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Design, synthesis, and structure–activity relationships of novel tetracyclic compounds as peripheral benzodiazepine receptor ligands ☆

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Abstract—The peripheral benzodiazepine receptor (PBR) is pharmacologically distinct from the central benzodiazepine receptor (CBR) and has been identified in a wide range of peripheral tissues as well as in the central nervous system. Although numerous studies have been performed of it, the physiological roles and functions of the PBR are still unclear. In the present study, in exploring new types of ligands for PBR, we found that a new series of compounds having a tetracyclic ring system, which were designed from FGIN-1-27, exhibited high affinities for PBR. We prepared and evaluated them for PBR affinities. The results of binding tests showed that **12e** and **12f** were the most potent PBR ligands among them (**12e**: $IC_{50} = 0.44$ nM, **12f**: $IC_{50} = 0.37$ nM). In this paper, we present the design, synthesis, and structure–activity relationships (SARs) of novel tetracyclic compounds.

1. Introduction

Benzodiazepine receptors can be subdivided into two types: central benzodiazepine receptors (CBR) and peripheral benzodiazepine receptors (PBR). The CBR is found only on neurons in the central nervous system,[1,2] coupled with the γ-aminobutyric acid $(GABA)_A$ receptor,[3] and mediates the classical pharmacological properties, that is, anxiolytic, anticonvulsant, sedative, and muscle relaxant, of the widely used benzodiazepines.[2] The PBR is a high-affinity binding site for diazepam in rat kidney[4] and is pharmacologically different from the CBR. The PBR has been detected in the outer mitochondrial membranes in several peripheral tissues[5,6] such as steroidogenic organs and blood cells.[7] The later study demonstrated PBR in the central nervous system.[8,9] It regulates translocation of cholesterol from the outer to the inner mitochondrial membranes[10,11] and is involved in numerous functions including biosynthesis of neurosteroids[12,13] such as pregnenolone sulfate, 3α-hydroxy-5α-pregnan-20-one, and 3α,21-dihydroxy-5α-pregnan-20-one. Neurosteroids modulate neurotransmitter-gated ion channel activity at $GABA_A$ receptors[14] and *N*-methyl-D-aspartate receptors,[15] an event which results in indirect modulation of GABAergic and glutamatergic transmission.

Several specific or nonspecific PBR ligands have been reported (Chart 1). Compound **1** (Ro5-4864),[16] 4′-chlorodiazepam, exhibits high affinity for the PBR and very low affinity for the CBR. Compound **2** (PK11195)[17,18] is an isoquinoline derivative and currently the most widely used specific probe for peripheral benzodiazepine receptors. Compound **3** (FGIN-1-27)[19,20] is a 2-aryl-3-indoleacetamide derivative and exhibits high affinity for the PBR with high selectivity over the CBR. Compound

Keywords: PBR (peripheral benzodiazepine receptor); Tetracyclic ring; SAR; PBR ligands.
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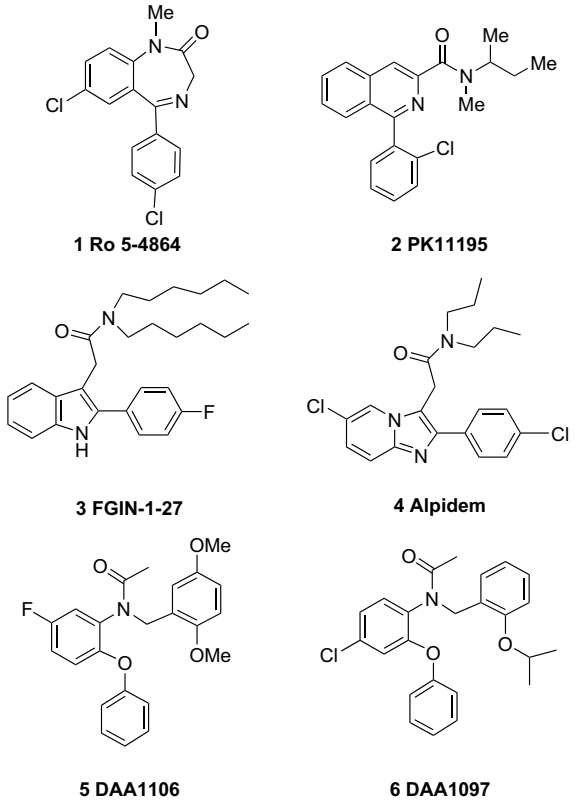


Chart 1.

4 (alpidem)[21] has an imidazopyridine skeleton in its structure and binds with high affinity to both PBR and CBR.

We have recently reported a series of potent and selective PBR ligands, for example, **5** (DAA1106)[22–24] and **6** (DAA1097)[22,23] designed from **1** (Ro5-4864) by opening the diazepine ring. In exploring further diverse PBR ligands, we have found novel potent PBR ligands having tetracyclic systems, designed from **3** (FGIN-1-27). In this paper, we present the design, synthesis, and structure–activity relationships of the new tetracyclic compounds.

2. Chemistry

Compounds **12**, **13**, **14**, and **16**, tetracyclic compounds having a thioether, were synthesized using the methods shown in Scheme 1. 4-Oxo-thiochroman-2-carboxylic acid **8** was obtained by reacting benzenethiol with furan-2,5-dione in the presence of Et_3N and with subsequent treatment with $AlCl_3$. Next, Fischer indolization was performed by H_2SO_4 in EtOH to yield ethylester **9**. Hydrolysis of the ester group and condensation with amines provided **12a**. Compound **12a** was also obtained by Fischer indolization of amide **11**, which had been synthesized by coupling carboxylic acid **8** with dihexylamine. Conversion of the thioether group in **12a** to the sulfonyl group was achieved by mCPBA. Treatment of **12a** with NaH and alkylhalide provided *N*-alkylated **14a** and **14b**. Compound **14c** was prepared by addition of a 4-chlorotetramethylene group to **12a** and followed by the substitution of the chloro group with dimethylamine. Carboxylic acid **15** was obtained from thiophenol **7** and pent-2-enedioic acid using the same method as for synthesis of **8** from **7**. Condensation of the carboxyl acid in **15** with amine and Fischer indolization under the same condition as used in the synthesis of **12a** from **11** gave acetamide derivative **16.**

The method of synthesis of **19** is shown in Scheme 2. Commercially available 2-benzylsuccinic acid **17** was used as a starting material. Stirring **17** in concd H_2SO_4 at room temperature for 9 h provided **18** in 45% yield.[25] Compound **19** was obtained from **18** using the same method as for synthesis of **16** from **15** in Scheme 1.

Synthesis of benzimidazolyl acetic amide **23a** was accomplished in three steps using benzene-1,2-diamine **20** as a starting material (Scheme 3). Heating **20** in polyphosphoric acid at 250 °C, followed by alkylation with bromoacetic acid using KOH as a base in DMSO, provided carboxylic acid **22**. Coupling with the corresponding amine via a mixed acid anhydride gave **23a**.

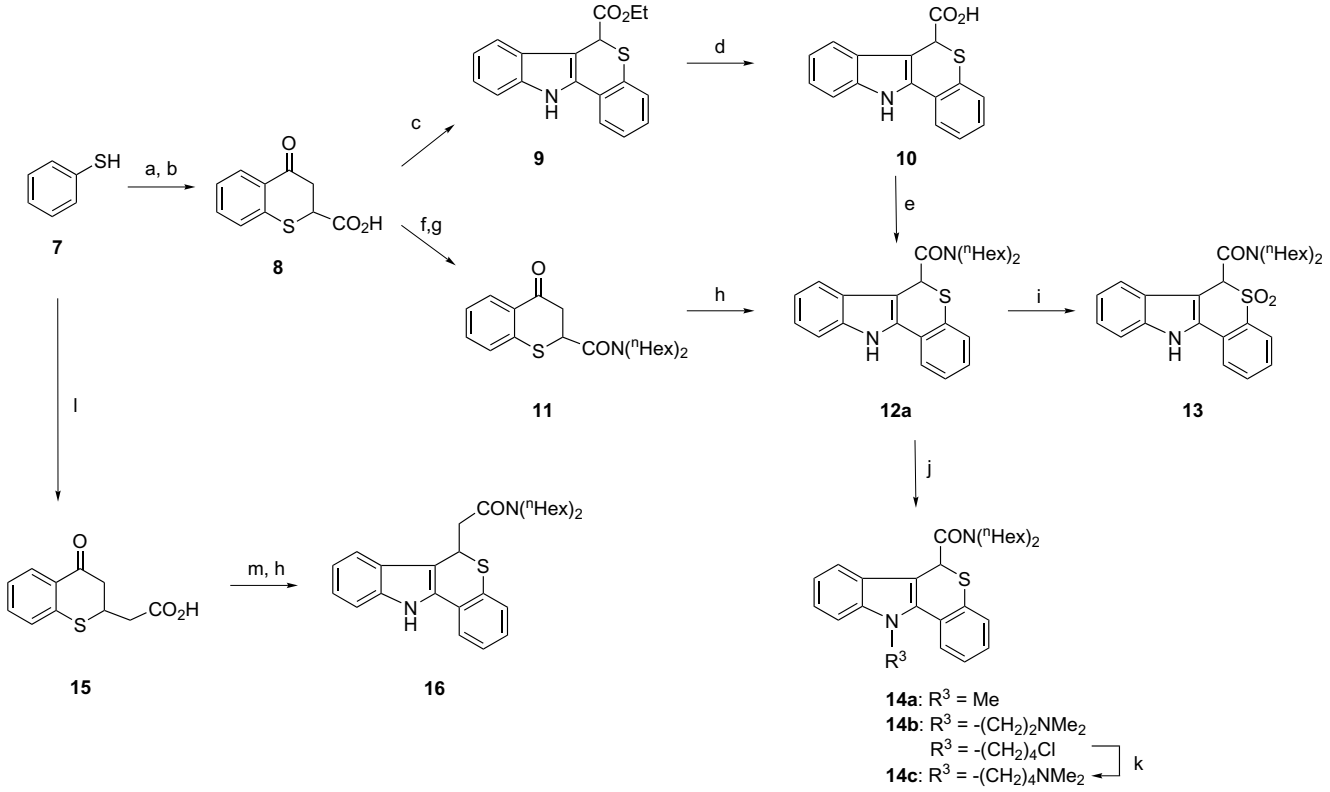
Preparation of **28**, a benzimidazolyl propionyl amide derivative, is shown in Scheme 4. Substitution reaction of 2-fluoronitrobenzene **24** with β-alaninamide **25** was performed in the presence of K_2CO_3 in DMF. Treatment of **26** with benzoyl chloride provided **27**. Reduction of the nitro group in **27** with iron powder was performed in acetic acid. Under the conditions, an imidazole ring was formed to provide **28** in good yield.

