Novel Indole-Based Peroxisome Proliferator-Activated Receptor Agonists: Design, SAR, Structural Biology, and Biological Activities

Neeraj Mahindroo,† Chien-Fu Huang,† Yi-Huei Peng,† Chiung-Chiu Wang,† Chun-Chen Liao,‡ Tzu-Wen Lien,† Santhosh Kumar Chittimalla,‡ Wei-Jan Huang,‡ Chia-Hua Chai,† Ekambaranellore Prakash,† Ching-Ping Chen,† Tsu-An Hsu,†,# Cheng-Hung Peng,† I-Lin Lu,† Ling-Hui Lee,† Yi-Wei Chang,† Wei-Cheng Chen,† Yu-Chen Chou,† Chiung-Tong Chen,† Chandra M. V. Goparaju,† Yuan-Shou Chen,† Shih-Jung Lan,† Ming-Chen Yu,† Xin Chen,† Yu-Sheng Chao,† Su-Ying Wu,*,† and Hsing-Pang Hsieh*,†

Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, 35 Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, Republic of China, and Department of Chemistry and Department of Chemical Engineering, National Tsing Hua University, Hsinchu 300, Taiwan, Republic of China

Received July 20, 2005

The synthesis and structure−activity relationship studies of novel indole derivatives as peroxisome proliferator-activated receptor (PPAR) agonists are reported. Indole, a druglike scaffold, was studied as a core skeleton for the acidic head part of PPAR agonists. The structural features (acidic head, substitution on indole, and linker) were optimized first, by keeping benzisoxazole as the tail part, based on binding and functional activity at PPARγ protein. The variations in the tail part, by introducing various heteroaromatic ring systems, were then studied. In vitro evaluation led to identification of a novel series of indole compounds with a benzisoxazole tail as potent PPAR agonists with the lead compound **14** (BPR1H036) displaying an excellent pharmacokinetic profile in BALB/c mice and an efficacious glucose lowering activity in KKA^y mice. Structural biology studies of **14** showed that the indole ring contributes strong hydrophobic interactions with PPARγ and could be an important moiety for the binding to the protein.

Introduction

Type 2 diabetes, a chronic disease characterized by the failure to respond to insulin, has assumed epidemic proportions, according to the WHO.[1] Currently more than 194 million people suffer from diabetes worldwide. The number is expected to exceed 333 million by 2025.[2] The single most important contributor to the pathogenesis of diabetes is obesity, which is increasing at a staggering pace with changing lifestyles and food habits.[3]

Most of the current therapies for type 2 diabetes were developed in the absence of defined molecular targets or an understanding of the disease pathogenesis and have a number of adverse effects, compromising the quality of life of the patients. But the emerging knowledge of the key pathogenic mechanisms has led to identification of a number of molecular drug targets during the last decade.[3] Peroxisome proliferator-activated receptors (PPAR), consisting of three subtypes with distinct genes, PPARα, PPARγ, and PPARδ, are members of the nuclear receptor family.[4] PPAR agonists (Figure 1) offer a promising approach to type 2 diabetes and the associated metabolic syndrome that includes obesity, hypertension, and dyslipidemia. PPARγ agonists, **1** (rosiglitazone) and **2** (pioglitazone), were launched in 1999 and registered increases in sales by 73.2% and 53.1%, respectively, in 2001, capturing 17% of the oral antidiabetic market within 2 years.[5,6] However, the mechanism-based side effects, including weight gain, fluid retention and edema, along with adipose tissue proliferation, fatty changes in bone marrow and significant increase in heart weight of rodents,[7,8] have triggered a reevaluation of the design of PPAR agonists. The addition of PPARα activity in PPARγ agonists could improve the profile of the PPAR agonists. A number of

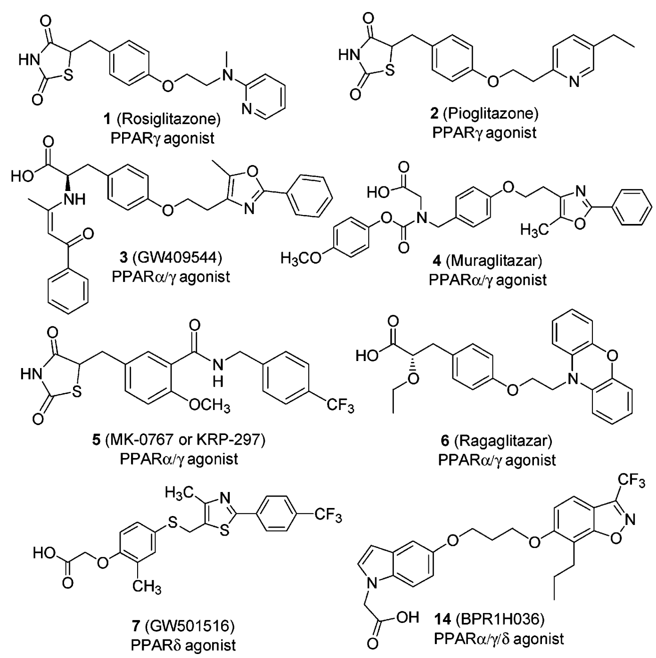


Figure 1. PPAR agonists.

* To whom correspondence should be addressed. S.-Y. Wu: phone 886-37-246-166 ext 35713; fax 886-37-586-456; e-mail suying@nhri.org.tw. H.-P. Hsieh: phone 886-37-246-166 ext 35708; fax 886-37-586-456; e-mail hphsieh@nhri.org.tw.

† National Health Research Institutes.

‡ Department of Chemistry, National Tsing Hua University.

\# Department of Chemical Engineering, National Tsing Hua University.

10.1021/jm0506930 CCC: $30.25
Published on Web 11/18/2005

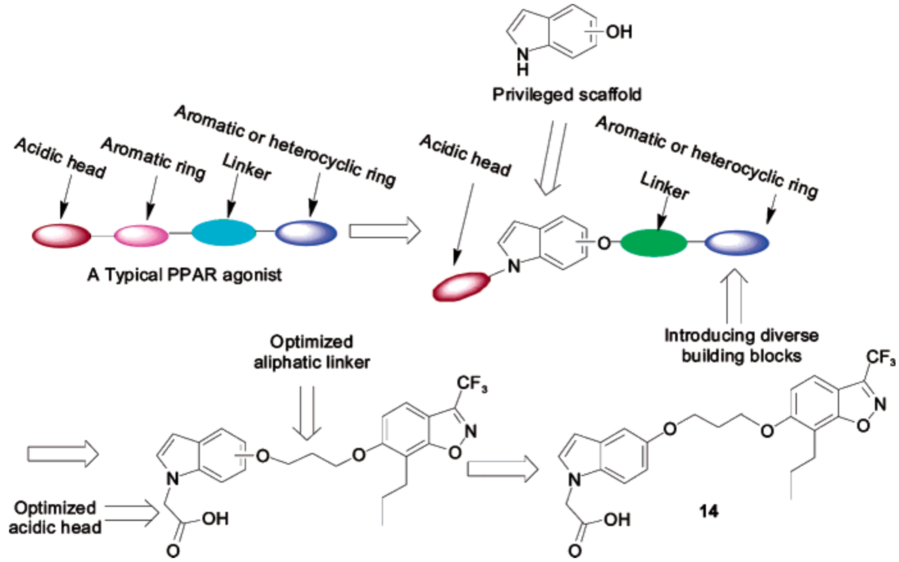


Figure 2. Design of indole-based PPAR agonists.

PPARα/γ dual agonists, such as **3**−**6**, have been reported in this class of compounds[9−11] and have shown robust insulin-sensitizing and hypolipidemic activities in clinical trials. The new drug application for muraglitazar (Bristol-Meyers Squibb/Merck) was filed with the US FDA in December 2004.[12,13]

PPARδ initially received the least attention of the three isoforms of PPAR nuclear receptors because of its ubiquitous expression and the unavailability of selective ligands. However, recently reported synthetic PPARδ agonists, such as **7**, have helped to reveal its role as a powerful regulator of fatty acid catabolism and energy homeostasis, retarding weight gain and improving insulin resistance, thus demonstrating their potential therapeutic value in diabetes and obesity.[14] Some PPAR pan-agonist compounds with potential antihyperglycemic, lipid-modulating, and insulin-sensitizing activity have been reported recently[15−17] and have evoked much interest with their evolving pharmacology. A PPAR pan-agonist GW677954 from GlaxoSmithKline is currently in Phase II clinical trials for type 2 diabetes,[18] while Plexxikon has completed the preclinical studies for its pan-agonist, PLX204 and plans to start clinical studies in 2005.[19]

Keeping in view the potential of PPAR agonist compounds as treatment for clinical conditions in metabolic disorders, we report the design, synthesis, structure−activity relationships (SAR), X-ray crystallographic studies, and biological studies of novel, highly potent indole-based PPAR agonists. A typical PPAR agonist usually has an acidic head attached to an aromatic scaffold, a linker, and a hetero-aromatic hydrophobic tail (Figure 2). Following a similar model, we designed novel indole-based PPAR agonists optimizing each essential part, acidic head, linker, and hydrophobic tail. Some indole 5-acetic acids with potent PPARγ agonist activity and high selectivity have been reported earlier.[20,21] We decided to use indole as a core skeleton for the acidic head, placing the acidic head at the N1-position, in place of the tyrosine-based acidic heads used in many of the reported PPAR agonists, and placing the tail part on the aromatic ring of indole attached through a linker. Indole, besides being a versatile drug-like scaffold, provided a simple synthetic pathway to the desired compounds starting from commercially available hydroxyindoles.

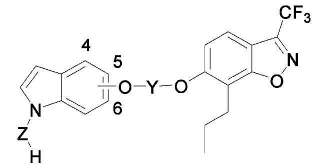
The 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol has been reported as a potent building block for hydrophobic tail in the literature.[16] We incorporated it as the tail part to study whether hydroxyindole with an acidic head at N1-position could be a replacement for the tyrosine-based scaffolds and to establish the initial structure−activity relationships to optimize the linker, substitution on the indole scaffold, and the acidic head. A series of compounds (**11**−**27**, Table 1) were synthesized with the 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol as a building block for the hydrophobic tail following the general synthetic scheme (Scheme 1).

Once the head and linker were optimized, compounds **28**−**35** (Table 2) with different hydrophobic tail parts were synthesized (Scheme 3) to optimize the tail part and to study the effect on the activity and selectivity of various heteroaromatic five,six-membered fused ring systems, such as benzisoxazole, indole, and benzofuran. The heteroaromatic building blocks were selected from either PPAR agonists reported in the literature or some newly synthesized indole and benzofuran-based compounds. The preliminary structure−activity relationships were studied on the basis of binding and functional assays for the human PPARγ protein. The potent compounds (Table 3) were evaluated for selectivity by determining hPPARα and hPPARδ functional activity. Based on the results from the binding and functional assays, compound **14** was selected for detailed biochemical, in vivo, pharmacokinetic, and structural biology studies.

