 mg/kg/day, $n = 13$), T_3 as a positive control (50 μg/kg/day, $n = 13$), or the vehicle or to be the untreated (control) .
* $P<0.05$ compared to the treated control.

Table 4
Effects of **11b′** and **11c′** on heart rate, heart weight, TSH, T_3, and T_4[a]

	Heart rate (bpm)	Heart weight (g)	TSH (μlU/ml)	Total-T_4 (μg/dl)	Total-T_3 (ng/dl)
Normal	404.8 ± 37.2	1.08 ± 0.04	2.8 ± 1.6	4.4 ± 1.2	68 ± 6
Control	383.0 ± 26.7	1.14 ± 0.12	3.0 ± 1.0	3.8 ± 0.9	62 ± 7
T_3	430.0 ± 19.3	1.25 ± 0.13	0.1 ± 0*	0.9 ± 0.4*	31 ± 9*
Low-dose **11c′**	401.8 ± 22.3	1.12 ± 0.06	2.7 ± 1.8	2.4 ± 1.0*	61 ± 14
High-dose **11c′**	424.3 ± 36.5	1.18 ± 0.08	1.3 ± 0.6	1.9 ± 0.5*	53 ± 8
Low-dose **11b′**	403.2 ± 32.5	1.13 ± 0.10	3.1 ± 1.3	2.8 ± 0.5	63 ± 11
High-dose **11b′**	415.8 ± 35.8	1.24 ± 0.07	0.9 ± 0.7	1.7 ± 0.3*	53 ± 8

[a] Cholestrol-fed rats were orally treated once daily for 1 week with **11b′** (30 and 150 mg/kg/day, $n = 13$), **11c′** (6 and 150 mg mg/kg/day, $n = 13$), T_3 as a positive control (50 μg/kg/day, $n = 13$), or the vehicle or to be left untreated (control).
* $P<0.05$ compared to the treated control.

T_3 are at least partly attributed to their binding affinity. Also, these cholesterol-lowering was found to be largely due to a decrease in LDL cholesterol, which is consistent with the reported results for GC-1 or T-681, another liver-selective thyromimetic, in mice.[19] As for plasma triglyceride level, **11c′** produced a significant reduction, while **11b′** induced a none significant downward trend. No change in triglyceride level was observed following treatment with T_3. Briefly, the safety profiles were examined (Table 4). Although heart rate and heart weight increased following treatment with T_3, **11b′**, or **11c′**, the increase in heart rate produced by the two analogues was less than 15%, which is considered the upper limit for clinical use,[20] even at the highest dose of 150 mg/kg. Unlike T_3 or GC-1,[20,21] **11b′** and **11 c′** had no effect on TSH at their cholesterol-lowering doses. This lack of effect on TSH seems to be a characteristic feature of **11b′** and **11c′**, because TRβ is thought to mediate cholesterol metabolism and TSH regulation. In addition, both analogues produced no significant reduction in plasma T_3 and T_4 levels at their cholesterol-lowering doses. These results demonstrate that **11b′** and **11c′** had marginal effects on cardiac function and HPT axis in cholesterol-fed rats.

In summary, a series of 1-benzylindole-based TRβ agonists were prepared and evaluated. Compounds **11b′** and **11c′** were found to have cholesterol-lowering effect in a rat model with marginal effects on cardiac function and HPT axis. Considering the previous work by Malm and co-workers,[17a] in which 1*H*-indole-2-carboxylic acid was designed as an inner ring isostere and found to be an excellent scaffold for TRβ selective ligand, our present work here additionally illustrates the potential use of indoles as inner ring isosteres. Further optimization of **11b′** and **11c′** requires understanding the mechanisms of TRβ selectivity and separation of these analogues cholesterol-lowering effect from cardiac and HPT axis toxicity. PK-PD relationship studies on this series of compounds should be informative and the results would be reported elsewhere.

3. Experimental section

3.1. Chemistry

3.1.1. General

All reagents and solvents were purchased commercially and used without further purification. The starting ethyl esters of 2-methylindole-3-carboxylic acid and 2-methylindole-3-acetic acid, and the 2-methyl-5-nitroindole are commercially available. Other 3-indoyl acid esters[22] and nitroindoles,[23] and the 3-isopropyl-4-methoxybenzylalcohol[24] were prepared according to the literature. Flash chromatography was performed using Silica Gel 60 (particle size 0.040–0.050 mm, Kanto Kagaku) or Hi-Flash columns (silica gel, particle size 0.070 mm, Yamazen Science). Melting points were determined on a cover glass with a Yanaco MP-J3 micro melting point apparatus and are given as uncorrected values. ^{1}H NMR spectra were recorded on a Bruker AVANCE III spectrometer operating at 400 MHz and 25 °C with tetramethylsilane as internal standard. Data are reported as follows: chemical shift in ppm (δ) relative to trimethylsilane, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), and coupling constant (Hz). Infrared spectra (IR) were recorded on a JASCO FT/IR-4200 spectrometer with a single-reflection diamond ATR unit. LC/MS spectra were determined on a Waters ZMD2000 equipped with a Waters 2690 injector and a PDA detector operating at 210–400 nm and interfaced with a Micromass ZMD mass spectrometer. High-resolution mass spectra (HRMS) were obtained using a Thermo LTQ Orbitrap.

3.1.2. 4-Bromo-2-isopropylphenol

To a solution of 2-isopropylphenol (50.0 g, 367 mmol) in MeCN (500 mL) at 0 °C was added NBS (71.9 g, 404 mmol). After stirring at room temperature for 4 h, the reaction mixture was concentrated under reduced pressure. The residue was suspended in hexane and the precipitates were filtered off. The filtrate was washed with water and brine, and then dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure to obtain the title compound (84.0 g, quantitative yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.10 (6H, d, J = 7.0 Hz), 3.17 (1H, m), 6.61 (1H, d, J = 8.4 Hz), 7.15 (1H, dd, J = 2.2, 8.4 Hz), 7.27 (1H, d, J = 2.2 Hz); MS (ESI) m/z 213, 215 $(M-H)^-$.

3.1.3. 1-Benzyloxy-4-bromo-2-isopropylbenzene

To a solution of 4-bromo-2-isopropylphenol (2.2 g, 7 mmol) in MeCN (10.0 mL) at room temperature were added benzyl chloride (1.7 g, 13 mmol), sodium bicarbonate (1.0 g, 12 mmol) and sodium iodide (0.2 g, 1 mmol). After stirring at 60 °C for 18 h, the reaction mixture was cooled to room temperature, and concentrated under reduced pressure. The residue was taken up with water and extracted with EtOAc, and the organic layers were washed with water and brine, and then dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure, and the residue was purified by flash chromatography on silica gel and eluted with hexane/EtOAc (1/0–9/1) to give the title compound (2.7 g, 86%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.21 (6H, d, J = 7.2 Hz), 3.37 (1H, m), 6.76 (1H, d, J = 8.4 Hz), 7.23 (1H, dd, J = 2.8, 8.4 Hz), 7.31–7.43 (6H, m); MS (ESI) m/z 303, 305 $(M-H)^-$.

3.1.4. 4-Bromo-2-isopropyl-1-triisopropylsilanyloxybenzene

To a solution of 4-bromo-2-isopropylphenol (84.8 g, 394 mmol) in DMF (848 mL) were added triisopropylsilyl chloride (101 mL, 472 mmol) and imidazole (53.7 g, 789 mmol). After stirring at room temperature for 24 h, the reaction mixture was diluted with hexane, washed with water and brine, and dried over Na_2SO_4. The solvent was then concentrated under reduced pressure, and the residue was crystallized with MeCN to give the title compound (98.0 g, 67%) as a white solid: mp 59–60 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 (18H, d, J = 7.3 Hz), 1.18 (6H, d, J = 7.0 Hz), 1.28 (3H, m), 3.32 (1H, m), 6.63 (1H, d, J = 8.4 Hz), 7.12 (1H, dd, J = 2.6, 8.4 Hz), 7.25 (1H, d, J = 2.6 Hz); MS (ESI) m/z 370, 372 $(M+H)^+$.