Synthesis of compound **34a**, a benzoimidazole derivative having a tetracyclic system, is shown in Scheme 5. Treatment of 2-cyanobenzyl bromide **29** and Boc-aminomalonic acid diethyl ester **30** with sodium ethoxide in ethanol provided **31**. Under the conditions for hydrolysis of both ester groups with NaOH, one of the carboxyl groups was removed. The resulting monocarboxylic acid was coupled with amine to give 2-cyanophenylalaninamide **32**. After removal of the Boc group, heating with 2-fluoronitrobenzene in the presence of K_2CO_3 in DMF gave **33**. Hydrogenation of the nitro group with PtO_2 as a catalyst followed by treatment with HCl gas in EtOH provided compound **34a**.

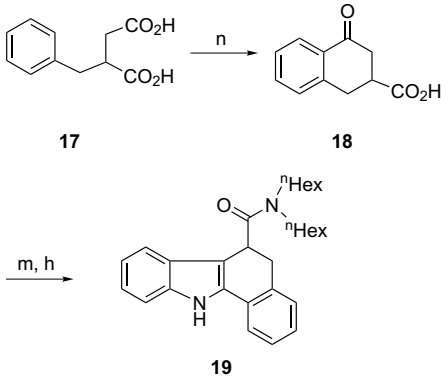
3. Results and discussion

Compounds **1**, **2**, **3**, **12a**–**12m**, **13a**–**13c**, **14a**–**14c**, **16**, **19**, **23a**, **23b**, **28**, and **34a**–**34c** were evaluated for binding affinities for PBR in mitochondria prepared from rat cerebral cortex against radioligand [^{3}H]PK11195,[19] and the obtained IC_{50} values are shown in Tables 1–3.

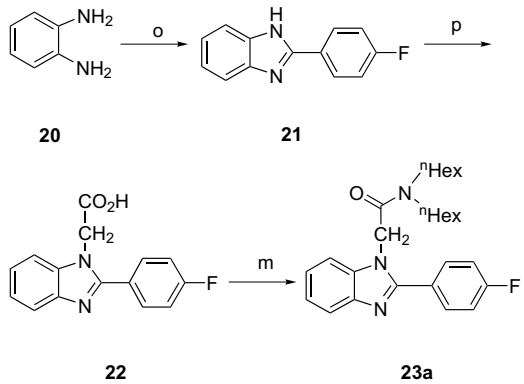
We designed a new tetracyclic compound from FGIN-1-27 (**3**) by closing the ring and accomplished the synthesis shown in Scheme 1. Compound **12a** exhibited high



Scheme 1. Reagents and conditions: (a) furan-2,5-dione, Et_3N, toluene; (b) $AlCl_3$, CH_2Cl_2; (c) $PhNHNH_2$, H_2SO_4, EtOH; (d) KOH, EtOH, H_2O; (e) *n*-Hex_2NH, EDC·HCl, HOBt, CH_2Cl_2; (f) $SOCl_2$, PhH; (g) *n*-Hex_2NH, Et_3N, CH_2Cl_2; (h) $PhNHNH_2$, $ZnCl_2$, heat; (i) mCPBA, CH_2Cl_2; (j) R^3Cl or R^3Br, NaH, DMF; (k) Me_2NH, MeOH; (l) pent-2-enedioic acid, Et_3N; (m) $ClCO_2Et$, *n*-Hex_2NH, Et_3N, THF. Method A: a, b, c, d, e; method B: a, b, f, g, h; method C: i; method D: j, k; method E: l, m, h.

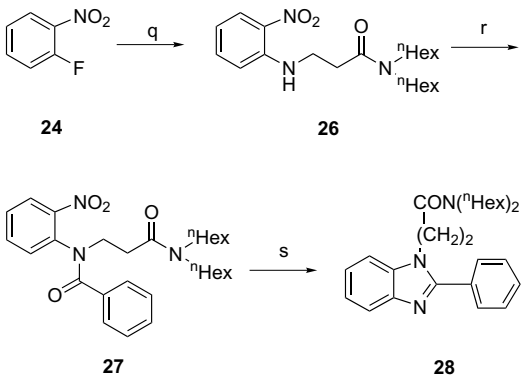


Scheme 2. Reagents and conditions: (n) H_2SO_4; (m) $ClCO_2Et$, (*n*-$Hex)_2NH$, Et_3N, THF; (h) $PhNHNH_2$, $ZnCl_2$, heat. Method F: n, m, h.

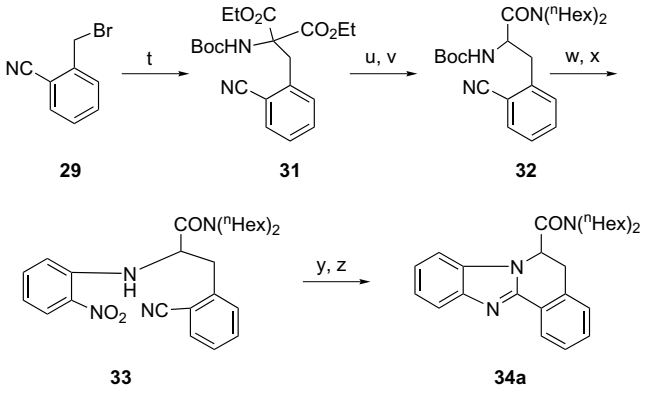


Scheme 3. Reagents and conditions: (o) 4-F–Ph–CO_2H, PPA; (p) $BrCH_2CO_2H$, KOH, DMSO; (m) $ClCO_2Et$, $(n\text{-Hex})_2NH$, Et_3N, THF. Method G: o, p, m.

affinity for PBR ($IC_{50} = 3.8$ nM) and no affinity for CBR ($IC_{50} > 1000$ nM) in [H^3]flunitrazepam binding. This finding encouraged us to explore a new type of PBR ligand using **12a** as a lead compound. Initially, conversion from R^1 and R^2 to various alkyl groups was examined. The results of the binding test for **12b**–**12i** demonstrated significant changes in affinities for PBR. Compounds **12c** (R^1, $R^2 = n$-Pr, H) and **12d** (R^1, $R^2 =$ Me, Me) exhibited lower PBR affinities than **12a**, and **12b** (R^1, $R^2 =$ H, H) had no affinity for PBR. Introducing an amino group into the alkyl chain significantly decreased PBR affinity (**12g**), while an ether group was tolerated (**12h**). The results of structural transformations at this site indicated that the most suitable group for this site was a diethylamino (**12e**) or dipropylamino (**12f**) group. Compounds **12e** and **12f** demonstrated significant increase on PBR affinity over **12a**, by about 10-fold. These findings suggested that R^1



Scheme 4. Reagents and conditions: (q) $NH_2CH_2CH_2CON(n\text{-Hex})_2$ (**25**), K_2CO_3, DMF; (r) PhCOCl, Et_3N, CH_2Cl_2; (s) Fe, AcOH. Method H: q, r, s.



Scheme 5. Reagents and conditions: (t) $BocNHCH(CO_2Et)_2$ **30**, EtONa, EtOH; (u) NaOH, EtOH, H_2O; (v) $(n\text{-Hex})_2NH$, EDC·HCl, HOBt, DMF; (w) TFA, CH_2Cl_2; (x) 2-fluoronitrobenzene, K_2CO_3, DMF; (y) H_2, PtO_2, MeOH; (z) HCl gas, EtOH. Method H: t–z.