Results and Discussion

Chemistry. The desired compounds with a 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol tail (**11**−**27**, Table 1) were synthesized starting from the commercially available hydroxyindoles **8** as shown in general synthetic Scheme 1. The hydroxyindole was alkylated with an appropriate bromochloroalkane in the presence of an equimolar amount of potassium hydroxide in DMSO or

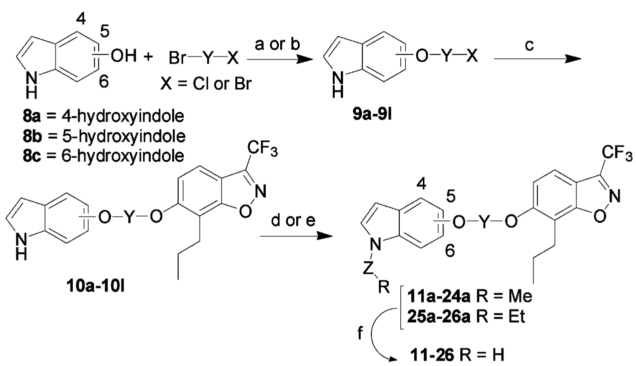
Table 1. In Vitro Human PPARγ Activities of **11**–**27**



compd	indole position	Y	Z	PPARγ (μM)[c] binding IC_{50}[a]	transactivation EC_{50}[b]
11	4	$(CH_2)_2$	CH_2CO_2	1.495	>10
12	5	$(CH_2)_2$	CH_2CO_2	0.422	0.690
13	4	$(CH_2)_3$	CH_2CO_2	1.986	2.393
14	5	$(CH_2)_3$	CH_2CO_2	0.152	0.230
15	6	$(CH_2)_3$	CH_2CO_2	1.047	1.350
16	4	$(CH_2)_4$	CH_2CO_2	0.774	1.981
17	5	$(CH_2)_4$	CH_2CO_2	0.105	0.280
18	4	$(CH_2)_5$	CH_2CO_2	1.784	>10
19	5	$(CH_2)_5$	CH_2CO_2	1.050	1.490
20	4	$CH_2CH(CH_3)CH_2$	CH_2CO_2	1.298	3.030
21	4	$(CH_2)_2CH(CH_3)(CH_2)_2$	CH_2CO_2	1.452	>10
22	5	$(CH_2)_2CH(CH_3)(CH_2)_2$	CH_2CO_2	1.368	>10
23	4	$(CH_2)_3$	$(CH_2)_2CO_2$	1.098	2.190
24	5	$(CH_2)_3$	$(CH_2)_2CO_2$	0.584	1.200
25	4	$(CH_2)_3$	$CH(C_2H_5)CO_2$	1.282	>10
26	5	$(CH_2)_3$	$CH(C_2H_5)CO_2$	1.709	5.900
27	5	$(CH_2)_3$	CH_2-tetrazole	1.123	1.490
rosiglitazone				0.092	0.220

[a] Concentration of the test compound required to displace 50% of tritiated ligand. [b] Concentration of test compound that produced 50% of the maximal reporter activity. [c] All data within ± 15% (n = 3).

Scheme 1. General Synthetic Scheme for **11**–**26**[a]



compd	indole position	Y	compd	indole position	Y
9a, 10a	4	$(CH_2)_2$	**9g, 10g**	5	$(CH_2)_4$
9b, 10b	5	$(CH_2)_2$	**9h, 10h**	4	$(CH_2)_5$
9c, 10c	4	$(CH_2)_3$	**9i, 10i**	5	$(CH_2)_5$
9d, 10d	5	$(CH_2)_3$	**9j, 10j**	4	$CH_2CH(CH_3)CH_2$
9e, 10e	6	$(CH_2)_3$	**9k, 10k**	4	$(CH_2)_2CH(CH_3)(CH_2)_2$
9f, 10f	4	$(CH_2)_4$	**9l, 10l**	5	$(CH_2)_2CH(CH_3)(CH_2)_2$

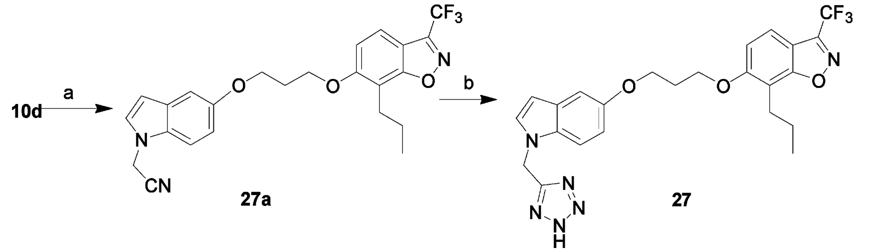
[a] Reagents: (a) KOH, DMSO, rt; (b) K_2CO_3, 2-butanone, reflux; (c) 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol, K_2CO_3, KI, DMF, 110 °C; (d) methyl-2-bromoalkylate, K_2CO_3, KI, CH_3CN, reflux; (e) ethyl acrylate, Cs_2CO_3, CH_3CN, rt; (f) LiOH, MeOH, H_2O, reflux.

potassium carbonate in 2-butanone, yielding **9a**–**9l**. Compounds **9a**–**9l** were coupled with the 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol, which was prepared by using a modification of the Adams et al. procedure,[22] to give **10a**–**10l**. The esters **11a**–**24a** were obtained by refluxing the appropriate compound from **10a**–**10l** with methyl-2-bromoalkylate in the presence of potassium carbonate and potassium iodide in acetonitrile. The esters **25a** and **26a** were prepared by stirring the mixture of **10c** and **10d**, respectively, with ethyl acrylate and potassium carbonate in acetonitrile at room temperature. Finally, compounds **11a**–**26a** were deprotected with lithium hydroxide in a methanol–water mixture to afford **11**–**26**. The tetrazole **27** was synthesized from **10d** in two steps as shown in Scheme 2. Compounds **28**–**35** (Table 2) with various heteroaromatic building blocks replacing 3-trifluoromethylbenzisoxazole as the tail part were synthesized as depicted in Scheme 3.

SAR Studies. The aim of the present study was to identify novel potent indole-based PPAR agonists with better profiles as possible drug candidates for type 2 diabetes. The synthesized compounds **11**–**35** were evaluated for the in vitro binding and transactivation at hPPARγ protein (Tables 1 and 2) to identify the optimum characteristics of the compounds for PPARγ agonist activity. The benzisoxazole analogues **11**–**27** (Table 1) displayed varying degrees of binding and transactivational activities thus establishing the viability of indole as a core skeleton for the acidic head. This set of compounds also elucidated the SAR for the acidic head and linker.

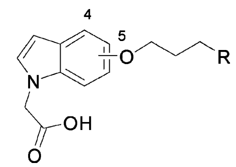
The 5-substituted indole analogue **14** with an *n*-propyl linker showed more potent binding and functional activity as compared to the 4- and 6-substituted analogues **13** and **15**, indicating that the 5-position of indole was most appropriate for the attachment to the linker with both 4-position and 6-position analogues showing weak activity. This variation in activity may be due to the most appropriate orientation provided by the 5-substituted indoles in binding with protein. Compounds **14** and **17**, with *n*-propyl and *n*-butyl linkers, respectively, attached to the 5-position of indole, exhibited potent binding as well as functional activities. The change in linker to ethyl as in compound **12** decreased the potency by 2-fold, while an *n*-pentyl linker (**19**) substantially decreased the activity. These results showed that an

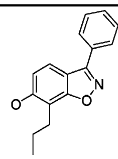
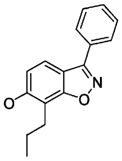
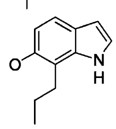
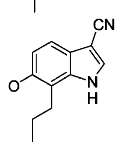
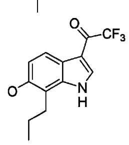
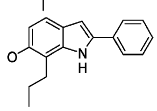
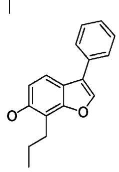
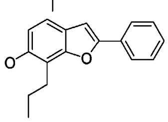
Scheme 2. Synthetic Scheme for **27**[a]



[a] Reagents: (a) chloroacetonitrile, KO^{t}Bu, KI, CH_3CN, reflux, 12 h; (b) TMS-N_3, Bu_2SnO, toluene, 110 °C, 4 h.

Table 2. In Vitro Human PPARγ Activities of **28**–**35**

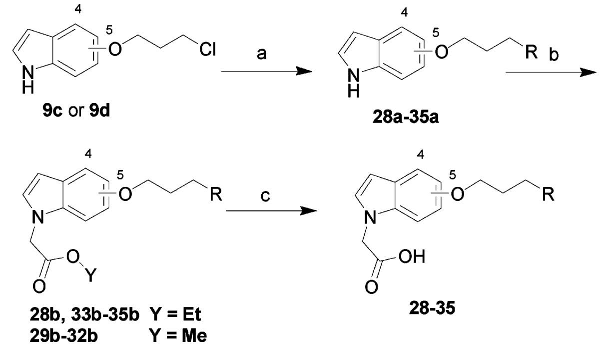


compd	indole position	R	PPARγ (μM)[c] binding IC_{50}[a]	PPARγ (μM)[c] transactivation EC_{50}[b]
28	4		0.127	0.430
29	5		0.120	0.290
30	5		4.809	1.810
31	5		0.759	9.170
32	5		2.405	>10
33	4		1.679	2.820
34	4		3.968	4.150
35	4		1.948	>10
Rosiglitazone			0.092	0.220

[a] Concentration of the test compound required to displace 50% of tritiated ligand. [b] Concentration of test compound that produced 50% of the maximal reporter activity. [c] All data within ± 15% (n=3).

n-propyl or n-butyl linker was most appropriate, while an ethyl linker slightly decreased the activity; any further increase in linker length was detrimental to activity. The 4-substituted indole analogues with ethyl, n-butyl, and n-pentyl linkers (**11**, **16**, and **18**, respectively) were also evaluated to see whether change in linker length could compensate for change in position on indole. All three compounds, **11**, **16**, and **18**, were less potent than their 5-position analogues, **12**, **17**, and **19**, respectively; but **16** with an n-butyl linker was more potent than **13** with an n-propyl linker, thus indicating that increase in linker length to n-butyl might restore some activity in case of 4-substituted indoles. Introduction of methyl substitution on the linker (**20**–**22**) led to further decrease in activity.

Scheme 3. General Synthetic Scheme for **28**–**35**[a]



[a] Reagents: (a) RH, K_2CO_3, KI, DMF, 110 °C; (b) alkyl-2-bromoacetate, K_2CO_3, KI, CH_3CN, reflux; (c) LiOH, MeOH, H_2O, reflux.

Table 3. In Vitro hPPARγ, hPPARα, and hPPARδ Transactivation of Selected Compounds

compd	transactivation PPARγ (μM)[a,b]	transactivation PPARα (μM)[a,b]	transactivation PPARδ (μM)[a,b]
12	0.690	0.311	0.125
13	2.393	0.186	0.610
14	0.230	0.014	0.010
15	1.350	1.819	0.080
16	1.981	>10	>10
17	0.280	1.037	0.243
27	0.898	0.474	0.370
28	0.430	0.002	0.320
29	0.290	0.018	0.080
30	1.810	1.209	0.420
31	9.170	5.211	2.620
32	>10	2.200	9.170
33	2.820	0.500	3.340
34	4.150	0.169	2.130
35	>10	>10	7.740
rosiglitazone	0.220		

[a] Concentration of test compound that produced 50% of the maximal reporter activity. [b] All data within ± 15% (n = 3).