3.1.5. (3-Isopropyl-4-triisopropylsilanyloxyphenyl)methanol

To a solution of 4-bromo-2-isopropyl-1-triisopropylsilanyloxybenzene (23.6 g, 64 mmol) in THF (236 mL) was added dropwise a solution of ^{t}BuLi (1.5 M in pentane, 100 mL, 150 mmol) at −78 °C over 1 h. After stirring at the same temperature for 1 h, paraformaldehyde (3.9 g) was added to the mixture, and the resulting mixture was stirred at −78 °C for an additional 1 h, and then warmed to room temperature for 3 h. The reaction mixture was subsequently diluted with Et_2O (300 mL), successively washed with 1 M hydrochloric acid, and brine, and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash chromatography on silica gel and eluted with hexane/EtOAc (1/0–4/1) to give the title compound (9.4 g, 46%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.12 (18H, d, J = 7.3 Hz), 1.21 (6H, d, J = 6.9 Hz), 1.32 (3H, m), 3.37 (1H, m), 4.59 (2H, d, J = 5.5 Hz), 6.75 (1H, d, J = 8.1 Hz), 7.02 (1H, dd, J = 1.2, 8.0 Hz), 7.20 (1H, d, J = 1.2 Hz); MS (ESI) m/z 305 $(M-H_2O)^+$, 321 $(M-H)^-$.

3.1.6. (4-Benzyloxy-3-isopropylphenyl)methanol

The title compound was prepared according to the procedure described in Section 3.1.5, using 1-benzyloxy-4-bromo-2-isopropylbenzene as white solid: mp 56–58 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (6H, d, J = 7.2 Hz), 3.41 (1H, m), 4.60 (2H, s), 5.07 (2H, s), 6.87 (1H, d, J = 8.0 Hz), 7.12 (1H, dd, J = 2.0, 8.4 Hz), 7.23–7.43 (6H, m); MS (ESI) m/z 239 $(M+H-H_2O)^+$, 255 $(M-H)^-$.

3.1.7. (4-Chloromethyl-2-isopropylphenoxy)triisopropylsilane (1b)

Thionyl chloride (1.6 mL, 22 mmol) was added dropwise to a solution of (3-isopropyl-4-triisopropylsilanyloxyphenyl)methanol (4.6 g, 14 mmol) in CH_2Cl_2 (46 mL), and the mixture was stirred at room temperature for 2 h. The mixture was next concentrated under reduced pressure to give **1b**, which was used immediately without further purification. 1-Benzyloxy-4-chloromethyl-2-isopropylbenzene (**1a**) was prepared from the corresponding alcohol in a similar manner and immediately used the next reaction.

3.1.8. Ethyl [1-(4-benzyloxy-3-isopropylbenzyl)-2-methyl-1*H*-indol-3-yl]acetate (3b)

To a suspension of NaH (60% in oil, 87.1 mg, 2.2 mmol) in DMF (2.0 mL) was added dropwise a solution of ethyl (2-methylindol-3-yl)acetate (430.0 mg, 2.0 mmol) in DMF (2.0 mL) at 0 °C. After stirring at 0 °C for 1 h, a DMF solution (2.0 mL) of freshly prepared **1a** (ca. 2.2 mmol) was added dropwise to the mixture at 0 °C, and the resulting mixture was stirred for an additional 3 h. The reaction was quenched with ice water, and then extracted with EtOAc. The organic layers were successively washed with water and brine, and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash chromatography on silica gel and eluted with hexane/EtOAc (9/1) to give the title compound (387 mg, 43%) as a white solid: mp 76–77 °C; ^{1}H NMR ($CDCl_3$) δ 1.18 (6H, d, J = 6.9 Hz), 1.22 (3H, t, J = 7.1 Hz), 2.35 (3H, s), 3.35 (1H, m), 3.73 (2H, s), 4.14 (2H, q, J = 7.1 Hz), 5.00 (2H, s), 5.25 (2H, s), 6.57 (1H, dd, J = 2.2, 8.4 Hz), 6.72 (1H, d, J = 8.4 Hz), 7.02 (1H, d, J = 2.2 Hz), 7.09–7.13 (2H, m), 7.24 (1H, m), 7.32 (1H, m), 7.36–7.40 (4H, m), 7.58 (1H, m); MS (ESI) m/z 456 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{30}H_{34}NO_3$ $(M+H)^+$ 456.2533, found 456.2528.

3.1.9. Ethyl 1-(4-benzyloxy-3-isopropylbenzyl)-2-methyl-1*H*-indole-3-carboxylate (3a)

The title compound was prepared according to the procedure described for **3b**, using ethyl 2-methyl-1*H*-indole-3-carboxylate as a white solid (88%): mp 89–91 °C, ^{1}H NMR ($CDCl_3$) δ 1.18 (6H, d, J = 6.9 Hz), 1.26 (3H, t, J = 7.1 Hz), 2.75 (3H, s), 3.35 (1H, m), 4.43 (2H, q, J = 7.1 Hz), 5.00 (2H, s), 5.29 (2H, s), 6.58 (1H, dd, J = 1.9, 8.4 Hz), 6.73 (1H, d, J = 8.4 Hz), 7.04 (1H, d, J = 1.9 Hz), 7.13–7.32 (4H, m), 7.36–7.40 (4H, m), 8.17 (1H, d, J = 7.5 Hz); MS (ESI) m/z 442 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{29}H_{32}NO_3$ $(M+H)^+$ 442.2377, found 442.2378.

3.1.10. Ethyl [1-(4-benzyloxy-3-isopropylbenzyl)-2-methyl-1*H*-indol-3-yl]propanoate (3c)

The title compound was prepared according to the procedure described for **3b**, using ethyl (2-methylindol-3-yl)propanoate as a foam (17%): ^{1}H NMR ($CDCl_3$) δ 1.18 (6H, d, J = 6.9 Hz), 1.26 (3H, t, J = 7.0 Hz), 2.32 (3H, s), 2.62 (2H, t, J = 7.8 Hz), 3.08 (2H, t, J = 7.8 Hz), 3.35 (1H, m), 4.11 (2H, q, J = 7.0 Hz), 5.00 (2H, s), 5.22 (2H, s), 6.54 (1H, dd, J = 2.2, 8.4 Hz), 6.71 (1H, d, J = 8.4 Hz), 7.00 (1H, d, J = 2.2 Hz), 7.05–7.12 (2H, m), 7.22 (1H, m), 7.30 (1H, m), 7.34–7.41 (4H, m), 7.54 (1H, m); MS (ESI) m/z 470 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{31}H_{36}NO_3$ $(M+H)^+$ 470.2690, found 470.2690.

3.1.11. Ethyl [1-(4-benzyloxy-3-isopropylbenzyl)-2-methyl-1*H*-indol-3-yl]butanoate (3d)

The title compound was prepared according to the procedure described for **3b**, using ethyl (2-methylindol-3-yl)butanoate as a foam (34%): ^{1}H NMR ($CDCl_3$) δ 1.18 (6H, d, J = 6.9 Hz), 1.23 (3H, t, J = 7.1 Hz), 1.97 (2H, m), 2.29 (3H, s), 2.32 (2H, t, J = 7.3 Hz), 2.80

(2H, t, J = 7.3 Hz), 3.35 (1H, m), 4.11 (2H, q, J = 7.1 Hz), 5.00 (2H, s), 5.23 (2H, s), 6.57 (1H, dd, J = 1.4, 8.4 Hz), 6.73 (1H, d, J = 8.4 Hz), 6.97 (1H, d, J = 1.4 Hz), 7.06–7.12 (2H, m), 7.22 (1H, m), 7.31 (1H, m), 7.34–7.41 (4H, m), 7.54 (1H, m); MS (ESI) m/z 484 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{32}H_{38}NO_3$ $(M+H)^+$ 484.2846, found 484.2843.