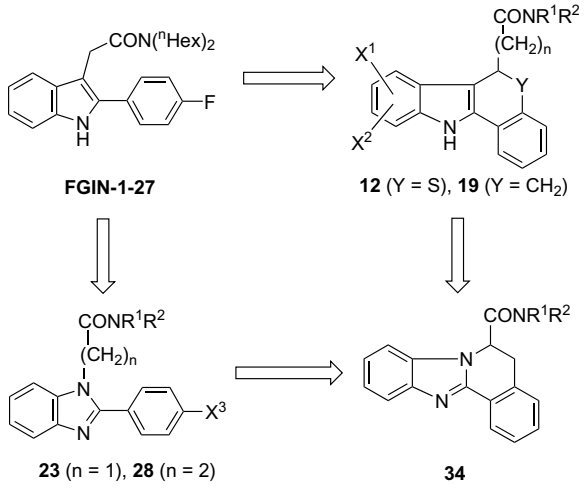


Chart 2.

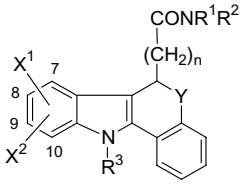
and R^2 groups play an important role in favoring conformation for binding to PBR. Introducing halogen groups or a methyl group to the benzene ring attached to the pyrrole resulted in a small decrease in PBR affinity (**12j**–**12m**). For compounds having a dihexylamino group or dipropylamino group, conversion of the sulfide group to a sulfonyl group was studied, and the changes obtained resulted in a slight increase in affinities for PBR (**13a** vs **12a** and **13c** vs **12f**). On the other hand, for compounds having a mono-alkyl amino group, oxidation of the sulfide group provided a slight decrease in affinity (**12c** vs **13b**). With introduction of an alkyl group including a basic group onto the indol ring, affinity for PBR disappeared completely (**14b** and **14c**). The conversion of thioether to methylene did not influence the affinity for PBR (**12a** vs **19**), suggesting that this structural change does not cause an important change in conformation of the molecule. Optical resolution of **12a** was performed by HPLC, and the (+)-enantiomer exhibited higher affinity than the (−)-enantiomer.

These tetracyclic compounds were highly lipophilic molecules, and they will probably show low solubility in water. Thus, we studied to find other PBR ligands having a basic part to improve the lipophilic property, and we extended our exploration to new types of PBR ligands having a different framework. Structural conversion from the indol ring in the structure of **3** (FGIN-1-27) to a benzimidazole ring was investigated, and **23a**–**23b** and **28** were obtained using the methods shown in Schemes 3 and 4, respectively. Contrary to our expectations, both **23a** ($X^3 = F$) and **23b** ($X^3 = H$) exhibited much lower affinities for PBR than compound **3** (**23a**: $IC_{50} = 310$ nM, **23b**: $IC_{50} = 230$ nM), and propionyl amide **28** exhibited no affinity for PBR. As a next structural conversion, a new tetracyclic compound, which was a hybrid compound between **23b** and **12a**, was designed and the method of synthesis was studied (Chart 2). Synthesis of **34a**–**34c** was achieved using the methods as shown in Scheme 5, and these compounds were evaluated for PBR affinities. Compounds **34a** (R^1, R^2 = hexyl) and **34c** (R^1, R^2 = propyl), a new type of tetracyclic compounds having a basic benzimidazole ring, exhibited higher affinities than **23b** (**34a**: $IC_{50} = 42$ nM, **34c**: $IC_{50} = 74$ nM). Surprisingly, **34b** (R^1, R^2 = ethyl) had no affinity for PBR. Taking into account the fact that **12e** (R^1, R^2 = ethyl) was one of the most potent PBR ligands among the aforementioned tetracyclic compounds, it is notable that the two different tetracyclic ring systems have different suitable alkyl groups.

4. Conclusions

In this paper, we have reported the synthesis and SARs of novel PBR ligands. We designed a new tetracyclic compound from FGIN-1-27 (**3**) and found that compound **12a** exhibited a high affinity for PBR ($IC_{50} = 3.8$ nM). The SAR study led us to more potent PBR ligands (**12e**: $IC_{50} = 0.44$ nM, **12f**: $IC_{50} = 0.37$ nM). In addition, we found that another type of PBR ligand, **34a** and **34c**, which have another basic tetracyclic ring

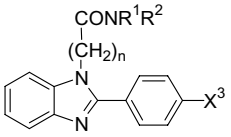
Table 1. Binding data (PBR) for **12a**–**12m**, **13a**–**13c**, **14a**–**14c**, **16**, **19**



Compd	Method	Y	R^3	X^1, X^2	*n*	NR^1R^2	IC_{50} (nM)[a]
12a	A, B	S	H	H, H	0	N(*n*-Hex)$_2$	3.8
(−)-**12a**	A	S	H	H, H	0	N(*n*-Hex)$_2$	42
(+)-**12a**	A	S	H	H, H	0	N(*n*-Hex)$_2$	2.2
12b	B	S	H	H, H	0	NH_2	>1000
12c	B	S	H	H, H	0	N(H)*n*-Pr	56
12d	B	S	H	H, H	0	NMe_2	39
12e	B	S	H	H, H	0	NEt_2	0.44
12f	B	S	H	H, H	0	N(*n*-Pr)$_2$	0.37
12g	A	S	H	H, H	0	N(*n*-Hex)((CH$_2$)$_2$N(H)*n*-Pr)	110
12h	A	S	H	H, H	0	N(*n*-Hex)((CH$_2$)$_2$O-*n*-Pr)	1.4
12i	B	S	H	H, H	0	N((CH$_2$)$_2$OMe)$_2$	5.0
12j	B	S	H	8-F, H	0	N(*n*-Hex)$_2$	14
12k	B	S	H	8-Cl, H	0	N(*n*-Hex)$_2$	20
12l	B	S	H	8-Me, H	0	N(*n*-Hex)$_2$	14
12m	B	S	H	8-F, 10-F	0	N(*n*-Hex)$_2$	22
13a	C	SO_2	H	H, H	0	N(*n*-Hex)$_2$	1.2
13b	C	SO_2	H	H, H	0	N(H)*n*-Pr	129
13c	C	SO_2	H	H, H	0	N(*n*-Pr)$_2$	1.1
14a	D	S	Me	H, H	0	N(*n*-Hex)$_2$	1.4
14b	D	S	$Me_2N(CH_2)_2$	H, H	0	N(*n*-Hex)$_2$	>1000
14c	D	S	$Me_2N(CH_2)_4$	H, H	0	N(*n*-Hex)$_2$	>1000
16	E	S	H	H, H	1	N(*n*-Hex)$_2$	250
19	F	CH_2	H	H, H	0	N(*n*-Hex)$_2$	1.4
Ro-5-4864 (**1**)							3.1
PK11195 (**2**)							1.1
FGIN-1-27 (**3**)							5.5

[a] IC_{50} values from duplicate determinations.

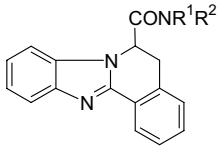
Table 2. Binding data (PBR) for **23a**, **23b**, and **28**



Compd	Method	*n*	NR^1R^2	X^3	IC_{50} (nM)[a]
23a	G	1	N(*n*-Hex)$_2$	F	310
23b	G	1	N(*n*-Hex)$_2$	H	230
28	H	2	N(*n*-Hex)$_2$	H	>1000

[a] IC_{50} values from duplicate determinations.

Table 3. Binding data (PBR) for **34a**–**34c**



Compd	Method	NR^1R^2	PBR affinity IC_{50} (nM)[a]
34a	I	N(*n*-Hex)$_2$	42
34b	I	NEt_2	>1000
34c	I	N(*n*-Pr)$_2$	74

[a] IC_{50} values from duplicate determinations.

system, showed moderate affinities for PBR (**34a**: $IC_{50} = 42$ nM, **34c**: $IC_{50} = 74$ nM). We believe that the SAR study will provide new opportunities to explore new PBR ligands with improved features, and the PBR ligands reported here will aid study of the physiological and biological roles of PBR.

5. Experimental section

Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (NMR) spectra were obtained using a Varian Gemini 2000 (200 MHz). Chemical shifts are reported in parts per million relative to tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on a Shimadzu Profile (EI and CI), JEOL JMS-SX102 (FAB) or Micromass Platform LC (IonSpray and ES). Elemental analyses were performed by a Perkin–Elmer 2400 (carbon, hydrogen, and nitrogen) and were within ±0.4% of the theoretical values. Silica gel [C-200, 100–200 mesh (Wako Pure Chemical)] was used for column chromatography, using the solvent systems (volume ratios) indicated below.