The increase in distance of carboxylic acid from the indole nitrogen (**23**, **24**) also resulted in decreased activity. Similarly, introduction of an ethyl substitution on the acidic head (**25**, **26**) resulted in decrease in activity. The bioisosteric replacement of the carboxylic acid with tetrazole (**27**) resulted in 4-fold decrease in binding activity, thus indicating that the acetic acid was the most appropriate acidic head and increase in bulk might decrease the activity. Compounds without an acidic head (**10c**, **10d**) were inactive both in binding and in functional assays, thus indicating that the acidic head was essential for activity. To summarize, the *n*-propyl or *n*-butyl linker, 5-substituted indole, and acetic acid head were most appropriate for potent PPARγ binding and functional activity. Compound **14** with an *n*-propyl linker substituted on the 5-position of indole and an acetic acid head showed the most potent binding and functional activities at PPARγ.

Next, we decided to replace the 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol with various building blocks to study the effect of various heteraromatic five,six-membered fused ring systems as the tail part (Table 2). The 3-phenyl-7-propyl-benzo[*d*]isoxazol-6-ol[17] and 3-phenyl-7-propylbenzofuran,[16] with reported potential contributions to PPARγ agonist activity, were selected from the literature. Some new indole- and benzofuran-based building blocks were synthesized to study the effect of substitution of groups such as hydrogen, cyano, $COCF_3$, and phenyl at the 3-position. The hydroxyl and propyl groups were retained at the 6- and 7-positions, respectively, in all the newly synthesized building blocks to mimic the 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol. These heteroaromatic systems also gave the flexibility to move the phenyl group to the 2-position.

Compounds **28** and **29** with a 7-propyl-3-phenylbenzo[*d*]isoxazol-6-ol tail showed potent activity in binding and functional assays with a 5-substituted indole, **29**, being slightly more potent than a 4-substituted one, **28**. The 3-phenyl substituted **28** was much more potent as compared to 3-trifluoromethyl substituted **13**, though a similar difference in potency was not seen in case of the corresponding 5-indole substituted analogues **29** and **14**. All other analogues (**30**−**35**) with the indole- and benzofuran-based tails showed substantial decreases in the functional activities. The 3-cyanoindole analogue exhibited submicromolar PPARγ binding activity but a weak functional activity in a cell-based transactivation assay. The benzisoxazole-based tails were the most suitable building blocks in the present study with the 3-trifluoromethyl (**14**) and 3-phenyl (**28**, **29**) substituted compounds, showing potent binding and transfection activities at all three subtypes. The structure−activity relationships are summarized in Figure 3.

Compounds with potent PPARγ functional activity and different tail groups were evaluated for PPARα and PPARδ functional activities to determine the selectivity (Table 3). The benzisoxazole analogues **14**, **28**, and **29** displayed potent activities at all three PPAR subtypes. The *n*-butyl linker analogue of **14**, **17**, showed potent PPARγ and PPARδ activities but a 70-fold decrease in PPARα activity. Compound **13** with the benzisoxazole tail attached to the 4-position of the indole through an *n*-propyl linker exhibited dual PPARα/δ activity. Compound **30** with the 7-propylindole as tail showed selectivity for PPARδ as compared to PPARα and PPARγ.

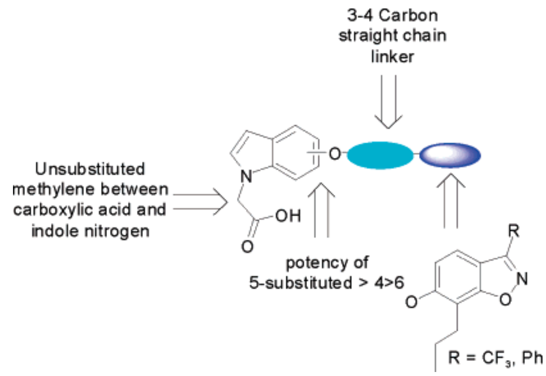


Figure 3. Structure−activity relationships.

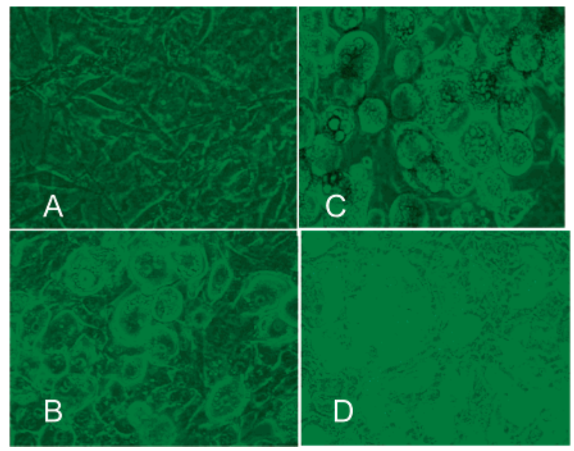


Figure 4. 3T3-L-1 adipocyte differentiation on treatment with (A) negative control, (B) positive control [1-methyl-3-isobutylxanthine (0.25 mM), dexamethasone (1 μM), and insulin (2 μM)], (C) **1** (4 μM), and (D) **14** (4 μM).

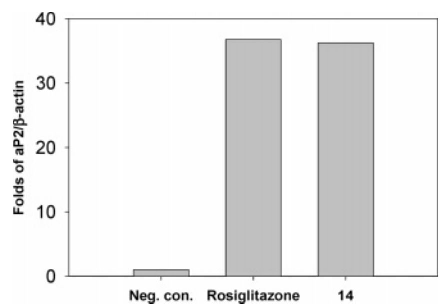


Figure 5. aP2 gene induction after treatment with negative control, **1** (10 μM), and **14** (10 μM).

Compounds **33** and **34** showed selective PPARα agonist activity. The results indicated that variation in the linker length, the position on indole, and the tail building block affects the selectivity toward the PPAR subtypes. The benzisoxazole building block, 5-substituted indole, and *n*-propyl linker favors potent PPARγ activity. Compound **14** with all these features exhibited PPARγ transactivation activity similar to that of rosiglitazone. It also displayed potent functional activities for PPARα and PPARδ. Based on the in vitro functional activities, it was selected for further biochemical, in vivo, pharmacokinetic, and structural biology studies.

Biological Studies. Compound **14** showed 3T3-L1 adipocyte differentiation (Figure 4) and aP2 gene induction (Figure 5) similar to those of the rosiglitazone. It also exhibited strong insulin sensitizer activity in a 2-deoxyglucose uptake assay (Figure 6). The overall in

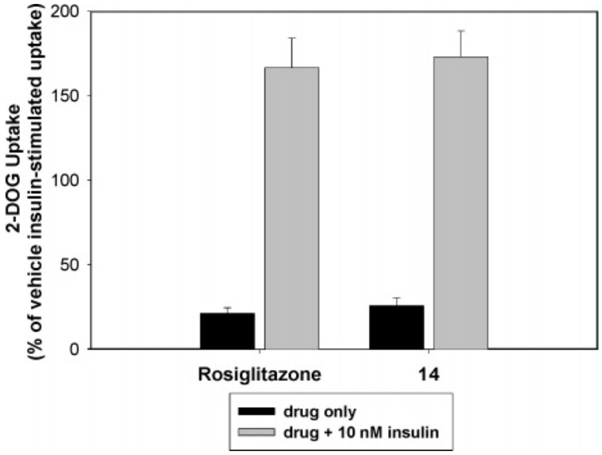


Figure 6. 3T3-L1 adipocytes (day 7 postdifferentiation) cultured in 12-well plates were supplemented with 10^{-5} M agonist in DMEM/FBS for 48 h and serum-starved in DMEM for 2 h. 2-Deoxyglucose uptake was measured over 5 min following stimulation ± 10 nM insulin for 30 min. Data are mean uptakes ± SE from one experiment performed in triplicate, normalized to vehicle insulin-stimulated uptake (mean insulin responses 7067.333 cpm/well).

Table 4. Pharmacokinetic Parameters of the Sodium Salt of **14** in Male BALB/c Mice[a]

pharmacokinetic parameter	po	iv
dose (mg/kg)	35.0	9.7
$AUC_{(0-t)}$ (ng h/mL)[b]	652 000 ± 60 900	181 000 ± 54 300
C_{max} (ng/mL)[c]	59 000 ± 9 830	
T_{max} (h)[d]	4.7 ± 1.2	
$T_{1/2}$ (h)[e]	7.8 ± 0.7	6.9 ± 0.4
V_{ss} (L/kg)[f]		0.40 ± 0.17
CL (mL/(min·kg))[g]		0.90 ± 0.34
F (%)[h]	100	

[a] Each value is mean ± SD, $n = 3$. [b] Estimated area under the plasma concentration vs time curve after intravenous and oral dosing, $t = 24$ h. [c] Maximum plasma concentration after oral dosing. [d] Time taken to achieve maximum concentration. [e] Half-life. [f] Volume of distribution during steady state. [g] Clearance. [h] Oral bioavailability.

vitro profile of **14** indicated that it could be a very promising antidiabetic candidate.

Pharmacokinetic studies conducted in the male BALB/c mice with the sodium salt of **14** indicated that it had 100% oral bioavailability and excellent pharmacokinetic parameters with a large AUC, low clearance, and reasonably long half-life (Table 4).

In the obese, insulin-resistant KKA^y mice with elevated plasma glucose levels, 10 days of treatment with the sodium salt of **14** showed a significant reduction in plasma glucose levels at 10 mg/kg and 30 mg/kg dose levels (Figure 7). The glucose levels returned to the pretreatment levels after the treatment was stopped.

Cocrystallization with hPPARγ. To further understand the interaction with the hPPARγ receptor, crystals of the hPPARγ ligand-binding domain (LBD) in a complex with **14** were prepared by cocrystallization. The cocrystal structure was solved to a resolution of 2.28 Å. The asymmetric unit contained two monomers, monomer A and monomer B. The electron density maps of the PPARγ LBD were clear, except for some disordered regions, including residues 264−273 in both monomers and the residues 468−477 in monomer B. The loop of residues 264−273 was located at the

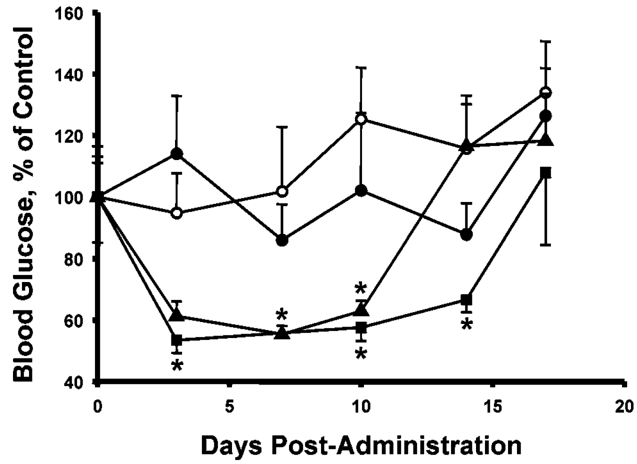


Figure 7. In vivo efficacy of the sodium salt of **14** in male KKA^y mice. The obese, insulin-resistant KKA^y mice were treated for 10 days (day 1−10) with control (○), 1 mg/(kg·day) (●), 10 mg/(kg·day) (▲), and 30 mg/(kg·day) (■) of sodium salt of **14**, and the blood glucose levels were monitored until day 17. Data are the mean ± SD of % of blood glucose level of control at each point ($n = 7$, * indicates $p < 0.05$ vs control).

entrance of the ligand-binding site and was highly flexible in all published structures. Its flexibility could allow access of a large ligand, such as **14**, to the binding pocket of PPARγ.