3.1.12. Ethyl [1-(4-benzyloxy-3-isopropylbenzyl)-2-methyl-1*H*-indol-3-yl]pentanoate (3e)

The title compound was prepared according to the procedure described for **3b**, using ethyl (2-methylindol-3-yl)pentanoate as a foam (12%): ^{1}H NMR ($CDCl_3$) δ 1.18 (6H, d, J = 6.9 Hz), 1.22 (3H, t, J = 7.1 Hz), 1.63–1.74 (4H, m), 2.29 (3H, s), 2.31 (2H, t, J = 6.9 Hz), 2.76 (2H, t, J = 6.9 Hz), 3.35 (1H, m), 4.11 (2H, q, J = 7.1 Hz), 5.00 (2H, s), 5.22 (2H, s), 6.55 (1H, dd, J = 2.2, 8.5 Hz), 6.72 (1H, d, J = 8.5 Hz), 6.99 (1H, d, J = 2.2 Hz), 7.03–7.11 (2H, m), 7.22 (1H, d, J = 7.7 Hz), 7.28 (1H, m), 7.34–7.40 (4H, m), 7.53 (1H, m); MS (ESI) m/z 498 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{33}H_{40}NO_3$ $(M+H)^+$ 498.3003, found 498.3004.

3.1.13. [1-(3-Isopropyl-4-hydroxybenzyl)-2-methyl-1*H*-indol-3-yl]acetic acid (4b)

To a solution of **3b** (339 mg, 0.7 mmol) in EtOAc (2.5 mL) and ethanol (2.5 mL) was added 10% Pd-C (135 mg). The mixture was stirred under hydrogen atmosphere at room temperature for 3 h. The Pd catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 1/0–4/1) to give a debenzylated intermediate (206 mg; MS (ESI) m/z 394 $(M+H)^+$, 392 $(M-H)^-$), which was then dissolved with methanol (2.0 mL), and treated with 1 M NaOH (1.1 mL). The mixture was heated at 60 °C for 2 h. After evaporation, the mixture was partitioned between 1 M hydrochloric acid and EtOAc, and the organic layers were washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was chromatographed on silica gel and eluted with hexane/EtOAc (1/1) to give **4b** (95 mg, 38%, 2 steps) as a foam: ^{1}H NMR ($CDCl_3$) δ 1.17 (6H, d, J = 6.9 Hz), 2.31 (3H, s), 3.12 (1H, m), 3.76 (2H, s), 5.21 (2H, s), 6.48 (1H, dd, J = 1.8, 8.4 Hz), 6.52 (1H, d, J = 8.4 Hz), 6.94 (1H, d, J = 1.8 Hz), 7.07–7.12 (2H, m), 7.21 (1H, m), 7.57 (1H, m); IR (ATR) 3498, 2960, 1698 cm^{-1}; MS (ESI) m/z 338 $(M+H)^+$, 336 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{21}H_{24}NO_3$ $(M+H)^+$ 338.1751, found 338.1752.

3.1.14. 1-(3-Isopropyl-4-hydroxybenzyl)-2-methyl-1*H*-indole-3-carboxylic acid (4a)

The title compound was prepared according to the procedure described for **4b**, using **3a** as a white solid (38%, 2 steps): mp 198–200 °C (dec), ^{1}H NMR (DMSO-d_6) δ 1.19 (6H, d, J = 6.9 Hz), 2.70 (3H, s), 3.12 (1H, m), 5.35 (2H, s), 6.52 (1H, dd, J = 1.8, 8.0 Hz), 6.64 (1H, d, J = 8.0 Hz), 7.01 (1H, d, J = 2.2 Hz), 7.10–7.16 (2H, m), 7.51 (1H, m), 8.00 (1H, m), 9.26 (1H, br s); IR (ATR) 3111, 1698 cm^{-1}; MS (ESI) m/z 324 $(M+H)^+$, 322 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{20}H_{22}NO_3$ $(M+H)^+$ 324.1594, found 324.1597.

3.1.15. [1-(3-Isopropyl-4-hydroxybenzyl)-2-methyl-1*H*-indol-3-yl]propanoic acid (4c)

The title compound was prepared according to the procedure described for **4b**, using **3c** as a foam (40%, 2 steps): ^{1}H NMR ($CDCl_3$) δ 1.19 (6H, d, J = 7.0 Hz), 1.22 (3H, t, J = 7.0 Hz), 2.34 (3H, s), 2.66 (2H, t, J = 7.3 Hz), 3.08 (2H, t, J = 7.3 Hz), 3.14 (1H, m), 5.19 (2H, s), 5.28 (1H, s), 6.43 (1H, d, J = 8.1 Hz), 6.51 (1H, d, J = 8.4 Hz), 6.97 (1H, s), 7.07–7.12 (2H, m), 7.24 (1H, m), 7.57 (1H, d, J = 6.6 Hz); IR (ATR) 3386, 2965, 1669 cm^{-1}; MS (ESI) m/z 352 $(M+H)^+$, 350 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{22}H_{26}NO_3$ $(M+H)^+$ 352.1907, found 352.1910.

3.1.16. [1-(3-Isopropyl-4-hydroxybenzyl)-2-methyl-1*H*-indol-3-yl]butanoic acid (4d)

The title compound was prepared according to the procedure described for **4b**, using **3d** as a foam (29%, 2 steps): ^{1}H NMR ($CDCl_3$) δ 1.21 (6H, d, J = 7.0 Hz), 2.07 (2H, m), 2.30 (3H, s), 2.39 (2H, t, J = 7.3 Hz), 2.83 (2H, t, J = 7.3 Hz), 3.17 (1H, m), 5.23 (2H, s), 6.49 (1H, dd, J = 1.2, 8.4 Hz), 6.56 (1H, d, J = 8.4 Hz), 6.96 (1H, br s), 7.10–7.14 (2H, m), 7.23 (1H, brd, J = 7.7 Hz), 7.55 (1H, d, J = 7.6 Hz); IR (ATR) 3380, 2959, 1702 cm^{-1}; MS (ESI) m/z 366 $(M+H)^+$, 364 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{23}H_{28}NO_3$ $(M+H)^+$ 366.2064, found 366.2064.

3.1.17. [1-(3-Isopropyl-4-hydroxybenzyl)-2-methyl-1*H*-indol-3-yl]pentanoic acid (4e)

The title compound was prepared according to the procedure described for **4b**, using **3e** as a foam (44%, 2 steps): ^{1}H NMR (DMSO-d_6) δ 1.07 (6H, d, J = 7.0 Hz), 1.45–1.70 (4H, m), 2.22 (2H, t, J = 7.0 Hz), 2.33 (3H, s), 2.69 (2H, t, J = 6.6 Hz), 3.21 (1H, m), 5.23 (2H, s), 6.52 (1H, dd, J = 2.2, 8.4 Hz), 6.63 (1H, d, J = 8.4 Hz), 6.89 (1H, d, J = 1.9 Hz), 6.95 (1H, t, J = 7.0 Hz), 7.01 (1H, t, J = 6.9 Hz), 7.33 (1H, d, J = 8.0 Hz), 7.44 (1H, d, J = 7.7 Hz), 9.09 (1H, br s), 11.8 (1H, br s); IR (ATR) 3394, 2929, 1703 cm^{-1}; MS (ESI) m/z 380 $(M+H)^+$, 378 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{24}H_{30}NO_3$ $(M+H)^+$ 380.2220, found 380.2223.