5.1. General methods for the synthesis of 12a, 12g, and 12h (method A)

5.1.1. (±)-4-Oxo-thiochroman-2-carboxylic acid (8). A mixture of benzenethiol **7** (82.6 g, 0.75 mol) and furan-2,5-dione (73.5 g, 0.75 mol) in toluene (180 mL) was stirred at 50 °C. After all materials were dissolved, Et_3N (10 drops) in toluene (10 mL) was added over 10 min keeping the temperature below 70 °C. After stirring at 70 °C for 20 min, the solvent was concentrated under reduced pressure to obtain crude 3-phenylthiodihydrofuran-2,5-dione. The residue was dissolved in CH_2Cl_2 (150 mL) and the mixture was cooled with an ice-cooling bath. $AlCl_3$ (150 g, 1.12 mol) was added and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted in CH_2Cl_2 (150 mL) and poured into ice-cooling concd HCl. After adding IPE into the mixture, a solid precipitated. The solid was collected by filtration, dried, and recrystallized from THF/IPE to obtain **8** (67.7 g, 43%) as a white crystal. ^{1}H NMR (DMSO-d_6) δ 2.95–3.22 (2H, m), 4.40 (1H, dd, $J = 4.6$, 5.9 Hz), 7.18–7.57 (3H, m), 7.94 (1H, dd, $J = 7.9$, 1.5 Hz); MS m/z (ESI) m/z 231 (M^++Na, 100%).

5.1.2. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid ethyl ester (9). To a solution of **8** (22.5 g, 108 mmol) and phenylhydrazine (10.7 mL, 108 mmol) in EtOH (100 mL), H_2SO_4 (15 mL) was added, and the mixture was heated at reflux for 5 h. After cooling to room temperature, the reaction mixture was poured into ice water (500 mL) and extracted with Et_2O. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was recrystallized from hexane/EtOAc to obtain **9** (21.8 g, 65%) as a solid. ^{1}H NMR ($CDCl_3$) δ 1.16 (3H, t, $J = 7.1$ Hz), 4.10 (2H, q, $J = 7.1$ Hz), 5.01 (1H, s), 6.97–7.42 (7H, m), 7.47–7.62 (1H, m), 8.46 (1H, br s); MS (EI) m/z 309 (M^+), 236 (M^+-73, 100%).

5.1.3. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid (10). To a solution of **9** (15.0 g, 48.5 mmol) in EtOH (100 mL), a solution of KOH (85%, 12.8 g, 194 mmol) in water (40 mL) was added. The mixture was heated at reflux for 2 h. The reaction mixture was acidified with 3 M HCl and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to obtain a solid. The solid was recrystallized from hexane/EtOH to obtain **10** (10.3 g, 75%) as a solid. ^{1}H NMR (DMSO-d_6) δ 5.20 (1H, s), 6.97–7.58 (7H, m), 7.72–7.83 (1H, m), 11.71 (1H, br s), 12.63 (1H, br s); MS (EI) m/z 281 (M^+), 236 (M^+-45, 100%).

5.1.4. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dihexylamide (12a). A solution of **10** (501 mg, 1.78 mmol) in THF (8.0 mL) was cooled to −15 °C. *N*-Methylmorpholine (180 mg, 1.78 mmol), isobutyl chloroformate (243 mg), and dihexylamine (330 mg, 1.78 mmol) were added and stirred at −15 °C for 4 h. The reaction was quenched with 0.5 M HCl and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [hexane/EtOAc, 5:1 (v/v), as eluent] and crystallized from EtOAc/hexane to obtain **12a** (0.43 g, 54%) as a crystal. Mp 138.5–140.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.68–1.89 (22H, m), 3.17–3.66 (4H, m), 5.33 (1H, s), 6.75–7.42 (8H, m), 8.99 (1H, br s); MS (CI, Pos) m/z 449 (M^++1, 100%); Anal. ($C_{28}H_{36}N_2OS$) C, H, N. Each enantiomer was obtained by using HPLC for optical resolution [Chiralpak AD (Daicel Chemical Industries, Ltd), 2×25 cm, mobile phase: hexane/EtOH = 3:7, flow rate: 5 mL/min]. (+)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dihexylamide [(+)-**12a**]: $[\alpha]_D^{26}$ +25.9 (*c* 0.207, EtOH); HPLC retention time: 37 min. (−)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dihexylamide [(−)-**12a**]: $[\alpha]_D^{26}$ −25.9 (*c* 0.180, EtOH); HPLC retention time: 20 min.

5.1.5. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid hexyl-(2-propoxyethyl)amide (12h). Starting from **10** and hexyl-(2-propoxyethyl)amine, **12h** was obtained as a crystal. Yield 55%; mp 165.0–167.0 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.70–1.91 (16H, m), 3.15–4.02 (8H, m), 5.36 (0.5H, s), 5.56 (0.5H, s), 6.73–7.49 (8H, m), 8.82–8.96 (1H, m); MS (ESI, Pos) m/z 473 (M^++Na, 100%); Anal. ($C_{27}H_{34}N_2O_2S$) C, H, N.

5.1.6. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid hexyl-(2-propylaminoethyl)amide (12g). To a mixture of **10** (844 mg, 3.00 mmol) and (2-hexylaminoethyl)propylcarbamic acid *tert*-butyl ester (1.72 g, 6.00 mmol) in CH_2Cl_2 (44 mL), HOBt·$1H_2O$ (552 mg, 3.60 mmol), and EDC·HCl (863 mg, 4.50 mmol) were added and the mixture was stirred at room temperature overnight. After evaporation of the solvent under reduced pressure, the residue was poured into EtOAc, and the organic phase was washed with H_2O, 5% $KHSO_4$ solution, satd $NaHCO_3$ solution and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [Chromatorex NHDM1020 (Fuji Silysia) as silica gel; hexane/EtOAc, 3:2 (v/v), as eluent] to obtain (±)-{2-[(6,11-dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carbonyl)hexylamino]ethyl}propylcarbamic acid *tert*-butyl ester (1.35 g, 82%) as an amorphous. ^{1}H NMR ($CDCl_3$) δ 0.60–1.90 (25H, m), 2.88–3.86 (8H, m), 5.28 (1H, s), 6.65–7.43 (8H, m), 9.02 (1H, br s); MS (ESI, Neg) m/z 548 (M^+-1, 100%).

(±)-{2-[(6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carbonyl)hexylamino]ethyl}propylcarbamic acid *tert*-butyl ester (600 mg) was dissolved in HCO_2H (4.2 mL) at room temperature for 5 h. HCO_2H was removed under reduced pressure and EtOAc was poured into the residue. The solution was washed with satd $NaHCO_3$ solution and brine, dried (Na_2SO_4), and evaporated under reduced pressure to obtain a solid. The solid was recrystallized from EtOAc to obtain **12g** (406 mg, 83%) as a crystal. Mp 135.5–138.0 °C; ^{1}H NMR ($CDCl_3$) δ

0.62–1.95 (16H, m), 2.18–3.82 (8H, m), 5.33 (0.5H, s), 5.52 (0.5H, s), 6.80–7.52 (8H, m), 8.72–8.90 (1H, m); MS (ESI, Pos) m/z 450 (M^++1, 100%); Anal. ($C_{27}H_{35}N_3OS$·0.1EtOAc) C, H, N.

5.2. General methods for the synthesis of 12b–f and 12i–m (method B)

5.2.1. (±)-4-Oxo-thiochroman-2-carboxylic acid dihexylamide (11). To a solution of **8** (20.0 g, 96.0 mmol) in benzene (200 mL), thionyl chloride (14.0 mL, 193 mmol) was added, and the mixture was heated at reflux for 3 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in dry CH_2Cl_2 (100 mL). The solution was added dropwise into a solution of dihexylamine (24.6 mL, 106 mmol) and Et_3N (20.0 mL, 143 mmol) in CH_2Cl_2 (200 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc, washed with water, 1 M HCl, satd $NaHCO_3$ solution and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [hexane/EtOAc, 3:1 (v/v), as eluent] and recrystallized from hexane to obtain **11** (29.2 g, 81%). ^{1}H NMR ($CDCl_3$) δ 0.68–1.82 (22H, m), 2.94–3.57 (6H, m), 4.26 (1H, dd, $J = 3.7$, 7.6 Hz), 7.09–745 (3H, m), 8.14 (1H, dd, $J = 1.4$, 8.2 Hz); MS (ESI, Neg) m/z 374 (M^+−1, 100%).

5.2.2. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dihexylamide (12a). A mixture of **11** (1.00 g, 2.67 mmol) and phenylhydrazine (0.26 mL, 2.64 mmol) was heated at 100 °C for 30 min. After drying under reduced pressure at 50 °C, $ZnCl_2$ (1.44 g, 10.6 mmol) was added and the mixture was heated at 170 °C for 5 min and then cooled to room temperature. To the reaction mixture, iced water was added, extracted with EtOAc, and the organic phase was washed with 1 M HCl, satd $NaHCO_3$ solution and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was crystallized from hexane/EtOAc to obtain **12a** (0.79 g, 66%) as a crystal.

5.2.3. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid amide (12b). Starting from 4-oxo-thiochroman-2-carboxylic acid amide and phenylhydrazine, **12b** was obtained as a crystal. Yield 36%; mp 241.0–242.5 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 5.05 (1H, s), 6.87–7.58 (9H, m), 7.78 (1H, dd, $J = 7.5$, 1.3 Hz), 11.7 (1H, br s); MS (CI, Pos) m/z 281 (M^++1), 236 (M^+−44, 100%); Anal. ($C_{16}H_{12}N_2OS$) C, H, N.