The electron density map of **14** bound in the binding site was very clear, and the model of the compound fitted well into the density map. It was found that two molecules of **14** bound to the two monomers (one to each) unlike other reported complex structures,[23,24] where only one molecule bound to the homodimer (one monomer contained the molecule, and the other monomer was unoccupied).

The overall structure of the PPARγ−**14** complex was very similar to the published PPARγ apo and complex structures, except for differences in some flexible loops, including residues 264−273. When the binding site of the PPARγ−**14** complex structure was compared to the published structures, the most significant movement was the shift of the residues 364 and 363 (Figure 8). The residue Met364 moved away from the active site upon ligand binding to accommodate the linker group of **14**. This movement consequently resulted in the shift of its adjacent residue, Phe363. In addition, residues 282, 286, and 289 also slightly shifted to enable better interaction with the ligand.

Protein−Ligand Interaction. Compound **14** bound to each monomer and adopted a very similar conformation in both, except for a minor rotation of its carboxylic acid head. The structure of monomer A in complex with **14** is presented to discuss the interaction between PPARγ and **14** (Figure 9).

The carboxylic acid head of **14** formed hydrogen bonds with Tyr473, His449, His323, and Ser289, where Tyr473 was located in the AF-2 helix. This hydrogen bonding pattern is conserved in most PPAR−agonist complex structures and is essential for the activity of the ligand. The indole ring of **14** was perpendicular to His449 and formed a hydrophobic interaction with it. This hydrophobic interaction was a unique feature in the PPARγ−**14** structures, because other reported complex structures had only a hydrogen bonding interaction with

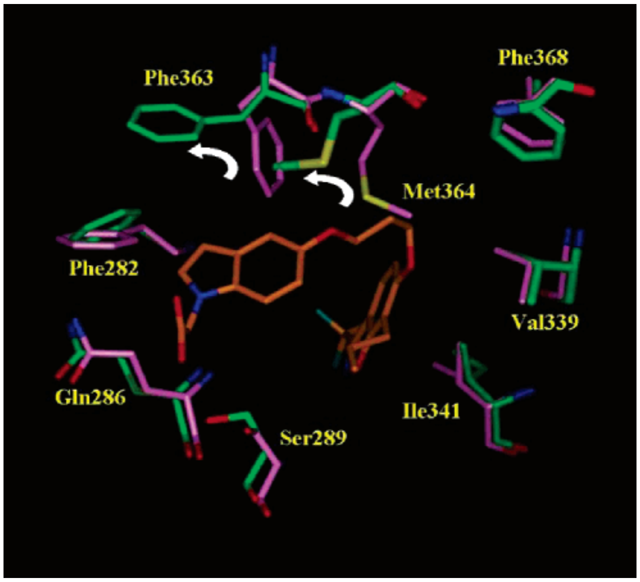


Figure 8. The shift in residues of PPARγ protein (purple before ligand binding, green after ligand binding) upon binding of ligand **14** (orange) with monomer A of PPARγ protein. The arrows depict the shift of residue Met364 away from the active site to accommodate the linker group of **14** and the consequent shift of residue Phe363.

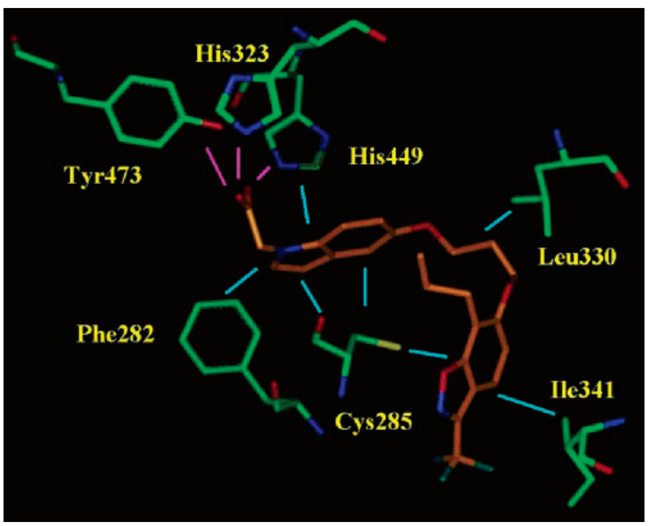


Figure 9. The structure of monomer A of PPARγ in the complex with **14** (orange) is depicted. The hydrogen bonding interactions are shown as solid lines in magenta, and the hydrophobic interactions are shown in cyan.

His449. In addition to His449, the indole ring formed hydrophobic interactions with Cys285 and Phe282. The indole ring contributed strong hydrophobic interactions with PPARγ and could be an important moiety for the binding of compound **14** to the protein. Moreover, the indole ring could position the carboxylic head into the proper orientation for interaction with the AF-2 helix. The linker of **14** was close to the hydrophobic pocket of PPAR that consisted of Leu330, Met334, Phe368, Val339, and Met364. Finally, the tail moiety of **14** extended toward the lower part of the binding site and formed a hydrophobic interaction with Cys285. The substituted propyl group provided an additional interaction with Arg288 and the substituted CF_3 group had close contacts with Ile281, Ile341, and Met348.

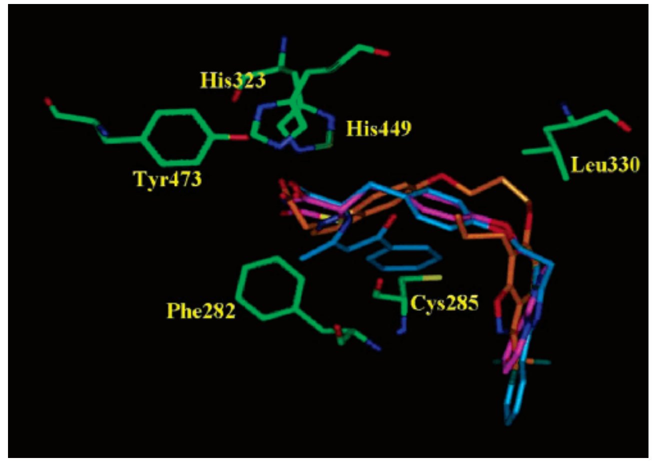


Figure 10. Superposition of the structures of **14** (orange), **1** (magenta), and **3** (blue) in the binding pocket of PPARγ.

Structural Comparison of 14, 1 (Rosiglitazone), and 3. Superimposition of PPARγ LBD in the complexes with **14**, **1**,[25] and **3**[26] revealed differences in the protein–ligand interactions (Figure 10). First, **14** formed both hydrogen bonding and hydrophobic interactions with His449, while the only interaction between rosiglitazone and His449 was a hydrogen bonding interaction. The additional hydrophobic interaction of compound **14** with His449 was caused by the particular position of the indole ring. The indole ring of **14** moved toward the AF-2 helix region and adopted a conformation perpendicular to His449. Moreover, the indole ring also formed a strong hydrophobic interaction with Phe282. This interaction was absent in the **1** and **3** structures. In addition, the linker of **14** was 4 Å from the hydrophobic pocket of PPARγ, 2.7 and 2.6 Å closer than that for **1** and **3**, respectively, thus providing additional hydrophobic interactions with the protein.

Conclusion

In conclusion, a novel indole-based series of PPAR agonist compounds were synthesized and the structure–activity relationships were elucidated on basis of binding and functional activities at the PPARγ protein. The *n*-propyl or *n*-butyl linker, 5-substituted indole, and the acetic acid head were most appropriate for potent PPARγ binding and functional activity with a benzisoxazole tail. The compounds with 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol and 7-propyl-3-phenylbenzo[*d*]isoxazol-6-ol tail part showed most potent activity. Based on the in vitro binding and transactivation studies, compound **14** was selected for the biochemical and in vivo evaluation. In vitro biochemical studies such as glucose uptake, adipocyte differentiation, and insulin sensitizer activity showed that **14** is an excellent candidate for further studies as an antidiabetic agent. The compound demonstrated highly efficacious glucose-lowering activity in in vivo studies in KKA^y mice, and an excellent pharmacokinetic profile. The structural biology studies of **14**, a compound belonging to a new chemical class with indole as a core skeleton for the acidic head unlike many tyrosine-based PPAR agonists, revealed that the indole ring contributed strong hydrophobic interactions with PPARγ and could be an important moiety for the binding to the protein. Compound

14 is currently undergoing additional evaluation to further assess the potential for development for the management of type 2 diabetes and syndrome X.

Experimental Section

Chemistry. Nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a Varian Mercury-300 spectrometer operating at 300 MHz, a Varian Mercury-400 at 400 MHz, or a Bruker DMX-600 at 600 MHz with chemical shift in parts per million (ppm, δ) downfield from TMS as an internal standard. High-resolution mass spectra (HRMS) were measured with a Finnigan (MAT-95XL) electron impact (EI) mass spectrometer. Analytical purity was assessed by RP-HPLC using an Agilent (Hewlett-Packard) 1100 series system equipped with a diode array spectrometer. Flash column chromatography was done using silica gel (Merck Kieselgel 60, No. 9385, 230-400 mesh ASTM). Reactions were monitored by TLC using Merck 60 F_{254} silica gel glass-backed plates (5 cm × 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) or by spraying with phosphomolybdic acid reagent (Aldrich) followed by heating at 80 °C. All solvents were dried according to standard procedures. All reagents were used as purchased without further treatment unless otherwise stated. All reactions were carried out under an atmosphere of dry nitrogen.

4-(2-Chloropropoxy)-1*H*-indole (9a). A mixture of 4-hydroxyindole (**8a**) (0.100 g, 0.75 mmol), K_2CO_3 (0.156 g, 1.13 mmol), 1-bromo-2-chloroethane (0.129 g, 0.90 mol), and 2-butanone (10 mL) was heated to reflux for 12 h and allowed to cool to ambient temperature. The solids were filtered, and the solvent was removed under vacuum to afford an oily residue. The oil was dissolved in ethyl acetate (30 mL), washed with water (2 × 25 mL), followed by brine (1 × 20 mL), and dried over anhydrous sodium sulfate, and the solvent was removed in vacuo. The residue was chromatographed over silica gel eluting with *n*-hexane/ethyl acetate (95:5) to give compound **9a** (99 mg, 67%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.89 (t, J = 6.0 Hz, 2H), 4.39 (t, J = 6.0 Hz, 2H), 6.51 (d, J = 6.9 Hz, 1H), 6.59−6.61 (m, 1H), 6.94 (d, J = 8.4 Hz, 1H), 7.00 (t, J = 2.7 Hz, 1H), 7.07 (t, J = 7.8 Hz, 1H), 8.03 (br s, 1H). MS (ESI *m/z*) 196.1 $(M + H)^+$.