3.1.18. {[1-(3-Isopropyl-4-hydroxybenzyl)-2-methyl-1*H*-indol-3-yl]acetylamino}acetic acid (5b)

To a solution of **4b** (985 mg, 2.9 mmol) in DMF (75 mL) were added Et_3N (985 μL, 7.0 mmol), HOBt (492 mg, 3.2 mmol), glycine ethyl ester HCl (448 mg, 3.2 mmol), and WSC (671 mg, 3.5 mmol) at room temperature. The mixture was stirred for 48 h and partitioned between water and EtOAc. The organic layers were successively washed with saturated aqueous $NaHCO_3$, 1 M hydrochloric acid, water, and brine, and dried over Na_2SO_4. After evaporation, the residue was triturated with hexane/Et_2O to give an ester intermediate (1.1 g; MS (ESI) m/z 423 $(M+H)^+$, 421 $(M-H)^-$), which was hydrolyzed in a similar manner as described in the preparation of **4b** to give **5b** (1.0 g, 87%) as a white solid: mp 117–120 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (6H, d, J = 6.9 Hz), 2.32 (3H, s), 3.13 (1H, m), 3.24 (2H, d, J = 4.0 Hz), 3.51 (2H, s), 5.24 (2H, s), 6.51 (1H, dd, J = 2.2, 8.4 Hz), 6.64 (1H, d, J = 8.0 Hz), 6.91–7.14 (4H, m), 7.35 (1H, d, J = 8.0 Hz), 7.46 (1H, d, J = 7.7 Hz), 9.32 (1H, br s); IR (ATR) 3302, 2959, 1731, 1651, 1509 cm^{-1}; MS (ESI) m/z 395 $(M+H)^+$, 393 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{23}H_{27}N_2O_4$ $(M+H)^+$ 395.1965, found 395.1954.

3.1.19. 2-[1-(4-hydroxy-3-isopropylbenzyl)-2-methyl-1*H*-indole-3-carboxamido]acetic acid (5a)

The title compound was prepared according to the procedure described for **5b**, using **4a** as a white solid (35%): mp 145–148 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.10 (6H, d, J = 7.0 Hz), 2.32 (3H, s), 3.13 (1H, m), 3.88 (2H, d, J = 5.2 Hz), 5.32 (2H, s), 6.51 (1H, d, J = 8.0 Hz), 6.65 (1H, d, J = 8.0 Hz), 7.03 (1H, s), 7.07–7.18 (2H, m), 7.48 (1H, d, J = 7.3 Hz), 7.62 (1H, br s), 7.86 (1H, d, J = 7.7 Hz), 9.20 (1H, br s); IR (ATR) 3328, 2958, 1721, 1591, 1543 cm^{-1}; MS (ESI) m/z 381 $(M+H)^+$, 379 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{22}H_{25}N_2O_4$ $(M+H)^+$ 381.1809, found 381.1812.

3.1.20. 1-(3-Isopropyl-4-triisopropylsilanyloxybenzyl)-4-nitro-1*H*-indole (7a)

To a suspension of NaH (60% in oil, 59.2 mg, 1.5 mmol) in DMF (1.0 mL) was added dropwise a solution of 4-nitro-1*H*-indole (200 mg, 1.2 mmol) in DMF (1.0 mL) at 5 °C. After stirring at the same temperature for 1 h, a DMF solution (2.0 mL) of **1b** (ca. 1.5 mmol) was added. The resulting mixture was stirred

for an additional 3 h, and the reaction was quenched with ice water, and then extracted with EtOAc. The organic layers were successively washed with water and brine, and dried over Na_2SO_4. After evaporation of the solvent, the resulting residue was purified by flash chromatography on silica gel (hexane/EtOAc = 9/1) to give the title compound (481 mg, 84%) as a yellow solid: mp 98–100 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (18H, d, J = 7.4 Hz), 1.09 (6H, d, J = 6.6 Hz), 1.27 (3H, m), 3.34 (1H, m), 5.29 (2H, s), 6.67 (1H, s), 7.05 (1H, s), 7.26 (3H, m), 7.38 (1H, d, J = 6.2 Hz), 7.66 (1H, d, J = 8.4 Hz), 8.13 (1H, d, J = 8.1 Hz); MS (ESI) m/z 467 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{27}H_{39}N_2O_3Si$ $(M+H)^+$ 467.2724, found 467.2720.

3.1.21. 1-(3-Isopropyl-4-triisopropylsilanyloxybenzyl)-2-methyl-4-nitro-1*H*-indole (7b)

The title compound was prepared according to the procedure described for **7a**, using 2-methyl-4-nitro-1*H*-indole as a yellow solid (45%): mp 116–118 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (18H, d, J = 7.4 Hz), 1.14 (6H, d, J = 7.0 Hz), 1.26 (3H, m), 2.46 (3H, s), 3.32 (1H, m), 5.29 (2H, s), 6.42 (1H, dd, J = 2.2, 8.4 Hz), 6.62 (1H, d, J = 8.1 Hz), 6.94 (1H, d, J = 2.2 Hz), 7.08 (1H, s), 7.13 (1H, t, J = 8.1 Hz), 7.54 (1H, d, J = 8.1 Hz), 8.10 (1H, d, J = 8.0 Hz); MS (ESI) m/z 481 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{28}H_{41}N_2O_3Si$ $(M+H)^+$ 481.2881, found 481.2875.

3.1.22. 1-(3-Isopropyl-4-triisopropylsilanyloxybenzyl)-7-methyl-4-nitro-1*H*-indole (7c)

The title compound was prepared according to the procedure described for **7a**, using 7-methyl-4-nitro-1*H*-indole as a yellow solid (82%): mp 102–104 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (18H, d, J = 6.9 Hz), 1.13 (6H, d, J = 7.4 Hz), 1.26 (3H, m), 2.63 (3H, s), 3.34 (1H, m), 5.55 (2H, s), 6.42 (1H, dd, J = 2.4, 8.3 Hz), 6.65 (1H, d, J = 8.3 Hz), 6.86 (1H, d, J = 2.4 Hz), 6.94 (1H, d, J = 8.1 Hz), 7.29 (1H, d, J = 3.1 Hz), 7.33 (1H, d, J = 3.1 Hz), 8.04 (1H, d, J = 8.1 Hz); MS (ESI) m/z 481 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{28}H_{41}N_2O_3Si$ $(M+H)^+$ 481.2881, found 481.2880.

3.1.23. 1-(3-Isopropyl-4-triisopropylsilanyloxybenzyl)-2-methyl-5-nitro-1*H*-indole (7d)

The title compound was prepared according to the procedure described for **7a**, using 2-methyl-5-nitro-1*H*-indole as a yellow solid (34%): mp 118–120 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (18H, d, J = 7.3 Hz), 1.14 (6H, d, J = 6.9 Hz), 1.26 (3H, m), 2.41 (3H, s), 3.32 (1H, m), 5.26 (2H, s), 6.45 (1H, dd, J = 2.2, 8.5 Hz), 6.48 (1H, s), 6.63 (1H, d, J = 8.0 Hz), 6.92 (1H, d, J = 2.2 Hz), 7.23 (1H, s), 8.02 (1H, dd, J = 2.2, 8.8 Hz), 8.50 (1H, d, J = 2.2 Hz); MS (ESI) m/z 481 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{28}H_{41}N_2O_3Si$ $(M+H)^+$ 481.2881, found 481.2877.

3.1.24. 1-(3-Isopropyl-4-triisopropylsilanyloxybenzyl)-1*H*-indol-4-ylamine (8a)

To a solution of **7a** (311 mg, 0.7 mmol) in THF (5.0 mL) and ethanol (5.0 mL) was added 10% Pd-C (93.3 mg). The mixture was stirred under hydrogen atmosphere at room temperature for 3 h. The Pd catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 1/0–4/1) to give the title compound (257 mg, 82%) as a foam: ^{1}H NMR ($CDCl_3$) δ 1.07 (18H, d, J = 7.3 Hz), 1.15 (6H, d, J = 6.9 Hz), 1.24 (3H, m), 3.33 (1H, m), 3.92 (2H, br s), 5.19 (2H, s), 6.39 (1H, d, J = 7.3 Hz), 6.41 (1H, d, J = 3.3 Hz), 6.64 (1H, d, J = 8.1 Hz), 6.70 (1H, dd, J = 2.2, 8.5 Hz), 6.81 (1H, d, J = 8.0 Hz), 6.98–7.02 (2H, m), 7.05 (1H, s); MS (ESI) m/z 437 $(M+H)^+$, 435 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{27}H_{41}N_2OSi$ $(M+H)^+$ 437.2983, found 437.2984.