5.2.4. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid propylamide (12c). Starting from 4-oxo-thiochroman-2-carboxylic acid propylamide and phenylhydrazine, **12c** was obtained as a crystal. Yield 71%; mp 172.0–174.0 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.59 (3H, t, $J = 7.4$ Hz), 1.10–1.40 (2H, m), 2.85–3.26 (2H, m), 4.96 (1H, s), 5.84–6.12 (1H, m), 7.05–7.64 (1H, m), 8.96 (1H, br s); MS (CI, Pos) m/z 323 (M^++1), 236 (M^+−86, 100%); Anal. ($C_{19}H_{18}N_2OS$) C, H, N.

5.2.5. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dimethylamide (12d). Starting from 4-oxo-thiochroman-2-carboxylic acid dimethylamide and phenylhydrazine, **12d** was obtained as a crystal. Yield 61%; mp 254.5–256.5 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 2.80 (3H, s), 3.32 (3H, s), 5.69 (1H, s), 6.92–7.50 (7H, m), 7.72–7.85 (1H, m), 11.64 (1H, br s); MS (CI, Pos) m/z 309 (M^++1), 236 (M^+−72, 100%); Anal. ($C_{18}H_{16}N_2OS$) C, H, N.

5.2.6. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid diethylamide (12e). Starting from 4-oxo-thiochroman-2-carboxylic acid diethylamide and phenylhydrazine, **12e** was obtained as a crystal. Yield 8%; mp 242.0–243.5 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.14 (3H, t, $J = 7.1$ Hz), 1.40 (3H, t, $J = 7.1$ Hz), 3.25–3.78 (4H, m), 5.34 (1H, s), 6.56–6.70 (1H, m), 6.74–7.40 (7H, m), 9.29 (1H, s); MS (CI, Pos) m/z 337 (M^++1), 236 (M^+−100, 100%); Anal. ($C_{20}H_{20}N_2OS$) C, H, N.

5.2.7. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dipropylamide (12f). Starting from 4-oxo-thiochroman-2-carboxylic acid dipropylamide and phenylhydrazine, **12f** was obtained as a crystal. Yield 25%; mp 196.0–197.0 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, t, $J = 7.5$ Hz), 1.05 (3H, t, $J = 7.5$ Hz), 1.45–1.96 (4H, m), 3.17–3.66 (4H, m), 5.34 (1H, s), 6.52–6.66 (1H, m), 6.76–7.39 (7H, m), 9.35 (1H, br s); MS (CI, Pos) m/z 365 (M^++1), 236 (M^+−128, 100%); Anal. ($C_{22}H_{24}N_2OS$) C, H, N.

5.2.8. (±)-6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid bis-(2-methoxyethyl)amide (12i). Starting from 4-oxo-thiochroman-2-carboxylic acid bis-(2-methoxyethyl)amide and phenylhydrazine, **12i** was obtained as a crystal. Yield 10%; mp 206.0–208.0 °C (EtOAc); ^{1}H NMR (DMSO-d_6) δ 3.12–4.01 (8H, m), 3.20 (3H, s), 3.41 (3H, s), 5.68 (1H, s), 6.92–7.48 (7H, m), 7.72–7.83 (1H, m), 11.64 (1H, br s); MS (CI, Pos) m/z 397 (M^++1), 236 (M^+−160, 100%); Anal. ($C_{22}H_{24}N_2O_3S$) C, H, N.

5.2.9. (±)-8-Fluoro-6,11-dihydro-5-thia-11-aza-benzo[*a*]-fluorene-6-carboxylic acid dihexylamide (12j). Starting from 4-oxo-thiochroman-2-carboxylic acid dihexylamide and 4-fluorophenylhydrazine, **12j** was obtained as a crystal. Yield 47%; mp 110.5–112.5 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.75–1.09 (6H, m), 1.11–1.95 (16H, m), 3.25–3.70 (4H, m), 5.27 (1H, s), 6.52–6.77 (3H, m), 6.82–7.03 (3H, m), 7.10–7.22 (1H, m), 9.66 (1H, br s); MS (CI, Pos) m/z 467 (M^++1), 254 (M^+−212, 100%); Anal. ($C_{28}H_{35}FN_2OS$) C, H, N.

5.2.10. (±)-8-Chloro-6,11-dihydro-5-thia-11-aza-benzo[*a*]-fluorene-6-carboxylic acid dihexylamide (12k). Starting from 4-oxo-thiochroman-2-carboxylic acid dihexylamide and 4-chlorophenylhydrazine, **12k** was obtained as a crystal. Yield 43%; mp 123.0–124.5 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.72–1.08 (6H, m), 1.10–1.99

(16H, m), 3.20–3.72 (4H, m), 5.27 (1H, s), 6.34–6.53 (2H, m), 6.62–6.95 (3H, m), 7.02–7.23 (2H, m), 10.11 (1H, br s); MS (FAB, Pos) *m/z* 483 (M^++1), 485 (M^++3), 270 (M^+−212, 100%); Anal. ($C_{28}H_{35}ClN_2OS$) C, H, N.

5.2.11. (±)-8-Methyl-6,11-dihydro-5-thia-11-aza-benzo[*a*]-fluorene-6-carboxylic acid dihexylamide (12l). Starting from 4-oxo-thiochroman-2-carboxylic acid dihexylamide and 4-methylphenylhydrazine, **12l** was obtained as a crystal. Yield 32%; mp 139.5–141.5 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.72–1.07 (6H, m), 1.10–1.90 (16H, m), 2.41 (3H, s), 3.15–3.67 (4H, m), 5.32 (1H, s), 6.74–7.27 (7H, m), 8.88 (1H, br s); MS (CI, Pos) *m/z* 463 (M^+, 100%); Anal. ($C_{29}H_{38}N_2OS$) C, H, N.

5.2.12. (±)-8,10-Difluoro-6,11-dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dihexylamide (12m). Starting from 4-oxo-thiochroman-2-carboxylic acid dihexylamide and 2,4-difluorophenylhydrazine, **12m** was obtained as a crystal. Yield 25%; mp 148.0–149.0 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.75–1.07 (6H, m), 1.12–1.97 (16H, m), 3.25–3.70 (4H, m), 5.19 (1H, s), 6.28–6.45 (1H, m), 6.54–6.74 (2H, m), 6.84–6.98 (1H, m), 7.10–7.31 (2H, m), 10.01 (1H, br s); MS (CI, Pos) *m/z* 485 (M^++31, 100%); Anal. ($C_{28}H_{34}F_2N_2OS$) C, H, N.

5.3. General methods for the synthesis of 13a–c (method C)

5.3.1. (±)-5,5-Dioxo-6,11-dihydro-5*H*-5λ^6-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dihexylamide (13a). A solution of mCPBA (>70%, 346 mg, 1.40 mmol) in CH_2Cl_2 (20 mL) was added to a solution of **12a** (300 mg, 0.669 mmol) in CH_2Cl_2 (20 mL) dropwise with cooling in an ice-water bath. The reaction mixture was stirred at room temperature for 1 h and then the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and the solution was washed with satd $NaHCO_3$ solution and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [hexane/EtOAc, 1:1 (v/v), as eluent] and crystallized from EtOAc/hexane to obtain **13a** (130 mg, 40%) as a crystal. Mp 156.0–157.0 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.78–2.10 (22H, m), 3.24–4.02 (4H, m), 5.77 (1H, s), 5.52 (0.5H, s), 6.66–7.11 (6H, m), 7.22–7.36 (1H, m), 7.72–7.83 (1H, m), 9.73 (1H, br s); MS (CI, Pos) *m/z* 481 (M^++1), 85 (M^+−395, 100%); Anal. ($C_{28}H_{36}N_2O_3S$) C, H, N.

5.3.2. (±)-5,5-Dioxo-6,11-dihydro-5*H*-5λ^6-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dipropylamide (13b). Starting from **12f**, **13b** was obtained as a crystal. Yield 31%; mp 296.5–298.0 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, t, J = 7.3 Hz), 1.18 (3H, t, J = 7.3 Hz), 1.40–1.78 (2H, m), 1.80–2.25 (2H, m), 3.20–3.98 (4H, m), 5.78 (1H, s), 6.60–7.12 (6H, m), 7.21–7.36 (1H, m), 7.78 (1H, dd, J = 7.7, 1.1 Hz), 9.72 (1H, br s); MS (CI, Pos) *m/z* 397 (M^++1), 128 (M^+−268, 100%); Anal. ($C_{19}H_{18}N_2O_3S$) C, H, N.