5-(2-Chloropropoxy)-1*H*-indole (9b). This compound was prepared as described in the case of **9a**, starting from 5-hydroxyindole (**8b**), giving a 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (t, J = 6.0 Hz, 2H), 4.25 (t, J = 6.0 Hz, 2H), 6.45−6.47 (m, 1H), 6.80 (dd, J = 2.4, 8.7 Hz, 1H), 7.11 (d, J = 2.7 Hz, 1H), 7.15 (t, J = 2.7 Hz, 1H), 7.25 (d, J = 8.7 Hz, 1H), 8.06 (br s, 1H). MS (ESI *m/z*) 196.1 $(M + H)^+$.

5-(3-Chloropropoxy)-1*H*-indole (9d). A mixture of 5-hydroxyindole (**8b**) (0.500 g, 3.76 mmol), powdered potassium hydroxide (0.211 g, 3.76 mmol), and DMSO (10 mL) was stirred at room temperature for 10 min, and then 1-bromo-3-chloropropane (0.590 g, 3.76 mmol) was added. This was stirred at room temperature for 0.5 h, and then water (15 mL) was added. The mixture was extracted with ethyl acetate (2 × 30 mL). The combined organic layer was washed with water (6 × 25 mL) followed by brine (2 × 20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the residue was flash chromatographed over silica gel eluting with *n*-hexane/ethyl acetate (95:5) to give compound **9d** (0.631 g, 80%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.21−2.29 (m, 2H), 3.77 (t, J = 6.3 Hz, 2H), 4.14 (t, J = 6.3 Hz, 2H), 6.45−6.47 (m, 1H), 6.84 (dd, J = 2.4, 9.0 Hz, 1H), 7.11 (d, J = 2.1 Hz, 1H), 7.17 (t, J = 2.1 Hz, 1H), 7.26 (d, J = 9.0 Hz, 1H), 8.05 (br s, 1H). MS (ESI *m/z*) 210.1 $(M + H)^+$.

4-(3-Chloropropoxy)-1*H*-indole (9c). This compound was prepared as described in the case of **9d**, starting from 4-hydroxyindole (**8a**), giving an 82% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.13−2.31 (m, 2H), 3.76 (t, J = 6.3 Hz, 2H), 4.21 (t, J = 6.0 Hz, 2H), 6.50 (d, J = 7.8 Hz, 1H), 6.60−6.62 (m, 1H), 6.94 (d, J = 8.4 Hz, 1H), 7.00 (t, J = 2.7 Hz, 1H), 7.07 (t, J = 7.8 Hz, 1H), 8.03 (br s, 1H). MS (ESI *m/z*) 210.1 $(M + H)^+$.

6-(3-Chloropropoxy)-1*H*-indole (9e). This compound was prepared as described in the case of **9d**, starting from 6-hydroxyindole (**8c**), giving an 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.24−2.33 (m, 2H), 3.81 (t, J = 5.7 Hz, 2H), 4.17 (t, J = 5.7 Hz, 2H), 6.49−6.51 (m, 1H), 6.81 (dt, J = 2.1, 8.7 Hz, 1H), 6.92 (s, 1H), 7.11−7.13 (m, 1H), 7.53 (d, J = 8.4 Hz, 1H), 8.05 (br s, 1H). MS (ESI *m/z*) 210.1 $(M + H)^+$.

4-(4-Chlorobutoxy)-1*H*-indole (9f). This compound was prepared as described in the case of **9d**, starting from 4-hydroxyindole, giving a 77% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03−2.07 (m, 4H), 3.66 (t, J = 6.3 Hz, 2H), 4.16 (t, J = 6.3 Hz, 2H), 6.50 (d, J = 7.8 Hz, 1H), 6.63−6.65 (m, 1H), 7.01 (d, J = 8.1 Hz, 1H), 7.06−7.12 (m, 2H), 8.15 (br s, 1H). MS (ESI *m/z*) 224.1 $(M + H)^+$.

5-(4-Chlorobutoxy)-1*H*-indole (9g). This compound was prepared as described in the case of **9d**, starting from 5-hydroxyindole (**8b**), giving an 85% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.82−1.97 (m, 4H), 3.54 (t, J = 6.3 Hz, 2H), 3.94 (t, J = 6.3 Hz, 2H), 6.40−6.42 (m, 1H), 6.81 (dd, J = 2.4, 9.0 Hz, 1H), 7.00 (t, J = 2.7 Hz, 1H), 7.05 (d, J = 2.4 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 7.90 (br s, 1H). MS (ESI *m/z*) 224.1 $(M + H)^+$.

4-(5-Chloropentyloxy)-1*H*-indole (9h). This compound was prepared as described in the case of **9d**, starting from 4-hydroxyindole (**8a**), giving an 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.66−1.74 (m, 2H), 1.85−1.96 (m, 4H), 3.58 (t, J = 6.3 Hz, 2H), 4.13 (t, J = 6.3 Hz, 2H), 6.50 (d, J = 7.2 Hz, 1H), 6.64−6.66 (m, 1H), 7.00 (d, J = 8.4 Hz, 1H), 7.06−7.12 (m, 2H), 8.13 (br s, 1H). MS (ESI *m/z*) 238.1 $(M + H)^+$.

5-(5-Chloropentyloxy)-1*H*-indole (9i). This compound was prepared as described in the case of **9d**, starting from 5-hydroxyindole (**8b**), giving an 85% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62−1.70 (m, 2H), 1.79−1.92 (m, 4H), 3.57 (t, J = 6.6 Hz, 2H), 4.01 (t, J = 6.3 Hz, 2H), 6.45−6.47 (m, 1H), 6.84 (dd, J = 2.4, 8.7 Hz, 1H), 7.08 (d, J = 2.4 Hz, 1H), 7.17 (t, J = 3.0 Hz, 1H), 7.27 (d, J = 8.7 Hz, 1H), 8.03 (br s, 1H). MS (ESI *m/z*) 238.1 $(M + H)^+$.

4-(3-Chloro-2-methylpropoxy)-1*H*-indole (9j). This compound was prepared as described in the case of **9d**, starting from 4-hydroxyindole (**8a**), giving a 50% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (d, J = 6.9 Hz, 3H), 2.47−2.54 (m, 1H), 3.75−3.85 (m, 2H), 4.13 (d, J = 6.0 Hz, 2H), 6.58 (d, J = 7.8 Hz, 1H), 6.69−6.71 (m, 1H), 7.04 (d, J = 8.1 Hz, 1H), 7.12−7.27 (m, 2H), 8.13 (br s, 1H). MS (ESI *m/z*) 224.1 $(M + H)^+$.

4-(5-Bromo-3-methylpentyloxy)-1*H*-indole (9k). This compound was prepared as described in the case of **9d**, starting from 4-hydroxyindole (**8a**), giving an 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (d, J = 6.3 Hz, 3H), 1.70−1.84 (m, 3H), 1.89−2.03 (m, 2H), 3.42−3.53 (m, 2H), 4.13−4.19 (m, 2H), 6.50 (d, J = 7.8 Hz, 1H), 6.64−6.65 (m, 1H), 7.00 (d, J = 8.1 Hz, 1H), 7.06−7.11 (m, 2H), 8.13 (br s, 1H). MS (ESI *m/z*) 297.2 $(M + H)^+$.

5-(5-Bromo-3-methylpentyloxy)-1*H*-indole (9l). This compound was prepared as described in the case of **9d**, starting from 4-hydroxyindole (**8a**), giving an 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (d, J = 6.0 Hz, 3H), 1.63−2.08 (m, 3H), 3.42−3.54 (m, 2H), 4.01−4.07 (m, 2H), 6.45−6.47 (m, 1H), 6.84 (dd, J = 2.4, 9.0 Hz, 1H), 7.09 (d, J = 2.1 Hz, 1H), 7.18 (t, J = 3.0 Hz, 1H), 7.26 (d, J = 9.0 Hz, 1H), 8.03 (br s, 1H). MS (ESI *m/z*) 297.2 $(M + H)^+$.

5-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]-1*H*-indole (10d). A mixture of **9d** (0.300 g, 1.44 mmol), 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol[22] (0.352 g, 1.44 mmol), potassium carbonate (0.297 g, 2.15 mmol), and potassium iodide (0.048 g, 0.29 mmol) in 5 mL of DMF was heated at 110 °C for 2 h. The mixture was cooled to room temperature and quenched with water (10 mL). The mixture was extracted with ethyl acetate (2 × 20 mL). The combined organic layer was washed with water (6 × 20 mL) followed by brine (2 × 20 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed in vacuo to give an oily residue, which was filtered through a short silica column eluting with *n*-hexane/dichloromethane (50:50) to give compound **10d** (0.468 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.62−1.72 (m, 2H), 2.34 (quintet, J = 6.0 Hz, 2H), 2.90 (t, J = 7.5 Hz, 2H), 4.23 (t, J = 6.0 Hz, 2H), 4.30 (t, J = 6.0 Hz, 2H), 6.44−6.45 (m, 1H), 6.86 (dd, J = 2.4, 8.7

Hz, 1H), 7.07 (d, J = 8.7 Hz, 1H), 7.11−7.15 (m, 2H), 7.25 (d, J = 8.7 Hz, 1H), 7.52 (d, J = 8.7 Hz, 1H), 8.07 (br s, 1H). MS (ESI m/z) 419.5 (M + H)$^+$.

4-[2-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)ethoxy]-1*H*-indole (10a). This compound was prepared as described in the case of **10d**, starting from **9a**, giving a 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.67−1.74 (m, 2H), 2.92 (t, J = 7.8 Hz, 2H), 4.48−4.58 (m, 4H), 6.48 (d, J = 3.0 Hz, 1H), 6.60 (d, J = 7.8 Hz, 1H), 6.94 (d, J = 8.1 Hz, 1H), 7.02−7.10 (m, 2H), 7.34 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 9.0 Hz, 1H), 8.04 (br s, 1H). MS (ESI m/z) 405.2 (M + H)$^+$.

5-[2-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)ethoxy]-1*H*-indole (10b). This compound was prepared as described in the case of **10d**, starting from **9b**, giving an 81% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.64−1.75 (m, 2H), 2.92 (t, J = 7.5 Hz, 2H), 4.39−4.47 (m, 4H), 6.48−6.50 (m, 1H), 6.90 (dd, J = 2.7, 8.7 Hz, 1H), 7.12 (d, J = 8.7 Hz, 1H), 7.17−7.19 (m, 2H), 7.29 (d, J = 9.0 Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H), 8.13 (br s, 1H). MS (ESI m/z) 405.2 (M + H)$^+$.