3.1.25. 1-(3-Isopropyl-4-triisopropylsilanyloxybenzyl)-2-methyl-1*H*-indol-4-ylamine (8b)

The title compound was prepared according to the procedure described for **8a**, using **7b** as a foam (54%): ^{1}H NMR ($CDCl_3$) δ 1.09 (18H, d, J = 7.3 Hz), 1.16 (6H, d, J = 7.0 Hz), 1.27 (3H, m), 2.36 (3H, s), 3.33 (1H, m), 3.49 (1H, br s), 5.16 (2H, s), 6.21 (1H, s), 6.38 (1H, d, J = 7.3 Hz), 6.48 (1H, dd, J = 2.2, 8.1 Hz), 6.59 (1H, d, J = 8.4 Hz), 6.75 (1H, d, J = 8.1 Hz), 6.94 (1H, t, J = 7.9 Hz), 7.01 (1H, d, J = 1.8 Hz); MS (ESI) m/z 451 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{28}H_{43}N_2OSi$ $(M+H)^+$ 451.3139, found 451.3134.

3.1.26. 1-(3-Isopropyl-4-triisopropylsilanyloxybenzyl)-7-methyl-1*H*-indol-4-ylamine (8c)

The title compound was prepared according to the procedure described for **8a**, using **7c** as a beige solid (71%): mp 121–123 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (18H, d, J = 7.3 Hz), 1.15 (6H, d, J = 6.9 Hz), 1.26 (3H, m), 2.48 (3H, s), 3.33 (1H, m), 3.81 (2H, br s), 5.47 (2H, s), 6.31 (1H, d, J = 7.7 Hz), 6.42 (1H, d, J = 2.3 Hz), 6.47 (1H, dd, J = 2.2, 8.4 Hz), 6.62 (1H, d, J = 8.1 Hz), 6.69 (1H, d, J = 7.7 Hz), 6.94 (1H, d, J = 2.2 Hz), 6.96 (1H, d, J = 2.9 Hz); MS (ESI) m/z 451 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{28}H_{43}N_2OSi$ $(M+H)^+$ 451.3139, found 451.3136.

3.1.27. 1-(3-Isopropyl-4-triisopropylsilanyloxybenzyl)-2-methyl-1*H*-indol-5-ylamine (8d)

The title compound was prepared according to the procedure described for **8a**, using **7d** as a beige solid (74%): mp 105–106 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (18H, d, J = 7.3 Hz), 1.15 (6H, d, J = 6.9 Hz), 1.25 (3H, m), 2.33 (3H, s), 3.32 (1H, m), 3.46 (1H, br s), 5.15 (2H, s), 6.12 (1H, s), 6.44 (1H, dd, J = 2.2, 8.4 Hz), 6.55 (1H, dd, J = 2.2, 8.4 Hz), 6.58 (1H, d, J = 8.4 Hz), 6.87 (1H, d, J = 2.2 Hz), 6.98 (1H, d, J = 2.2 Hz), 7.03 (1H, d, J = 8.7 Hz); MS (ESI) m/z 451 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{28}H_{43}N_2OSi$ $(M+H)^+$ 451.3139, found 451.3134.

3.1.28. Ethyl *N*-[1-(3-isopropyl-4-triisopropylsilanyloxybenzyl)-1*H*-indol-4-yl]oxamate (9a)

A mixture of **8a** (139.0 mg, 0.3 mmol) in diethyl oxalate (3.0 mL) was stirred at 100 °C for 3 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel, (hexane/EtOAc = 1/0–4/1) to give the title compound (126 mg, 74%) as a pale yellow solid: mp 115–116 °C; ^{1}H NMR ($CDCl_3$) δ 1.06 (18H, d, J = 7.3 Hz), 1.13 (3H, m), 1.35 (3H, t, J = 7.3 Hz), 3.31 (1H, m), 4.33 (2H, q, J = 7.3 Hz), 5.21 (2H, s), 6.51 (1H, d, J = 3.3 Hz), 6.64 (1H, d, J = 8.5 Hz), 6.68 (1H, dd, J = 2.2, 8.4 Hz), 7.01 (1H, d, J = 2.2 Hz), 7.11 (1H, d, J = 3.3 Hz), 7.17 (1H, d), 7.90 (1H, m), 9.16 (1H, br s); MS (ESI) m/z 537 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{31}H_{45}N_2O_4Si$ $(M+H)^+$ 537.3143, found 537.3152.

3.1.29. Ethyl *N*-[1-(3-isopropyl-4-triisopropylsilanyloxybenzyl)-2-methyl-1*H*-indol-4-yl]oxamate (9b)

The title compound was prepared according to the procedure described for **9a**, using **8b** as a foam (81%): ^{1}H NMR ($CDCl_3$) δ 1.07 (18H, d, J = 7.3 Hz), 1.15 (6H, d, J = 7.3 Hz), 1.26 (3H, m), 1.46 (3H, t, J = 7.1 Hz), 2.40 (3H, s), 3.33 (1H, m), 4.45 (2H, q, J = 7.1 Hz), 5.23 (2H, s), 6.33 (1H, s), 6.45 (1H, dd, J = 2.4, 8.3 Hz), 6.61 (1H, d, J = 8.3 Hz), 6.96 (1H, d, J = 2.4 Hz), 7.13 (2H, m), 7.86–7.90 (1H, m), 9.13(1H, s); MS (ESI) m/z 551 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{32}H_{47}N_2O_4Si$ $(M+H)^+$ 551.3300, found 551.3301.

3.1.30. Ethyl *N*-[1-(3-isopropyl-4-triisopropylsilanyloxybenzyl)-7-methyl-1*H*-indol-4-yl]oxamate (9c)

The title compound was prepared according to the procedure described for **9a**, using **8c** as a yellow solid (85%): mp

136–138 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (18H, d, J = 7.3 Hz), 1.15 (6H, d, J = 7.0 Hz), 1.25 (3H, m), 1.46 (3H, t, J = 7.1 Hz), 2.55 (3H, s), 3.32 (1H, m), 4.45 (2H, q, J = 7.1 Hz), 5.23 (2H, s), 6.33 (1H, s), 6.44 (1H, dd, J = 2.2, 8.4 Hz), 6.61 (1H, d, J = 8.1 Hz), 6.96 (1H, d, J = 2.2 Hz), 7.12–7.15 (2H, m), 7.91 (1H, d, J = 2.2 Hz), 9.14 (1H, s); MS (ESI) m/z 551 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{32}H_{47}N_2O_4Si$ $(M+H)^+$ 551.3300, found 551.3301.

3.1.31. Ethyl *N*-[1-(3-isopropyl-4-triisopropylsilanyloxybenzyl)-2-methyl-1*H*-indol-5-yl]oxamate (9d)

The title compound was prepared according to the procedure described for **9a**, using **8d** as a white solid (75%): mp 150–151 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (18H, d, J = 7.4 Hz), 1.15 (6H, d, J = 7.0 Hz), 1.26 (3H, m), 1.42 (3H, t, J = 7.1 Hz), 2.52 (3H, s), 3.31 (1H, m), 4.42 (2H, q, J = 7.1 Hz), 5.20 (2H, s), 6.31 (1H, s), 6.43 (1H, dd, J = 2.2, 8.4 Hz), 6.60 (1H, d, J = 8.4 Hz), 6.95 (1H, d, J = 2.2 Hz), 7.20 (1H, d, J = 8.8 Hz), 7.26 (1H, dd, J = 2.2, 8.8 Hz), 7.91 (1H, d, J = 2.2 Hz), 8.89 (1H, br s); MS (ESI) m/z 551 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{32}H_{47}N_2O_4Si$ $(M+H)^+$ 551.3300, found 551.3303.