5.3.3. 5,5-Dioxo-6,11-dihydro-5*H*-5λ^6-thia-11-aza-benzo[*a*]-fluorene-6-carboxylic acid propylamide (13c). Starting from **12c**, **13c** was obtained as a crystal. Yield 31%; mp 266.0–267.0 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, t, J = 7.1 Hz), 1.26–1.52 (2H, m), 2.90–3.09 (2H, m), 5.69 (1H, s), 7.05–7.32 (2H, m), 7.43–7.65 (3H, m), 7.73–8.06 (3H, m), 8.57–8.72 (1H, br t), 12.13 (1H, br s); MS (CI, Pos) *m/z* 355 (M^++1), 205 (M^+−149, 100%); Anal. ($C_{22}H_{24}N_2O_3S$) C, H, N.

5.4. General methods for the synthesis of 14a–c (method D)

5.4.1. (±)-11-(2-Dimethylaminoethyl)-6,11-dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dihexylamide hydrochloride (14b). To a suspension of NaH (53 mg, 1.33 mmol) in dry DMF (5 mL), a solution of **12a** in dry DMF (10 mL) dropwise was added and the mixture was stirred at room temperature for 30 min. A solution of 2-(dimethylamino)ethylchloride hydrochloride (96 mg, 0.67 mmol) was added and the mixture was heated at 80 °C for 3 h. The reaction was quenched with H_2O and extracted with EtOAc. The organic phase was washed with H_2O and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [CH_2Cl_2/EtOH, 15:1 (v/v), as eluent] to obtain the free base of **14b**. Treatment of the free base with 4 M HCl/EtOAc in EtOAc to give **14b** (10 mg, 2.7%) and crystallized from Et_2O/hexane to obtain a solid. Mp 89.5–90.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.60–1.90 (22H, m), 2.38–2.72 (6H, m), 2.95–3.68 (6H, m), 4.90–5.19 (3H, m), 7.08–7.82 (8H, m), 13.20 (1H, br s); MS (CI, Pos) *m/z* 520 (M^++1), 307 (M^+−212, 100%); HRMS calculated 520.3362, found 520.3373.

5.4.2. (±)-11-Methyl-6,11-dihydro-5-thia-11-aza-benzo-[*a*]fluorene-6-carboxylic acid dihexylamide (14a). Compound **14a** was synthesized using the same procedure as **14b** using MeI instead of 2-(dimethylamino)ethylchloride hydrochloride. Yield 37%; mp 111.0–112.0 °C (hexane); ^{1}H NMR ($CDCl_3$) δ 0.70–1.90 (22H, m), 3.11–3.67 (4H, m), 4.01 (3H, s), 5.12 (1H, s), 7.03–7.49 (7H, m), 7.73 (1H, dd, J = 7.9, 1.3 Hz); MS (CI, Pos) *m/z* 463 (M^++1), 250 (M^+−212, 100%); Anal. ($C_{29}H_{38}N_2OS$) C, H, N.

5.4.3. (±)-11-(4-Dimethylaminobutyl)-6,11-dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dihexylamide (14c). To a suspension of NaH (37 mg, 0.93 mmol) in dry DMF (5 mL), a solution of **12a** in dry DMF (10 mL) dropwise was added and the mixture was stirred at room temperature for 25 min. A solution of 1-bromo-4-chlorobutane (0.11 mL, 0.96 mmol) in dry DMF (5 mL) was added with a cooling ice-water bath and the mixture was heated at room temperature for

30 min. The reaction was quenched with H_2O and extracted with EtOAc. The organic phase was washed with H_2O and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [CH_2Cl_2/EtOH, 15:1 (v/v), as eluent] to obtain a solid. The solid was recrystallized from hexane to provide 11-(4-chlorobutyl)-6,11-dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dihexylamide (175 mg, 53%) as a crystal. ^{1}H NMR ($CDCl_3$) δ 0.68–2.25 (26H, m), 3.08–3.64 (4H, m), 3.53 (2H, t, $J = 6.4$ Hz), 4.25–4.53 (2H, m), 5.09 (1H, s), 7.02–7.52 (7H, m), 7.63 (1H, dd, $J = 1.2$, 7.8 Hz); MS (CI, Pos) m/z 539 (M^++1, 100%). A solution of 11-(4-chloro-butyl)-6,11-dihydro-5-thia-11-aza-benzo[*a*]fluorene-6-carboxylic acid dihexylamide (0.150 g, 0.28 mmol) and 50% aqueous dimethylamine solution (1.46 mL, 8.83 mmol) in MeOH (20 mL) was heated at reflux for two days. H_2O was added into the reaction mixture and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to obtain a solid. The solid was recrystallized from hexane to obtain **14c** (0.12 g, 77%) as a crystal. Mp 50.0–52.0 °C; ^{1}H NMR ($CDCl_3$) δ 0.72–2.10 (26H, m), 2.21 (6H, s), 2.20–2.36 (2H, m), 3.08–3.65 (4H, m), 4.23–4.50 (2H, m), 5.10 (1H, s), 7.02–7.49 (7H, m), 7.61–7.72 (1H, m); MS (EI) m/z 547 (M^+), 335 (M^+−212, 100%); Anal. ($C_{34}H_{49}N_3OS$) C, H, N.

5.5. Method E

5.5.1. (±)-2-(6,11-Dihydro-5-thia-11-aza-benzo[*a*]fluoren-6-yl)-*N*,*N*-hexylacetamide (16). A solution of (4-oxo-thiochroman-2-yl)acetic acid **15** (0.89 g, 4.0 mmol), which was prepared from benzenethiol and glutaconic acid using the same method as shown for preparation of **8**, and Et_3N (1.96 mL, 14.1 mmol) in dry THF (30 mL) was cooled to −40 °C and ethyl chloroformate (0.42 mL, 4.4 mmol), and then a solution of dihexylamine (1.03 mL, 4.4 mmol) in dry THF (10 mL) was added. The reaction mixture was stirred at room temperature for 2.5 h, quenched with H_2O, and extracted with EtOAc. The organic phase was washed with 5% HCl and brine, dried (Na_2SO_4), and evaporated under reduced pressure to obtain crude *N*,*N*-dihexyl-2-(4-oxo-thiochroman-2-yl)acetamide. Phenylhydrazine (0.39 mL, 4.0 mmol) was added to the crude *N*,*N*-dihexyl-2-(4-oxo-thiochroman-2-yl)acetamide and the mixture was heated at 100 °C, and then the water generated was removed under reduced pressure. $ZnCl_2$ (2.19 g, 16.0 mmol) was added and the mixture was heated at 170 °C for 5 min and cooled to 80 °C. After the reaction mixture was dissolved in acetone (30 mL), Et_2O and H_2O were added and separated. The organic phase was washed with 5% HCl and brine, dried (Na_2SO_4), and evaporated under reduced pressure to obtain a solid. The solid was recrystallized from hexane/EtOAc to obtain **16** (0.56 g, 30%) as a crystal. Mp 155.0–157.0 °C; ^{1}H NMR ($CDCl_3$) δ 0.68–1.61 (22H, m), 2.65–3.00 (4H, m), 3.12–3.42 (2H, m), 5.06 (1H, dd, $J = 9.5$, 5.1 Hz), 7.08–7.30 (4H, m), 7.37–7.49 (3H, m), 7.60–7.69 (1H, m), 8.33 (1H, br s); MS (CI, Pos) m/z 463 (M^++1, 100%); Anal. ($C_{29}H_{38}N_2OS$) C, H, N.

5.6. Method F

5.6.1. (±)-4-Oxo-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (18). 2-Benzylsuccinic acid (5.00 g, 24.0 mmol) was added to concd H_2SO_4 and the mixture was stirred at room temperature for 9 h. The reaction mixture was poured into ice and the resulting precipitate was collected by filtration and washed with H_2O. The precipitate was recrystallized from EtOH/H_2O to obtain **18** (2.1 g, 45%) as a crystal. ^{1}H NMR ($CDCl_3$) δ 2.72–3.39 (5H, m), 7.19–7.60 (3H, m), 7.95–8.13 (1H, m), 13.25 (1H, br s); MS (ESI, Neg) m/z 189 (M^+−1), 145 (M^+−45, 100%).

5.6.2. (±)-5,11-Dihydro-6*H*-benzo[*a*]carbazole-6-carboxylic acid dihexylamide (19). Compound **19** was obtained from **18** using the same method as for synthesis of **12a** from **11**. Yield 56%; mp 152.0–153.0 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.70–1.84 (22H, m), 3.01 (1H, dd, $J = 15.5$, 5.9 Hz), 3.25–3.67 (5H, m), 4.38 (1H, dd, $J = 12.6$, 5.9 Hz), 6.97–7.42 (8H, m), 8.40 (1H, br s); MS (CI, Pos) m/z 431 (M^++1), 218 (M^+−212, 100%); Anal. ($C_{29}H_{38}N_2O$) C, H, N.

5.7. General methods for the synthesis of 23a and 23b (method G)

5.7.1. 2-(4-Fluorophenyl)-1*H*-benzoimidazole (21). A mixture of benzene-1,2-diamine (7.72 g, 71 mmol), 4-fluorobenzoic acid (10.0 g, 71 mmol), and polyphosphoric acid (150 g) was heated at 250 °C for 4 h. After cooling to room temperature, with addition of water to the reaction mixture a solid precipitated. The solid was collected by filtration, washed with H_2O, 10% Na_2CO_3, and H_2O again, and purified by recrystallization to obtain **21** (13.2 g, 87%). ^{1}H NMR (DMSO-d_6) δ 7.13–7.28 (2H, m), 7.30–7.72 (4H, m), 8.13–8.32 (2H, m), 12.91 (1H, br s); MS (ESI) m/z 213 (M^++1, 100%).