4-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]-1*H*-indole (10c). This compound was prepared as described in the case of **10d**, starting from **9c**, giving a 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.62−1.74 (m, 2H), 2.41 (quintet, J = 6.0 Hz, 2H), 2.90 (t, J = 7.5 Hz, 2H), 4.32−4.37 (m, 4H), 6.54 (d, J = 7.2 Hz, 1H), 6.62−6.64 (m, 1H), 7.00 (d, J = 8.4 Hz, 1H), 7.05−7.12 (m, 3H), 7.51 (d, J = 8.7 Hz, 1H), 8.16 (br s, 1H). MS (ESI m/z) 419.5 (M + H)$^+$.

6-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]-1*H*-indole (10e). This compound was prepared as described in the case of **10d**, starting from **9e**, giving a 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, J = 7.2 Hz, 3H), 1.63−1.71 (m, 2H), 2.34 (quintet, J = 6.0 Hz, 2H), 2.90 (t, J = 7.5 Hz, 2H), 4.21 (t, J = 6.0 Hz, 2H), 4.30 (t, J = 6.0 Hz, 2H), 6.45−6.47 (m, 1H), 6.79 (dd, J = 2.1, 8.7 Hz, 1H), 6.87 (s, 1H), 7.04−7.07 (m, 2H), 7.49 (d, J = 8.7 Hz, 1H), 7.52 (d, J = 8.7 Hz, 1H), 8.04 (br s, 1H). MS (ESI m/z) 419.5 (M + H)$^+$.

4-[4-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)butoxy]-1*H*-indole (10f). This compound was prepared as described in the case of **10d**, starting from **9f**, giving a 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, J = 7.5 Hz, 3H), 1.65−1.75 (m, 2H), 2.07−2.17 (m, 4H), 2.91 (t, J = 7.5 Hz, 2H), 4.17−4.26 (m, 4H), 6.52 (d, J = 7.2 Hz, 1H), 6.59−6.61 (m, 1H), 6.93 (d, J = 8.4 Hz, 1H), 7.00−7.11 (m, 3H), 7.52 (d, J = 8.4 Hz, 1H), 8.15 (br s, 1H). MS (ESI m/z) 433.1 (M + H)$^+$.

5-[4-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)butoxy]-1*H*-indole (10g). This compound was prepared as described in the case of **10d**, starting from **9g**, giving a 76% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3H), 1.63−1.73 (m, 2H), 1.95−2.08 (m, 4H), 2.90 (t, J = 7.5 Hz, 2H), 4.07−4.15 (m, 4H), 6.43−6.45 (m, 1H), 6.85 (dd, J = 2.4, 8.7 Hz, 1H), 7.00 (d, J = 8.7 Hz, 1H), 7.10−7.13 (m, 2H), 7.23 (d, J = 8.7 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 8.08 (br s, 1H). MS (ESI m/z) 433.1 (M + H)$^+$.

4-[5-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)pentyloxy]-1*H*-indole (10h). This compound was prepared as described in the case of **10d**, starting from **9h**, giving a 73% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3H), 1.65−1.82 (m, 4H), 1.92−1.99 (m, 4H), 2.92 (t, J = 7.5 Hz, 2H), 4.11−4.18 (m, 4H), 6.51 (d, J = 7.5 Hz, 1H), 6.62−6.64 (m, 1H), 6.94−7.11 (m, 4H), 7.52 (d, J = 8.7 Hz, 1H), 8.14 (br s, 1H). MS (ESI m/z) 447.5 (M + H)$^+$.

5-[5-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)pentyloxy]-1*H*-indole (10i). This compound was prepared as described in the case of **10d**, starting from **9i**, giving a 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, J = 7.5 Hz, 3H), 1.64−1.76 (m, 4H), 1.85−1.96 (m, 4H), 2.90 (t, J = 7.5 Hz, 2H), 4.01−4.10 (m, 4H), 6.43−6.45 (m, 1H), 6.84 (dd, J = 2.4, 8.7 Hz, 1H), 6.99 (d, J = 8.7 Hz, 1H), 7.09−7.13 (m, 2H), 7.22 (d, J = 8.7 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 8.09 (br s, 1H). MS (ESI m/z) 447.5 (M + H)$^+$.

4-[2-Methyl-3-(7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]-1*H*-indole (10j). This compound was prepared as described in the case of **10d**, starting from **9j**, giving a 55% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 7.5 Hz, 3H), 1.21 (d, J = 6.9 Hz, 3H), 1.55−1.69 (m, 2H), 2.53−2.59 (m, 1H), 2.79−2.85 (m, 2H), 4.07−4.21 (m, 4H), 6.46 (d, J = 7.5 Hz, 1H), 6.54−6.56 (m, 1H), 6.84 (d, J = 8.4 Hz, 1H), 6.92−7.04 (m, 3H), 7.44 (d, J = 8.4 Hz, 1H), 8.10 (br s, 1H). MS (ESI m/z) 433.4 (M + H)$^+$.

4-[3-Methyl-5-(7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)pentyloxy]-1*H*-indole (10k). This compound was prepared as described in the case of **10d**, starting from **9k**, giving a 73% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, J = 7.5 Hz, 3H), 1.10 (d, J = 6.6 Hz, 3H), 1.64−1.84 (m, 4H), 1.98−2.11 (m, 3H), 2.89 (t, J = 7.5 Hz, 2H), 4.15−4.23 (m, 4H), 6.50 (d, J = 7.5 Hz, 1H), 6.58−6.60 (m, 1H), 6.91−7.08 (m, 4H), 7.49 (d, J = 8.7 Hz, 1H), 8.11 (br s, 1H). MS (ESI m/z) 461.5 (M + H)$^+$.

5-[3-Methyl-5-(7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)pentyloxy]-1*H*-indole (10l). This compound was prepared as described in the case of **10d**, starting from **9l**, giving a 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3H), 1.07 (d, J = 6.6 Hz, 3H), 1.65−1.78 (m, 4H), 1.88−2.08 (m, 3H), 2.90 (t, J = 7.5 Hz, 2H), 4.04−4.18 (m, 4H), 6.43−6.45 (m, 1H), 6.82 (dd, J = 2.4, 8.7 Hz, 1H), 7.03 (d, J = 9.0 Hz, 1H), 7.08 (d, J = 2.1 Hz, 1H), 7.16−7.24 (m, 2H), 7.51 (d, J = 8.7 Hz, 1H), 8.03 (br s, 1H). MS (ESI m/z) 461.5 (M + H)$^+$.

Methyl 2-{5-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoate (14a). A mixture of compound **10d** (0.100 g, 0.24 mmol), methyl 2-bromoacetate (0.109 g, 0.72 mmol, 0.07 mL), potassium carbonate (0.050 g, 0.36 mmol), and potassium iodide (0.008 g, 0.05 mmol) in 15 mL of acetonitrile was heated at reflux for 12 h. The mixture was cooled to room temperature and filtered to remove suspended salts. The solvent was removed in vacuo, and the residue was partitioned between dichloromethane and water. The organic layer was washed with water (2 × 20 mL) followed by brine (2 × 20 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed, and the residue chromatographed over silica gel eluting with *n*-hexane/ethyl acetate (95:5) to give the desired methyl ester **14a** (77 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3H), 1.63−1.72 (m, 2H), 2.34 (quintet, J = 6.0 Hz, 2H), 2.91 (t, J = 7.5 Hz, 2H), 3.73 (s, 3H), 4.23 (t, J = 6.0 Hz, 2H), 4.32 (t, J = 6.0 Hz, 2H), 4.82 (s, 2H), 6.46 (d, J = 3.3 Hz, 1H), 6.87 (dd, J = 2.4, 8.7 Hz, 1H), 7.05−7.14 (m, 4H), 7.54 (d, J = 8.7 Hz, 1H). MS (ESI m/z) 491.4 (M + H)$^+$.

Methyl 2-{4-[2-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)ethyloxy]indol-1-yl}ethanoate (11a). This compound was prepared as described in the case of **14a**, starting from **10a**, giving a 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 7.5 Hz, 3H), 1.67−1.75 (m, 2H), 2.92 (t, J = 7.8 Hz, 2H), 3.74 (s, 3H), 4.50−4.56 (m, 4H), 4.84 (s, 2H), 6.58−6.60 (m, 2H), 6.90 (d, J = 8.1 Hz, 1H), 6.98 (d, J = 2.7 Hz, 1H), 7.12−7.17 (m, 2H), 7.57 (d, J = 8.7 Hz, 1H). MS (ESI m/z) 477.2 (M + H)$^+$.

Methyl 2-{5-[2-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)ethyloxy]indol-1-yl}ethanoate (12a). This compound was prepared as described in the case of **14a**, starting from **10b**, giving a 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.65−1.75 (m, 2H), 2.92 (t, J = 7.5 Hz, 2H), 3.67 (s, 3H), 4.39−4.47 (m, 4H), 4.82 (s, 2H), 6.46 (m, 1H), 6.90 (dd, J = 2.7, 8.7 Hz, 1H), 7.08−7.29 (m, 4H), 7.56 (d, J = 8.7 Hz, 1H). MS (ESI m/z) 477.2 (M + H)$^+$.

Methyl 2-{4-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoate (13a). This compound was prepared as described in the case of **14a**, starting from **10c**, giving a 64% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.60−1.74 (m, 2H), 2.41 (quintet, J = 6.0 Hz, 2H), 2.91 (t, J = 7.5 Hz, 2H), 3.78 (s, 3H), 4.35 (t, J = 6.0 Hz, 4H), 4.83 (s, 2H), 6.56 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 3.3 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 3.0 Hz, 1H), 7.07−7.15 (m, 2H), 7.53 (d, J = 8.7 Hz, 1H). MS (ESI *m/z*) 491.2 $(M + H)^+$.

Methyl 2-{6-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]-isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoate (15a). This compound was prepared as described in the case of **14a**, starting from **10e**, giving a 64% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.61−1.72 (m, 2H), 2.35 (quintet, J = 6.0 Hz, 2H), 2.91 (t, J = 7.5 Hz, 2H), 3.72 (s, 3H), 4.23 (t, J = 6.0 Hz, 2H), 4.32 (t, J = 6.0 Hz, 2H), 4.78 (s, 2H), 6.48 (dd, J = 0.6, 3.0 Hz, 1H), 6.72 (s, 1H), 6.80 (dd, J = 2.4, 8.4 Hz, 1H), 6.97 (d, J = 3.3 Hz, 1H), 7.08 (d, J = 9.0 Hz, 1H), 7.49 (d, J = 8.7 Hz, 1H), 7.54 (d, J = 8.7 Hz, 1H). MS (ESI *m/z*) 491.4 $(M + H)^+$.