3.1.32. Ethyl *N*-[1-(3-isopropyl-4-triisopropylsilanyloxybenzyl)-2-methyl-1*H*-indol-4-yl]malonamate (9b')

Ethyl malonyl chloride (248 μL, 1.9 mmol) was added to a mixture of **8b** (829.8 mg, 1.8 mmol) and Et_3N (284 μL, 2.0 mmol) in CH_2Cl_2 (40 mL) at 0 °C. The mixture was stirred at room temperature for 30 min and diluted with EtOAc. The resulting mixture was washed with water and brine, and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash chromatography on silica gel (hexane/EtOAc = 4/1) to give the title compound (737.6 mg, 71%) as a foam: ^{1}H NMR ($CDCl_3$) δ 1.07 (18H, d, J = 7.0 Hz), 1.15 (6H, d, J = 6.8 Hz), 1.25 (3H, m), 1.35 (3H, t, J = 7.1 Hz), 2.39 (3H, s), 3.31 (1H, m), 3.55 (2H, s), 4.31 (2H, q, J = 7.1 Hz), 5.21 (2H, s), 6.37 (1H, s), 6.45 (1H, d, J = 8.4 Hz), 6.59 (1H, d, J = 8.4 Hz), 6.97 (1H, s), 7.05–7.11 (2H, m), 7.82 (1H, d, J = 7.3 Hz), 9.66 (1H, s); MS (ESI) m/z 565 $(M+H)^+$; HRMS (ESI) m/z calcd for $C_{33}H_{49}N_2O_4Si$ $(M+H)^+$ 565.3456, found 565.3455.

3.1.33. Ethyl *N*-[1-(3-isopropyl-4-triisopropylsilanyloxybenzyl)-7-methyl-1*H*-indol-4-yl]malonamate (9c')

The title compound was prepared according to the procedure described for **9b'**, using **8c** as a white solid (62%): mp 118–120 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (18H, d, J = 4.9 Hz), 1.14 (6H, d, J = 7.0 Hz), 1.26 (3H, m), 1.35 (3H, m), 2.53 (3H, s), 3.33 (1H, m), 3.55 (2H, s), 4.30 (2H, q, J = 7.3 Hz), 5.50 (2H, s), 6.42 (1H, dd, J = 2.2, 8.4 Hz), 6.59–6.62 (2H, m), 6.86 (1H, d, J = 7.6 Hz), 6.91 (1H, d, J = 2.2 Hz), 7.05 (1H, d, J = 3.3 Hz), 7.73 (1H, d, J = 7.7 Hz), 9.69 (1H, s)); MS (ESI) m/z 565 $(M+H)^+$, 563 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{33}H_{49}N_2O_4Si$ $(M+H)^+$ 565.3456, found 565.3455.

3.1.34. Ethyl *N*-[1-(4-hydroxy-3-isopropylbenzyl)-1*H*-indol-4-yl]oxamate (10a)

A mixture of **9a** (112.0 mg, 0.2 mmol) and tetrabutylammonium fluoride (1 M in THF, 230 μL, 0.2 mmol) in THF (1.0 mL) was stirred at room temperature for 30 min. The reaction mixture was then diluted with EtOAc, successively washed with water and brine, and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash chromatography on silica gel hexane/EtOAc = 1/0–4/1) to give the title compound (33.1 mg, 42%) as a white solid: mp 145–146 °C; ^{1}H NMR ($CDCl_3$) δ 1.20 (6H, d, J = 6.9 Hz), 1.45 (3H, t, J = 7.3 Hz), 3.17 (1H, m), 4.45 (2H, q, J = 7.3 Hz), 5.24 (2H, s), 6.54 (1H, d, J = 3.3 Hz), 6.65 (1H, d, J = 4.5 Hz), 6.74 (1H, dd, J = 2.2, 10.2 Hz), 7.04 (1H, d, J = 2.2 Hz), 7.13 (1H, d, J = 3.3 Hz), 7.19–7.20 (2H, m), 7.92 (1H, m), 9.18 (1H, br s); IR (ATR) 3359, 2960, 1761, 1692, 1536 cm^{-1}; MS (ESI) m/z 381$(M+H)^+$, 379 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{22}H_{25}N_2O_4$ $(M+H)^+$ 381.1809, found 381.1809.

3.1.35. Ethyl *N*-[1-(4-hydroxy-3-isopropylbenzyl)-2-methyl-1*H*-indol-4-yl]oxamate (10b)

The title compound was prepared according to the procedure described for **10a**, using **9b** as a yellow solid (23%): mp 143–145 °C; ^{1}H NMR ($CDCl_3$) δ 1.19 (6H, d, J = 6.9 Hz), 1.46 (3H, t, J = 6.9 Hz), 2.40 (3H, s), 3.15 (1H, m), 4.45 (2H, q, J = 6.9 Hz), 4.66 (1H, s), 5.24 (2H, s), 6.34 (1H, s), 6.50 (1H, dd, J = 2.2, 8.1 Hz), 6.59 (1H, d, J = 8.1 Hz), 6.95 (1H, d, J = 2.2 Hz), 7.09–7.15 (2H, m), 7.87 (1H, dd, J = 1.8, 6.6 Hz), 9.12 (1H, br s); IR (ATR) 3339, 2959, 1687, 1538 cm^{-1}; MS (ESI) m/z 395 $(M+H)^+$, 393 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{23}H_{27}N_2O_4$ $(M+H)^+$ 395.1965, found 395.1954.

3.1.36. Ethyl *N*-[1-(4-hydroxy-3-isopropylbenzyl)-7-methyl-1*H*-indol-4-yl]oxamate (10c)

The title compound was prepared according to the procedure described for **10a**, using **9c** as a yellow solid (12%): mp 138–139 °C; ^{1}H NMR ($CDCl_3$) δ 1.19 (6H, d, J = 7.0 Hz), 1.46 (3H, t, J = 7.1 Hz), 2.55 (3H, s), 3.16 (1H, m), 4.45 (2H, q, J = 7.1 Hz), 4.69 (1H, s), 5.51 (2H, s), 6.48 (1H, dd, J = 2.2, 8.1 Hz), 6.54 (1H, d, J = 3.3 Hz), 6.62 (1H, d, J = 8.1 Hz), 6.88 (1H, d, J = 2.6 Hz), 6.90 (1H, d, J = 8.4 Hz), 7.08 (1H, d, J = 3.3 Hz), 7.80 (1H, d, J = 7.7 Hz), 9.14 (1H, br s); IR (ATR) 3316, 2958, 1764, 1682, 1541 cm^{-1}; MS (ESI) m/z 395 $(M+H)^+$, 393 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{23}H_{27}N_2O_4$ $(M+H)^+$ 395.1965, found 395.1956.

3.1.37. Ethyl *N*-[1-(4-hydroxy-3-isopropylbenzyl)-2-methyl-1*H*-indol-4-yl]malonamate (10b′)

The title compound was prepared according to the procedure described for **10a**, using **9b** as a foam (88%): ^{1}H NMR ($CDCl_3$) δ 1.20 (6H, d, J = 7.0 Hz), 1.35 (3H, t, J = 7.4 Hz), 2.39 (3H, s), 3.16 (1H, m), 3.55 (2H, s), 4.30 (2H, q, J = 7.4 Hz), 4.83 (1H, s), 5.22 (2H, s), 6.37 (1H, s), 6.49 (1H, dd, J = 2.2, 8.0 Hz), 6.58 (1H, d, J = 8.4 Hz), 6.96 (1H, d, J = 2.2 Hz), 7.02–7.11 (2H, m), 7.81 (1H, d, J = 6.6 Hz), 9.68 (1H, s); IR (ATR) 3312, 2960, 1717, 1659, 1551 cm^{-1}; MS (ESI) m/z 409 $(M+H)^+$, 407 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{24}H_{29}N_2O_4$ $(M+H)^+$ 409.2122, found 409.2113.