5.7.2. [2-(4-Fluorophenyl)benzoimidazol-1-yl]acetic acid (22). A mixture of KOH (5.57 g, 94 mmol) and DMSO (100 mL) was stirred at room temperature for 15 min, and then **21** (5.00 g, 24.0 mmol) was added to the mixture. After stirring at room temperature for 1 h, bromoacetic acid (3.60 g, 26 mmol) was added and the mixture was stirred at room temperature for 15 h. The reaction was quenched with H_2O and washed with EtOAc three times. The aqueous phase was acidified with 3 M HCl and washed with EtOAc twice. Next, the aqueous phase was neutralized with 10% NaOH and a solid was precipitated. The solid was collected by filtration and washed with H_2O to provide **22** (4.3 g, 67%). ^{1}H NMR (DMSO-d_6) δ 5.10 (2H, s), 7.17–7.86 (8H, m), 13.33 (1H, br s); MS (FAB, Pos) m/z 271 (M^++1).

5.7.3. 2-[2-(4-Fluorophenyl)benzoimidazol-1-yl]-*N*,*N*-dihexylacetamide (23a). To a solution of **22** (1.00 g, 3.70 mmol) and Et_3N (1.81 mL, 13.0 mmol) in THF (29 mL), $ClCO_2Et$ (0.39 mL, 4.1 mmol) was added, and

then a solution of dihexylamine (0.95 mL, 4.1 mmol) in THF (10 mL) at −40 °C, and the mixture was stirred at room temperature for 1.5 h. The reaction was quenched with H_2O and extracted with EtOAc. The organic phase was washed with H_2O and brine, dried (Na_2SO_4), and evaporated under reduced pressure to give a solid. The solid was recrystallized from hexane to obtain **23a** (0.48g, 30%). Mp 90.0–91.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.80–1.05 (6H, m), 1.14–1.73 (16H, m), 3.17–3.49 (4H, m), 4.91 (2H, m), 7.10–7.39 (5H, m), 7.64–7.90 (3H, m); MS (FAB, Pos) m/z 438 (M^++1, 100%); Anal. ($C_{27}H_{36}FN_3O$) C, H, N.

5.7.4. *N*,*N*-Dihexyl-2-(2-phenylbenzoimidazol-1-yl)acetamide (23b). Starting from (2-phenylbenzoimidazol-1-yl)acetic acid, **23b** was obtained as a crystal. Yield 45%; mp 83.5–85.0 °C (hexane); ^{1}H NMR ($CDCl_3$) δ 0.78–1.02 (6H, m), 1.12–1.80 (16H, m), 3.22 (2H, t, $J = 7.5$ Hz), 3.40 (2H, t, $J = 7.5$ Hz), 4.92 (2H, s), 7.18–7.59 (6H, m), 7.65–7.91 (3H, m); MS (EI) m/z 419 (M^+), 43 (M^+−376, 100%); Anal. ($C_{27}H_{37}N_3O$) C, H, N.

5.8. Method H

5.8.1. *N*,*N*-Dihexyl-3-(2-nitrophenylamino)propionamide (26). β-Alanine (10.0 g, 112 mmol) was dissolved in a mixture of 1,4-dioxane (220 mL) and 0.5 M NaOH solution (220 mL). After cooling with an ice-cooling bath, Boc_2O (27.0 g, 123 mmol) was added to the solution. The solution was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and to the residue 5% $KHSO_4$ solution was added and extracted with CH_2Cl_2, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was crystallized from EtOAc/hexane and the crystal was collected by filtration to obtain 3-*tert*-butoxycarbonylaminopropionic acid (17.7 g, 91%). ^{1}H NMR ($CDCl_3$) δ 1.37 (9H, m), 2.34 (2H, t, $J = 7.1$ Hz), 2.95–3.22 (2H, m), 6.65–6.90 (1H, m), 12.16 (1H, br s). To the solution of 3-*tert*-butoxycarbonylaminopropionic acid (14.1 g, 81.5 mmol) and dihexylamine (19.0 mL, 81.5 mmol) in CH_2Cl_2 (100 mL), EDC·HCl (16.4 g, 85.6 mmol) was added, and the mixture was stirred overnight. The solution was washed with 5% $KHSO_4$ solution, satd $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and evaporated under reduced pressure to obtain crude 3-*tert*-butoxycarbonylamino-*N*,*N*-dihexylpropionamide (29.9 g, quant.). To a solution of crude 3-*tert*-butoxycarbonylamino-*N*,*N*-dihexylpropionamide (7.75 g, 21.8 mmol) in CH_2Cl_2 (17 mL), trifluoroacetic acid (17 mL) was added. The mixture was stirred at room temperature for 3.5 h. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$. The solution was washed with satd $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated to obtain crude 3-amino-*N*,*N*-dihexylpropionamide (**25**). The product was used in the next step without further purification. A mixture of **25**, synthesized using the method described above, 2-fluoronitrobenzene (3.07 g, 21.8 mmol) and K_2CO_3 (3.61 g) in DMF (50 mL) was heated at 150 °C for 3 h. The resulting orange solution was diluted with EtOAc, and the solution obtained was washed with H_2O and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [hexane/EtOAc, 3:1 (v/v), as eluent] to obtain **26** (7.79 g) as an orange oil. ^{1}H NMR ($CDCl_3$) δ 0.75–1.65 (22H, m), 2.69 (4H, t, $J = 6.9$ Hz), 3.10–3.39 (4H, m), 3.61–3.79 (2H, m), 6.57–6.71 (1H, m), 6.87–6.99 (1H, m), 7.36–7.52 (1H, m), 8.06–8.29 (2H, m); MS (ESI, Pos) m/z 400 (M^++Na, 100%).

5.8.2. *N*-(2-Dihexylcarbamoylethyl)-*N*-(2-nitrophenyl)-benzamide (27). To a solution of **26** (2.31 g, 6.12 mmol) and Et_3N (1.71 mL, 12.2 mmol) in CH_2Cl_2 (20 mL), benzoyl chloride (0.75 mL, 6.43 mmol) was added, and the mixture was heated at reflux for 5 h. The reaction mixture was poured into EtOAc, washed with 0.5 M HCl, satd $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [hexane/EtOAc, 3:1 (v/v), as eluent] to obtain **27** (1.38 g, 47%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.72–1.80 (22H, m), 2.65–3.50 (6H, m), 3.97–4.46 (2H, m), 6.99–7.90 (9H, m); MS (FAB, Pos) m/z 482 (M^++1, 100%).

5.8.3. *N*,*N*-Dihexyl-3-(2-phenylbenzoimidazol-1-yl)propionamide hydrochloride (28). To a solution of **27** (433 mg, 0.899 mmol) in CH_3CO_2H (10.0 mL), iron powder (151 mg, 2.70 mmol) was added, and the mixture was heated at 120 °C for 2 h. The insoluble material was removed by filtration with a Celite filter, and the filtrate was evaporated under reduced pressure. The residue was dissolved in EtOAc and the solution was washed with satd $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [hexane/EtOAc, 2:1 (v/v), as eluent] to obtain a free base of **28** (0.38 g, 97%) as a colorless oil. Treatment of a solution of the free base of **28** in EtOAc with 4 M HCl/EtOAc and crystallized from diisopropylether to provide **28** (HCl salt) as a white crystal. Mp 101.0–103.0 °C; ^{1}H NMR (DMSO-d_6) δ 0.75–0.91 (6H, m), 1.00–1.43 (16H, m), 2.92 (2H, t, $J = 7.3$ Hz), 2.95–3.20 (4H, m), 4.68 (2H, t, $J = 7.3$ Hz), 7.53–7.97 (8H, m), 8.07–8.18 (1H, m); MS (CI, Pos) m/z 434 (M^++1, 100%); Anal. ($C_{28}H_{40}ClN_3O$) C, H, N.

5.9. General methods for the synthesis of 12b–f and 12i–m (method I)

5.9.1. 2-*tert*-Butoxycarbonylamino-2-(2-cyanobenzyl)malonic acid diethyl ester (31). Aminomalonic acid diethyl ester hydrochloride (25.1 g, 119 mmol) was dissolved in a mixture of 1 M NaOH solution (119 mL, 119 mmol) and 1,4-dioxane (100 mL). A solution of Boc_2O (28.5 g, 130 mmol) in 1,4-dioxane (50 mL) was added dropwise to the solution and the mixture was stirred at room temperature overnight. The solvent was removed by evaporation under reduced pressure and the residue was dissolved in EtOAc. The solution was washed with 5% $KHSO_4$ solution, satd $NaHCO_3$ solution and brine, dried ($MgSO_4$), and evaporated under reduced pressure

to provide crude 2-*tert*-butoxycarbonylaminomalonic acid diethyl ester **30** (33.2 g, quant.).