Methyl 2-{4-[4-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)butoxy]indol-1-yl}ethanoate (16a). This compound was prepared as described in the case of **14a**, starting from **10f**, giving a 67% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, J = 7.5 Hz, 3H), 1.67−1.74 (m, 2H), 2.08−2.13 (m, 4H), 2.91 (t, J = 7.5 Hz, 2H), 3.73(s, 3H), 4.17−4.25 (m, 4H), 4.83 (s, 2H), 6.54 (d, J = 7.8 Hz, 1H), 6.62 (dd, J = 0.6, 3.0 Hz, 1H), 6.85 (d, J = 7.8 Hz, 1H), 6.97 (d, J = 3.3 Hz, 1H), 7.05 (d, J = 8.7 Hz, 1H), 7.12 (t, J = 7.8 Hz, 1H), 7.53 (d, J = 8.7 Hz, 1H). MS (ESI *m/z*) 505.4 $(M + H)^+$.

Methyl 2-{5-[4-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)butoxy]indol-1-yl}ethanoate (17a). This compound was prepared as described in the case of **14a**, starting from **10g**, giving a 68% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, J = 7.5 Hz, 3H), 1.63−1.74 (m, 2H), 2.00−2.09 (m, 4H), 2.91 (t, J = 7.5 Hz, 2H), 3.73 (s, 3H), 4.09 (t, J = 6.0 Hz, 2H), 4.17 (t, J = 6.0 Hz, 2H), 4.81 (s, 2H), 6.47 (dd, J = 0.6, 3.0 Hz, 1H), 6.88 (dd, J = 2.4, 8.7 Hz, 1H), 7.04−7.06 (m, 2H), 7.10−7.14 (m, 2H), 7.54 (d, J = 8.4 Hz, 1H). MS (ESI *m/z*) 505.4 $(M + H)^+$.

Methyl 2-{4-[5-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)pentyloxy]indol-1-yl}ethanoate (18a). This compound was prepared as described in the case of **14a**, starting from **10h**, giving a 64% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3H), 1.66−1.78 (m, 4H), 1.92−1.99 (m, 4H), 2.91 (t, J = 7.5 Hz, 2H), 3.73 (s, 3H), 4.10−4.18 (m, 4H), 4.83 (s, 2H), 6.53 (d, J = 7.5 Hz, 1H), 6.64 (dd, J = 0.6, 3.0 Hz, 1H), 6.85 (d, J = 8.7 Hz, 1H), 6.97 (d, J = 3.3 Hz, 1H), 7.04 (d, J = 9.0 Hz, 1H), 7.11 (t, J = 8.1 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H). MS (ESI *m/z*) 519.2 $(M + H)^+$.

Methyl 2-{5-[5-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)pentyloxy]indol-1-yl}ethanoate (19a). This compound was prepared as described in the case of **14a**, starting from **10i**, giving a 68% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, J = 7.5 Hz, 3H), 1.67−1.77 (m, 4H), 1.85−1.96 (m, 4H), 2.92 (t, J = 7.5 Hz, 2H), 3.73 (s, 3H), 4.04 (t, J = 6.3 Hz, 2H), 4.11 (t, J = 6.3 Hz, 2H), 4.81(s, 2H), 6.46 (d, J = 3.0 Hz, 1H), 6.88 (dd, J = 2.4, 9.0 Hz, 1H), 7.02−7.05 (m, 2H), 7.10 (d, J = 2.1 Hz, 1H), 7.13 (d, J = 9.0 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H). MS (ESI *m/z*) 519.2 $(M + H)^+$.

Methyl 2-{4-[2-Methyl-3-(7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoate (20a). This compound was prepared as described in the case of **14a**, starting from **10j**, giving a 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 7.5 Hz, 3H), 1.30 (d, J = 6.9 Hz, 3H), 1.55−1.65 (m, 2H), 2.53−2.59 (m, 1H), 2.84 (t, J = 7.5 Hz, 2H), 3.65 (s, 3H), 4.07−4.21 (m, 4H), 4.76 (s, 2H), 6.48 (d, J = 7.8 Hz, 1H), 6.57 (dd, J = 0.6, 3.0 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 3.3 Hz, 1H), 6.99−7.08 (m, 2H), 7.45 (d, J = 8.7 Hz, 1H). MS (ESI *m/z*) 505.1 $(M + H)^+$.

Methyl 2-{4-[3-Methyl-5-(7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)pentyloxy]indol-1-yl}ethanoate (21a). This compound was prepared as described in the case of **14a**, starting from **10k**, giving a 63% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.09 (d, J = 6.6 Hz, 3H), 1.64−1.78 (m, 4H), 1.98−2.10 (m, 3H), 2.89 (t, J = 7.5 Hz, 2H), 3.73 (s, 3H), 4.15−4.23 (m, 4H), 4.83 (s, 2H), 6.52 (d, J = 7.5 Hz, 1H), 6.60 (d, J = 3.3 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 6.95 (d, J = 3.0 Hz, 1H), 7.03 (d, J = 8.7 Hz, 1H), 7.11 (t, J = 8.4 Hz, 1H), 7.51 (d, J = 8.4 Hz, 1H). MS (ESI *m/z*) 533.2 $(M + H)^+$.

Methyl 2-{5-[3-Methyl-5-(7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)pentyloxy]indol-1-yl}ethanoate (22a). This compound was prepared as described in the case of **14a**, starting from **10l**, giving a 66% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3H), 1.07 (d, J = 6.6 Hz, 3H), 1.65−1.78 (m, 4H), 1.88−2.08 (m, 3H), 2.90 (t, J = 7.5 Hz, 2H), 3.73 (s, 3H), 4.04−4.17 (m, 4H), 4.81 (s, 2H), 6.44 (d, J = 3.3 Hz, 1H), 6.84 (dd, J = 2.4, 8.7 Hz, 1H), 7.01−7.12 (m, 4H), 7.52 (d, J = 8.7 Hz, 1H). MS (ESI *m/z*) 533.2 $(M + H)^+$.

Ethyl 3-{4-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}propanoate (23a). A mixture of **10c** (0.030 g, 0.07 mmol), ethyl acrylate (0.022 g, 0.22 mmol, 0.02 mL), and cesium carbonate (0.047 g, 0.14 mmol) in 10 mL of acetonitrile was stirred overnight. The mixture was filtered to remove suspended salts. The solvent was removed in vacuo, and the residue was partitioned between dichloromethane and water. The organic layer was washed with water (2 × 20 mL) followed by brine (2 × 20 mL) and then dried over anhydrous sodium sulfate. The solvent was removed, and the residue was chromatographed over silica gel eluting with hexane/ethyl acetate (95:5) to give the desired ethyl ester **23a** (25 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 7.2 Hz, 3H), 1.12 (t, J = 7.2 Hz, 3H), 1.54−1.64 (m, 2H), 2.33 (quintet, J = 6.0 Hz, 2H), 2.71 (t, J = 6.6 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 4.02 (q, J = 7.2 Hz, 2H), 4.25−4.36 (m, 6H), 6.45−6.48 (m, 2H), 6.89 (d, J = 8.1 Hz, 1H), 6.95 (d, J = 3.1 Hz, 1H), 6.99−7.04 (m, 2H), 7.44 (d, J = 8.7 Hz, 1H). MS (ESI *m/z*) 519.2 $(M + H)^+$.

Ethyl 3-{5-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}propanoate (24a). This compound was prepared as described in the case of **23a**, starting from **10d**, giving a 68% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, J = 7.5 Hz, 3H), 1.22 (t, J = 7.5 Hz, 3H), 1.65−1.77 (m, 2H), 2.37 (quintet, J = 6.0 Hz, 2H), 2.81 (t, J = 6.6 Hz, 2H), 2.93 (t, J = 7.5 Hz, 2H), 4.12 (q, J = 7.2 Hz, 2H), 4.25 (t, J = 6.0 Hz, 2H), 4.34 (t, J = 6.0 Hz, 2H), 4.43 (t, J = 6.6 Hz, 2H), 6.40 (d, J = 3.0 Hz, 1H), 6.89 (dd, J = 2.4, 8.7 Hz, 1H), 7.09−7.12 (m, 3H), 7.25 (d J = 8.4 Hz, 1H), 7.56 (d, J = 8.7 Hz, 1H). MS (ESI *m/z*) 519.2 $(M + H)^+$.

Methyl 2-Ethyl-2-{4-[3-(7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoate (25a). This compound was prepared as described in the case of **14a**, starting from **10c**, giving a 35% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−1.00 (m, 6H), 1.66−1.76 (m, 2H), 2.14−2.31 (m, 2H), 2.38 (quintet, J = 6.0 Hz, 2H), 2.91 (t, J = 7.5 Hz, 2H), 3.72 (s, 3H), 4.30−4.41 (m, 4H), 4.89 (dd, J = 6.0, 9.3 Hz, 1H), 6.59 (d, J = 7.5 Hz, 1H), 6.70 (d, J = 3.3 Hz, 1H), 6.99 (d, J = 8.1 Hz, 1H), 7.10−7.17 (m, 2H), 7.20 (d, J = 3.3 Hz, 1H), 7.56 (d, J = 8.1 Hz, 1H). MS (ESI *m/z*) 519.2 $(M + H)^+$.

Methyl 2-Ethyl-2-{5-[3-(7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoate (26a). This compound was prepared as described in the case of **14a**, starting from **10d**, giving a 38% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88−0.97 (m, 6H), 1.61−1.72 (m, 2H), 2.13−2.38 (m, 3H), 2.90 (t, J = 7.5 Hz, 2H), 3.68 (s, 3H), 4.23 (t, J = 6.0 Hz, 2H), 4.31 (t, J = 6.0 Hz, 2H), 4.81 (dd, J = 6.3, 9.0 Hz, 1H), 6.47 (d, J = 3.3 Hz, 1H), 6.86 (dd, J = 2.4, 8.7 Hz, 1H), 7.07−7.10 (m, 2H), 7.23−7.25 (m, 2H), 7.53 (d, J = 8.7 Hz, 1H). MS (ESI *m/z*) 519.2 $(M + H)^+$.

2-{5-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoic acid (14). The mixture of compound **14a** (0.075 g, 0.153 mmol) and LiOH (0.015 g, 0.612 mmol) in methanol and water mixture (4:1) was refluxed for 2 h. The solvent was removed in vacuo, and 0.5 N HCl was added to the residue before extraction with ether (2 × 20 mL). The combined organic layer was washed with water (2 × 20 mL) followed by brine (2 × 10 mL). The solvent was removed in vacuo, and the residue was chromatographed over a short column of silica gel eluting with dichloromethane/methanol (98:2) to give the desired acid **14** (0.064 g, 83%, mp 113−116

°C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3H), 1.65−1.73 (m, 2H), 2.34 (quintet, J = 6.0 Hz, 2H), 2.91 (t, J = 7.5 Hz, 2H), 4.23 (t, J = 6.0 Hz, 2H), 4.32 (t, J = 6.0 Hz, 2H), 4.79 (s, 2H), 6.45 (d, J = 3.0 Hz, 1H), 6.87 (dd, J = 1.5, 8.7 Hz, 1H), 7.06−7.16 (m, 4H), 7.54 (d, J = 8.7 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{24}H_{23}F_3N_2O_5$ 476.1559, found 476.1552.