3.1.38. Ethyl *N*-[1-(4-hydroxy-3-isopropylbenzyl)-7-methyl-1*H*-indol-4-yl]malonamate (10c′)

The title compound was prepared according to the procedure described for **10a**, using **9c** as a white solid (87%): mp 141–142 °C; ^{1}H NMR ($CDCl_3$) δ 1.19 (6H, d, J = 7.0 Hz), 1.34 (3H, t, J = 7.1 Hz), 2.52 (3H, s), 3.16 (1H, m), 3.55 (2H, s), 4.30 (2H, q, J = 7.1 Hz), 4.93 (1H, s), 5.49 (2H, s), 6.46 (1H, dd, J = 2.0, 8.2 Hz), 6.59 (1H, d, J = 3.3 Hz), 6.60 (1H, d, J = 8.1 Hz), 6.85 (1H, d, J = 7.7 Hz), 6.90 (1H, d, J = 1.2 Hz), 7.04 (1H, d, J = 3.3 Hz), 7.72 (1H, d, J = 8.1 Hz), 9.70 (1H, s); IR (ATR) 3262, 2957, 1719, 1652, 1621, 1556 cm^{-1}; MS (ESI) m/z 409 $(M+H)^+$, 407 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{24}H_{29}N_2O_4$ $(M+H)^+$ 409.2122, found 409.2120.

3.1.39. Ethyl *N*-[1-(4-hydroxy-3-isopropylbenzyl)-2-methyl-1*H*-indol-5-yl]oxamate (10d)

The title compound was prepared according to the procedure described for **10a**, using **9c** as a pale yellow solid (30%): mp 126–129 °C; ^{1}H NMR ($CDCl_3$) δ 1.18 (6H, d, J = 7.0 Hz), 1.41 (3H, t, J = 7.0 Hz), 2.35 (3H, s), 3.18 (1H, m), 4.40 (2H, q, J = 7.0 Hz), 5.45 (2H, s), 5.57 (1H, s), 6.28 (1H, s), 6.45 (1H, dd, J = 1.9, 8.1 Hz), 6.61 (1H, d, J = 8.5 Hz), 6.93 (1H, d, J = 1.9 Hz), 7.15 (1H, d, J = 8.8 Hz), 7.24 (1H, dd, J = 2.2, 9.1 Hz), 7.89 (1H, s), 8.92 (1H, br s); IR (ATR) 1736, 1683 cm^{-1}; MS (ESI) m/z 395 $(M+H)^+$, 393 $(M-H)^-$; HRMS (ESI) m/z calcd for $C_{23}H_{27}N_2O_4$ $(M+H)^+$ 395.1965, found 395.1959.

3.1.40. *N*-[1-(4-Hydroxy-3-isopropylbenzyl)-1*H*-indol-4-yl]oxamic acid (11a)

A mixture of **10a** (33.0 mg, 0.1 mmol) and 1 M NaOH (173 μL, 0.2 mmol) in EtOH (0.5 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was taken up with water, acidified with 1 M hydrochloric acid, and extracted with EtOAc. The organic layer was washed with water and brine, and dried over Na_2SO_4. After evaporation of the solvent, the residue was crystallized with Et_2O/hexane to give the title compound (22 mg, quant.) as an off-white solid: mp 153–155 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.11 (6H, d, J = 6.9 Hz), 3.12 (1H, m), 5.27 (2H, s), 6.56 (1H, d, J = 3.0 Hz), 6.67 (1H, d, J = 8.4 Hz), 6.81 (1H, dd, J = 1.2, 8.4 Hz), 7.09 (1H, t, J = 7.7 Hz), 7.13 (1H, d, J = 1.2 Hz), 7.36 (1H, d, J = 8.1 Hz), 7.42 (1H, d, J = 7.7 Hz), 7.44 (1H, d, J = 3.0 Hz), 9.27 (1H, s), 10.35 (1H, s); IR (ATR) 1748, 1691 cm^{-1}; MS (ESI) m/z 353 (M+H)$^+$, 351 (M−H)$^-$; HRMS (ESI) m/z calcd for $C_{20}H_{21}N_2O_4$ (M+H)$^+$ 353.1496, found 353.1498.

3.1.41. *N*-[1-(4-Hydroxy-3-isopropylbenzyl)-2-methyl-1*H*-indol-4-yl]oxamic acid (11b)

The title compound was prepared according to the procedure described for **11a**, using **10b** as a foam (quant.): ^{1}H NMR (DMSO-d_6) δ 1.09 (6H, d, J = 6.9 Hz), 2.37 (3H, s), 3.13 (1H, m), 5.25 (2H, s), 6.34 (1H, s), 6.53 (1H, dd, J = 2.2, 8.4 Hz), 6.64 (1H, d, J = 8.4 Hz), 6.98 (1H, d, J = 2.2 Hz), 7.01 (1H, t, J = 7.7 Hz), 7.26 (1H, d, J = 8.1 Hz), 7.36 (1H, d, J = 7.7 Hz), 9.15 (1H, s), 10.19 (1H, s); IR (ATR) 1741, 1685, 1552 cm^{-1}; MS (ESI) m/z 367 (M+H)$^+$, 365 (M−H)$^-$; HRMS (ESI) m/z calcd for $C_{21}H_{23}N_2O_4$ (M+H)$^+$ 367.1652, found 367.1656.

3.1.42. *N*-[1-(4-Hydroxy-3-isopropylbenzyl)-7-methyl-1*H*-indol-4-yl]oxamic acid (11c)

The title compound was prepared according to the procedure described for **11a**, using **10b** as a yellow solid (95%): mp 145–146 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.08 (6H, d, J = 6.6 Hz), 2.46 (3H, s), 3.13 (1H, m), 5.43 (2H, s), 6.40 (1H, dd, J = 2.4, 8.2 Hz), 6.56 (1H, d, J = 3.3 Hz), 6.65 (1H, d, J = 8.1 Hz), 6.78 (1H, d, J = 8.1 Hz), 6.87 (1H, d, J = 2.2 Hz), 7.30 (1H, d, J = 7.3 Hz), 7.32 (1H, d, J = 3.3 Hz), 9.22 (1H, s), 10.15 (1H, s); IR (ATR) 1752, 1692, 1548 cm^{-1}; MS (ESI) m/z 367 (M+H)$^+$, 365 (M−H)$^-$; HRMS (ESI) m/z calcd for $C_{21}H_{23}N_2O_4$ (M+H)$^+$ 367.1652, found 367.1654.

3.1.43. *N*-[1-(4-Hydroxy-3-isopropylbenzyl)-2-methyl-1*H*-indol-4-yl]malonic acid (11b′)

The title compound was prepared according to the procedure described for **11a**, using **10b'** as a beige solid (quant.): mp 139–141 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.09 (6H, d, J = 7.0 Hz), 2.37 (3H, s), 2.88 (2H, s), 3.12 (1H, m), 5.23 (2H, s), 6.39 (1H, s), 6.49 (1H, dd, J = 2.0, 8.2 Hz), 6.63 (1H, d, J = 8.1 Hz), 6.91 (1H, t, J = 7.9 Hz), 6.95 (1H, s), 7.03 (1H, d, J = 8.1 Hz), 7.78 (1H, d, J = 7.7 Hz), 9.14 (1H, s); IR (ATR) 1761, 1692, 1574 cm^{-1}; MS (ESI) m/z 381 (M+H)$^+$, 379 (M−H)$^-$; HRMS (ESI) m/z calcd for $C_{22}H_{25}N_2O_4$ (M+H)$^+$ 381.1809, found 381.1813.