Na (492 mg, 21.4 mmol) was dissolved in EtOH (20 mL). To the solution, crude **30** (5.90 g, 21.4 mmol) and 2-cyanobenzyl bromide (4.00 g, 20.4 mmol) were added. The mixture was heated at reflux for 3.5 h. EtOH was removed by evaporation under reduced pressure and the residue was dissolved in EtOAc. The solution was washed with 5% $KHSO_4$ solution, satd $NaHCO_3$ solution and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [hexane/EtOAc, 5:1 (v/v), as eluent] to obtain **31** (8.50 g, quant.) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.29 (6H, t, $J = 7.1$ Hz), 1.47 (9H, s), 3.84 (2H, s), 4.02–4.46 (4H, m), 5.73 (1H, br s), 7.17–7.70 (4H, m); MS (ESI, Neg) m/z 389 (M^+-1, 100%).

5.9.2. (±)-2-*tert*-Butoxycarbonylamino-3-(2-cyanophenyl)-*N*,*N*-dihexylpropionamide (32). To a solution of **31** (1.17 g, 3.00 mmol) in a mixture of EtOH (20 mL) and H_2O (0.5 mL), NaOH (360 mg, 9.00 mmol) was added, and the solution was heated at reflux for 2 h. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in EtOAc and the solution was washed with 5% $KHSO_4$ solution, satd $NaHCO_3$ solution and brine, dried ($MgSO_4$), and evaporated under reduced pressure to provide crude 2-*tert*-butoxycarbonylamino-3-(2-cyanophenyl)propionic acid (0.81 g). To a mixture of crude 2-*tert*-butoxycarbonylamino-3-(2-cyanophenyl)propionic acid and dihexylamine (0.67 g, 3.6 mmol) in DMF (8.0 mL) were added HOBt (0.55 g, 3.6 mmol) and EDC·HCl (0.69 g, 3.6 mmol). After stirring at room temperature overnight, the reaction mixture was poured into EtOAc, washed with 5% $KHSO_4$ solution, satd $NaHCO_3$ solution and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [hexane/EtOAc, 4:1 (v/v), as eluent] to obtain **32** (1.01 g, 74%). ^{1}H NMR ($CDCl_3$) δ 0.72–1.68 (22H, m), 1.29 (9H, s), 2.79–3.70 (6H, m), 4.76–5.00 (1H, m), 5.27–5.48 (1H, m), 7.17–7.70 (4H, m); MS (FAB, Pos) m/z 458 (M^++1), 402 (M^+-55, 100%).

5.9.3. (±)-3-(2-Cyanophenyl)-*N*,*N*-dihexyl-2-(2-nitrophenylamino)propionamide (33). To a solution of **32** (0.98 g, 2.14 mmol) in CH_2Cl_2 (1.65 mL), trifluoroacetic acid (1.65 mL, 21.4 mmol) was added. The mixture was stirred at room temperature for 1.5 h. The solvent and trifluoroacetic acid were removed by evaporation under reduced pressure. The residue was dissolved in $CHCl_3$ and the resulting solution was washed with satd $NaHCO_3$ solution and brine, dried (Na_2SO_4), and evaporated under reduced pressure to obtain crude 2-amino-3-(2-cyanophenyl)-*N*,*N*-dihexylpropionamide (0.88 g). The crude 2-amino-3-(2-cyanophenyl)-*N*,*N*-dihexylpropionamide was dissolved in DMF (8 mL). 2-Fluoronitrobenzene (302 mg, 2.14 mmol) and K_2CO_3 (355 mg) were added to the solution. The mixture was heated at 150 °C for 2 h. The reaction mixture was diluted with EtOAc, washed with H_2O and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [hexane/EtOAc, 5:1 (v/v), as eluent] to obtain **33** (1.01 g, 74%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.67–1.68 (22H, m), 2.76–3.65 (6H, m), 4.81–5.05 (1H, m), 6.51–6.97 (2H, m), 7.10–7.75 (4H, m), 8.04–8.23 (1H, m), 8.50–8.72 (1H, m); MS (CI, Pos) m/z 479 (M^++1, 100%).

5.9.4. (±)-5,6-Dihydrobenzo[4,5]imidazo[2,1-*a*]isoquinoline-6-carboxylic acid dihexylamide (34a). To a solution of **33** (95 mg, 0.20 mmol) in MeOH (3.0 mL), PtO_2 (10 mg) was added. Under an H_2 atmosphere, the mixture was stirred at room temperature for 2 h. The catalyst was removed by filtration using a Celite filter, and the filtrate was concentrated under reduced pressure. The residue was passed through a silica gel short column to obtain crude 2-(2-aminophenylamino)-3-(2-cyanophenyl)-*N*,*N*-dihexylpropionamide (84 mg) as a dark red oil. The crude 2-(2-aminophenylamino)-3-(2-cyanophenyl)-*N*,*N*-dihexylpropionamide was dissolved in EtOH (5 mL). HCl gas was passed through the solution until it was saturated with HCl. The mixture was then stirred at room temperature for 4 h. The reaction mixture was poured into satd $NaHCO_3$ solution and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography [hexane/EtOAc, 2:1 (v/v), as eluent] and crystallized from EtOAc/hexane to obtain **34a** (24 mg, 24%) as a white crystal. Mp 134.5–136.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.72–1.90 (22H, m), 3.02–3.72 (6H, m), 5.40–5.54 (1H, m), 7.07–7.50 (6H, m), 7.75–7.91 (1H, m), 8.27–8.42 (1H, m); MS (FAB, Pos) m/z 432 (M^++1, 100%); Anal. ($C_{28}H_{37}N_3O$) C, H, N.

5.9.5. (±)-5,6-Dihydrobenzo[4,5]imidazo[2,1-*a*]isoquinoline-6-carboxylic acid diethylamide (34b). Starting from (±)-3-(2-cyanophenyl)-*N*,*N*-diethyl-2-(2-nitrophenylamino)-propionamide, **34b** was obtained as a crystal. Yield 15%; mp 206.5–207.5 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 1.12 (3H, t, $J = 7.0$ Hz), 1.41 (3H, t, $J = 7.0$ Hz), 3.17–3.78 (6H, m), 5.47 (1H, dd, $J = 6.8$, 4.8 Hz), 7.10–7.51 (6H, m), 7.75–7.90 (1H, m), 8.30–8.42 (1H, m); MS (CI, Pos) m/z 320 (M^++1, 100%); Anal. ($C_{20}H_{21}N_3O$) C, H, N.

5.9.6. (±)-5,6-Dihydrobenzo[4,5]imidazo[2,1-*a*]isoquinoline-6-carboxylic acid dipropylamide (34c). Starting from (±)-3-(2-cyanophenyl)-*N*,*N*-dipropyl-2-(2-nitrophenylamino)propionamide, **34c** was obtained as a crystal. Yield 14%; mp 138.5–140.5 °C (EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, t, $J = 7.4$ Hz), 1.08 (3H, t, $J = 7.4$ Hz), 1.35–1.97 (4H, m), 2.99–3.74 (6H, m), 5.48 (1H, dd, $J = 6.8$, 4.4 Hz), 7.08–7.50 (6H, m), 7.75–7.91 (1H, m), 8.28–8.42 (1H, m); MS (CI, Pos) m/z 348 (M^++1, 100%); Anal. ($C_{22}H_{25}N_3O$) C, H, N.

5.9.7. Peripheral benzodiazepine receptor (PBR) binding. Crude mitochondrial fractions prepared from rat cerebral cortex were used as a receptor sample, and [^{3}H]PK11195 was used as a [^{3}H]-labeled ligand. A binding assay using the [^{3}H]-labeled ligand was carried out according to the method reported by Romeo et al.[19]

Preparation of receptor sample: Rats were decapitated, the whole brain rapidly removed and the cerebral cortex homogenized in 10 vol of 10 mM HEPES buffer (pH 7.4) containing 0.32 M sucrose, using a Teflon homogenizer, and then centrifuged at 900*g* for 5 min. The supernatant was centrifuged at 12,000*g* for 10 min. The pellet (crude mitochondrial fraction) was washed with 50 mM HEPES buffer (pH 7.4) once, and suspended in 50 mM HEPES buffer (pH 7.4) at a protein concentration of 0.3 mg/mL.

PBR binding assay: The crude mitochondrial preparation (1 mL) was incubated with [^{3}H]PK11195 (2 nM) and the test sample for 90 min at 4 °C, and the reaction was stopped by rapid filtration through a GF/B glass filter presoaked with 0.3% polyethyleneimine, after which the filter was washed three times with 3 mL of the buffer. Radioactivity was quantified in a liquid scintillation spectrometer. Nonspecific binding was determined in the presence of 10 μM PK11195. Specific binding was determined by subtracting nonspecific from total binding. [^{3}H]PK11195 (2 nM) was reacted with each concentration of the test sample under the above-mentioned conditions to obtain an inhibition curve, and the concentration (IC_{50}) of the test sample exhibiting 50% inhibition of [^{3}H]PK11195 binding was obtained from the inhibition curve.

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