3.1.44. *N*-[1-(4-Hydroxy-3-isopropylbenzyl)-7-methyl-1*H*-indol-4-yl]malonic acid (11c′)

The title compound was prepared according to the procedure described for **11a**, using **10c′** as a white solid (quant.): mp 157–158 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.07 (6H, d, J = 6.6 Hz), 2.46 (3H, s), 3.13 (1H, m), 3.48 (2H, s), 5.48 (2H, s), 6.39 (1H, dd, J = 2.2, 8.4 Hz), 6.54 (1H, d, J = 2.2 Hz), 6.64 (1H, d, J = 8.4 Hz), 6.82 (1H, s), 6.86 (1H, s), 7.31 (1H, d, J = 2.9 Hz), 7.74 (1H, s), 9.14 (1H, s), 9.68 (1H, s); IR (ATR) 1740, 1609, 1556 cm^{-1}; MS (ESI) m/z 381 (M+H)$^+$, 379 (M−H)$^-$; HRMS (ESI) m/z calcd for $C_{22}H_{25}N_2O_4$ (M+H)$^+$ 381.1809, found 381.1812.

3.1.45. *N*-[1-(4-Hydroxy-3-isopropylbenzyl)-2-methyl-1*H*-indol-5-yl]oxamic acid (11d)

The title compound was prepared according to the procedure described for **11a**, using **10d** as a foam (quant.): ^{1}H NMR (DMSO-d_6) δ 1.08 (6H, d, J = 7.0 Hz), 2.35 (3H, s), 3.12 (1H, m), 5.22 (2H, s), 6.23 (1H, s), 6.52 (1H, dd, J = 1.5, 8.0 Hz), 6.64 (1H, d, J = 8.0 Hz), 6.94 (1H, d, J = 1.5 Hz), 7.33 (2H, s), 7.88 (1H, s), 9.14 (1H, s), 10.39 (1H, s); MS (ESI) m/z 367 (M+H)$^+$, 365 (M−H)$^-$; HRMS (ESI) m/z calcd for $C_{21}H_{23}N_2O_4$ (M+H)$^+$ 367.1652, found 367.1655.

3.2. Biology

3.2.1. TH receptor binding assay

Human TRα1 and TRβ1 ligand binding domains were expressed in Escherichia coli as a fused protein (recombinant human TRα1 or recombinant human TRβ1) having TRα1 and TRβ1 ligand binding domains at the C-terminus of His-Patch Thioredoxin using the His-Patch Thioredoxin fusing protein expressing system (Invitorogen). The fused protein was produced by culturing Escherichia coli (JM 109) transformed with the prepared plasmid, and adding IPTG during proliferation to induce expression. ^{125}I-T_3 (Perkin Elmer, Cat. No. NEX110X, AS62450) was added to the binding solution (0.16 nM) containing recombinant human TRα1 or recombinant human TRβ1, and the mixture was allowed to stand at room temperature for 1–3 h to form a complex of recombinant human TRβ1 or recombinant human TRα1 and ^{125}I-T_3. The complex solution (60 μL) was then added to all wells of a 96-well plate and supplemented with a dilution series solution (90 μL), made so that the test compound or non-labeled T_3 represents 1.67-fold the final concentration in the binding solution. The plate was incubated at 25 °C for 2–3 h, so that the formation of a complex with ^{125}I-T_3 may be inhibited by a test compound or non-labeled T_3. Separately, SephadexG25 (80 μL) was added to each well of a MultiScreen-HV (MILLIPORE, Cat. No. MANVN4550, Lot. No. F4SN86613) plate and supplemented with sufficient amount of the binding solution to form a gel. The mixture was then centrifuged at 600*g* for 1 min to prepare a column of SephadexG25. Twenty-five μL of the mixed solution containing the test compound or non-labeled T_3 was passed through the SephadexG25 column and immediately centrifuged at 600*g* for 1 min. Similarly, 25 μL of the untreated binding solution was passed through the SephadexG25 column and immediately centrifuged at 600*g* for 1 min. The separated solution containing ^{125}I-T_3 complex and passed through the column twice was recovered into an Isoplate (PS) (Perkin Elmer, Cat. No. 1450–514). Optiphase Super Mix (200 μL, Perkin Elmer, Cat. No. 1200–439) was then added to each well containing the separated solution, and the radioactivity was measured with 1450 MICROBETA TRILUX (Perkin Elmer). The amount of each recombinant human TRα1 or recombinant human TRβ1 complex and ^{125}I-T_3 remaining at each concentration of test compound or T_3 (i.e., remaining complex amount) was obtained by conversion of the value acquired by subtracting the remaining radioactivity from the measured radioactivity. Binding was calculated by the following equation:Binding rate = remaining complex amount/total complex amount

Results are reported as mean of the binding rates × 100 (IC_{50} values) obtained from four separate experiments. IC_{50} values were obtained by linear approximation using the binding rate and a logarithmic value of each test compound or T_3 concentration, and by determining the concentration at binding rate = 0.5. For linear approximation, only concentrations of test compound or T_3 with a binding rate of 0.1–0.9 were used.

3.2.2. Reporter gene assay

Cells were seeded in 96-well culture plates and grown in Dulbecco's Modified Eagle Medium (DMEM, Sigma, Japan) containing 10% FBS (Sigma, Japan) and an appropriate amount of penicillin/

streptomycin (Sigma, Japan) for 24 h at 37 °C (5% CO_2). The following day, a plasmid was added to DMEM containing 6% FuGENE, and the solution was 15 min later diluted with 20-fold volume of DMEM containing 10% FBS. The cells were then transfected with the prepared solution and incubated for 24 h at 37 °C (5% CO_2). The supernatant was removed and replaced with a solution of T_3 or a test compound in DMEM containing 10% FBS at the required concentrations, and the cells were incubated for 8–10 h at 37 °C (5% CO_2). SEAP activity was measured by reading *p*–nitrophenol levels generated from 4-nitrophenyl phosphate with a Tecan Spectra Fluor Plus reader (Tecan Group Ltd, Männedorf, Switzerland). The assay mixtures contained 10 μL of the supernatant and 100 μL of the medium (1 mM $MgCl_2$, 0.1 M Na_2CO_3, pH 9.8 buffer, 2 mg/mL of *p*-nitrophenyl phosphate disodium). After a 30 min-incubation at room temperature, the reaction was stopped with 100 μL of 0.1 N NaOH solution containing 40 mM EDTA, and the absorbance was measured at 405 nm.

Results are reported as mean of the test compound concentration that produces half-maximal effect (EC_{50} values) obtained from four separate experiments. EC_{50} values were calculated from maximal effects of T_3 as 100%, by linear approximation using the activity rate, and a logarithmic value of each concentration of test compound or T_3, and by determining the concentration at EC_{50} = 50%. For linear approximation, only concentrations of test compound or T_3 with a binding rate of 0.1–0.9 were used.

3.2.3. Rat model

Animals care and experimental procedures were approved by the Animal Experimental Committee of Sanwa Kagaku Kenkyusho Co., Ltd. Male Sprague Dawley rats (Charles River, Kanagawa, Japan) were fed a diet containing 1.5% cholesterol and 0.5% cholic acid (Oriental Yeast, Tokyo, Japan) for 1 week, after which they were divided into 6 groups to be orally (gavage) treated once daily for 1 week with **11b′** (30 and 150 mg/kg/day, $n = 13$), **11c′** (6 and 150 mg/kg/day, $n = 13$), T_3 as a positive control (50 μg/kg/day, $n = 13$), or the vehicle (5% tragacanth, Wako, Osaka), or to be left untreated (control). Cholesterol-feeding was continued throughout the study. After 3–6 h of the final treatment, blood pressure and heart rate were measured according to the method of Tail Cuff et al. Then, the animals were sacrificed, and blood was collected from the inferior vena cava and analyzed for lipids, TSH, T_4, and T_3. Plasma total cholesterol, free cholesterol, LDL-cholesterol, free fatty acid, and triglyceride levels were measured by enzyme assays, and HDL-cholesterol level was directly measured with a chemical assay kit. TSH, T_4, and T_3 levels were determined by a chemiluminescent immunoassay (CLIA).

3.3. Statistical analysis

Differences between animal groups were analyzed by Student *t*-test. $P < 0.05$ was assumed significant. Data are given as the mean ± SEM.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.11.001.

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