2-{4-[2-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)ethoxy]indol-1-yl}ethanoic acid (11). This compound was prepared as described in the case of **14**, starting from **11a**, giving a 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 0.90 (t, J = 7.5 Hz, 3H), 1.65−1.76 (m, 2H), 2.92 (t, J = 7.8 Hz, 3H), 4.47−4.60 (m, 4H), 4.80 (s, 2H), 6.48 (d, J = 3.0 Hz, 1H), 6.59 (d, J = 7.8 Hz, 1H), 6.93 (d, J = 8.1 Hz, 1H), 7.02−7.10 (m, 2H), 7.33 (d, J = 9.0 Hz, 1H), 7.63 (d, J = 8.7 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{23}H_{21}O_5N_2F_3$ 462.1403, found 462.1388.

2-{5-[2-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)ethoxy]indol-1-yl}ethanoic acid (12). This compound was prepared as described in the case of **14**, starting from **12a**, giving a 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.66−1.76 (m, 2H), 2.93 (t, J = 7.5 Hz, 2H), 4.40−4.48 (m, 4H), 4.82 (s, 2H), 6.49 (d, J = 2.7 Hz, 1H), 6.92 (dd, J = 2.4, 8.7 Hz, 1H), 7.09−7.20 (m, 4H), 7.58 (d, J = 8.4 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{23}H_{21}O_5N_2F_3$ 462.1403, found 462.1403.

2-{4-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoic acid (13). This compound was prepared as described in the case of **14**, starting from **13a**, giving a 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 0.94 (t, J = 7.5 Hz, 3H), 1.65−1.72 (m, 2H), 2.41 (quintet, J = 6.0 Hz, 2H), 2.91 (t, J = 7.5 Hz, 2H), 4.34−4.38 (m, 4H), 4.82 (s, 2H), 6.56 (d, J = 7.2 Hz, 1H), 6.63 (dd, J = 0.9, 3.0 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 7.00 (d, J = 3.0 Hz, 1H), 7.09−7.15 (m, 2H), 7.54 (d, J = 8.4 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{24}H_{23}O_5N_2F_3$ 476.1559, found 476.1528.

2-{6-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoic Acid (15). This compound was prepared as described in the case of **14**, starting from **15a**, giving a 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 7.5 Hz, 3H), 1.63−1.71 (m, 2H), 2.34 (quintet, J = 6.0 Hz, 2H), 2.89 (t, J = 7.5 Hz, 2H), 4.22(d, J = 6.0 Hz, 2H), 4.30 (t, J = 6.0 Hz, 2H), 4.79 (s, 2H), 6.48 (d, J = 3.0 Hz, 1H) 6.70 (d, J = 1.8 Hz, 1H), 6.80 (dd, J = 2.1, 8.7 Hz, 1H), 6.94 (d, J = 3.0 Hz, 1H), 7.07 (d, J = 8.7 Hz, 1H), 7.47−7.54 (m, 2H). HRMS (EI$^+$ *m/z*) calcd for $C_{24}H_{23}O_5N_2F_3$ 476.1559, found 476.1549.

2-{4-[4-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)butoxy]indol-1-yl}ethanoic Acid (16). This compound was prepared as described in the case of **14**, starting from **16a**, giving a 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 0.99 (t, J = 7.5 Hz, 3H), 1.70−1.78 (m, 2H), 2.12−2.18 (m, 4H), 2.94 (t, J = 7.2 Hz, 2H), 4.23−4.33 (m, 4H), 4.88 (s, 2H), 6.54−6.60 (m, 2H), 6.92 (d, J = 8.1 Hz, 1H), 7.05−7.12 (m, 2H), 7.22 (d, J = 9.0 Hz, 1H), 7.61 (d, J = 8.1 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{25}H_{25}O_5N_2F_3$ 490.1716, found 490.1725.

2-{5-[4-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)butoxy]indol-1-yl}ethanoic Acid (17). This compound was prepared as described in the case of **14**, starting from **17a**, giving a 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.62−1.75 (m, 2H), 1.94−2.10 (m, 4H), 2.89 (t, J = 7.5 Hz, 2H), 4.05 (t, J = 5.4 Hz, 2H), 4.14 (t, J = 6.0 Hz, 2H), 4.63 (s, 2H), 6.38 (d, J = 3.0 Hz, 1H), 6.83 (dd, J = 2.4, 8.7 Hz, 1H), 6.90 (d, J = 2.1 Hz, 1H), 7.00−7.05 (m, 3H), 7.52 (d, J = 8.7 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{25}H_{25}O_5N_2F_3$ 490.1716, found 490.1720.

2-{4-[5-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)pentyloxy]indol-1-yl}ethanoic Acid (18). This compound was prepared as described in the case of **14**, starting from **18a**, giving a 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 0.94 (t, J = 7.5 Hz, 3H), 1.66−1.83 (m, 4H), 1.92−2.02 (m, 4H), 2.91 (t, J = 7.5 Hz, 2H), 4.14−4.21 (m, 4H), 4.87 (s, 2H), 6.50−6.53 (m, 2H), 6.87 (d, J = 8.1 Hz, 1H), 7.02−7.10 (m, 2H), 7.20 (d, J = 9.0 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{26}H_{27}O_5N_2F_3$ 504.1872, found 504.1872.

2-{5-[5-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)pentyloxy]indol-1-yl}ethanoic Acid (19). This compound was prepared as described in the case of **14**, starting from **19a**, giving a 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, J = 7.5 Hz, 3H), 1.65−1.78 (m, 4H), 1.86−1.97 (m, 4H), 2.92 (t, J = 7.5 Hz, 2H), 4.05 (t, J = 6.3 Hz, 2H), 4.14 (t, J = 6.3 Hz, 2H), 4.81(s, 2H), 6.46 (dd, J = 0.6, 3.0 Hz, 1H), 6.88 (dd, J = 2.4, 9.0 Hz, 1H), 7.07−7.11 (m, 3H), 7.16 (d, J = 9.0 Hz, 1H), 7.56 (d, J = 8.7 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{26}H_{27}O_5N_2F_3$ 504.1872, found 504.1868.

2-{4-[2-Methyl-3-(7-propyl-3-trifluoromethylbenzo[*d*]-isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoic Acid (20). This compound was prepared as described in the case of **14**, starting from **20a**, giving a 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J = 7.5 Hz, 3H), 1.21 (d, J = 6.9 Hz, 3H), 1.55−1.67 (m, 2H), 2.52−2.58 (m, 1H), 2.83 (t, J = 7.5 Hz, 2H), 4.07−4.20 (m, 4H), 4.78 (s, 2H), 6.48 (d, J = 7.8 Hz, 1H), 6.57 (d, J = 3.0 Hz, 1H), 6.77 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 3.3 Hz, 1H), 6.99−7.08 (m, 2H), 7.44 (d, J = 8.7 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{25}H_{25}O_5N_2F_3$ 490.1716, found 490.1716.

2-{4-[3-Methyl-5-(7-propyl-3-trifluoromethylbenzo[*d*]-isoxazol-6-yloxy)pentyloxy]indol-1-yl ethanoic Acid (21). This compound was prepared as described in the case of **14**, starting from **21a**, giving a 94% yield. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 0.95 (t, J = 7.5 Hz, 3H), 1.11 (d, J = 6.3 Hz, 3H), 1.66−1.84 (m, 4H), 1.98−2.13 (m, 3H), 2.90 (t, J = 7.5 Hz, 2H), 4.17−4.23 (m, 4H), 4.72 (s, 2H), 6.49−6.54 (m, 2H), 6.87 (d, J = 7.8 Hz, 1H), 6.96−7.11 (m, 3H), 7.54 (d, J = 8.4 Hz, 1H). MS (ESI *m/z*) 519.2 (M + H)$^+$.

2-{5-[3-Methyl-5-(7-propyl-3-trifluoromethylbenzo[*d*]-isoxazol-6-yloxy)pentyloxy]indol-1-yl ethanoic Acid (22). This compound was prepared as described in the case of **14**, starting from **22a**, giving a 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 0.96 (t, J = 7.5 Hz, 3H), 1.09 (d, J = 6.3 Hz, 3H), 1.67−1.80 (m, 4H), 1.91−2.08 (m, 3H), 2.91 (t, J = 7.5 Hz, 2H), 4.05−4.22 (m, 4H), 4.74 (s, 2H), 6.39 (d, J = 3.0 Hz, 1H), 6.82 (dd, J = 2.4, 8.7 Hz, 1H), 7.06−7.15 (m, 4H), 7.55 (d, J = 9.0 Hz, 1H). MS (ESI *m/z*) 519.2 (M + H)$^+$.

3-{4-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}propanoic Acid (23). This compound was prepared as described in the case of **14**, starting from **23a**, giving a 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 7.5 Hz, 3H), 1.54−1.64 (m, 2H), 2.33 (quintet, J = 6.0 Hz, 2H), 2.74 (t, J = 6.9 Hz, 2H), 2.83 (t, J = 7.5 Hz, 2H), 4.25−4.36 (m, 6H), 6.45−6.48 (m, 2H), 6.89 (d, J = 8.1 Hz, 1H), 6.96 (t, J = 1.5 Hz, 1H), 7.00−7.07 (m, 2H), 7.45 (d, J = 9.0 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{25}H_{25}O_5N_2F_3$ 490.1716, found 490.1714.

3-{5-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}propanoic Acid (24). This compound was prepared as described in the case of **14**, starting from **24a**, giving a 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.62−1.74 (m, 2H), 2.34 (quintet, J = 6.0 Hz, 2H), 2.85 (t, J = 6.6 Hz, 2H), 2.90 (t, J = 7.5 Hz, 2H), 4.23 (t, J = 6.0 Hz, 2H), 4.31 (t, J = 6.0 Hz, 2H), 4.39 (t, J = 6.6 Hz, 2H), 6.38 (dd, J = 0.6, 3.0 Hz, 1H), 6.87 (dd, J = 2.4, 8.7 Hz, 1H), 7.06−7.10 (m, 3H), 7.21 (d J = 9.0 Hz, 1H), 7.53 (d, J = 8.7 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{25}H_{25}O_5N_2F_3$ 490.1716, found 490.1724.

2-Ethyl-2-{4-[3-(7-propyl-3-trifluoromethylbenzo[*d*]-isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoic Acid (25). This compound was prepared as described in the case of **14**, starting from **25a**, giving a 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81−0.88 (m, 6H), 1.54−1.66 (m, 2H), 2.04−2.26 (m, 2H), 2.34 (quintet, J = 6.0 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 4.24−4.29 (m, 4H), 4.78 (dd, J = 6.0, 9.9 Hz, 1H), 6.47 (d, J = 7.8 Hz, 1H), 6.59 (d, J = 3.3 Hz, 1H), 6.86 (d, J = 8.1 Hz, 1H), 6.99−7.05 (m, 3H), 7.44 (d, J = 8.1 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{26}H_{27}O_5N_2F_3$ 504.1872, found 504.1875.

2-Ethyl-2-{5-[3-(7-propyl-3-trifluoromethylbenzo[*d*]-isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoic Acid (26). This compound was prepared as described in the case of **14**, starting from **26a**, giving a 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88−0.96 (m, 6H), 1.61−1.72 (m, 2H), 2.12−2.38 (m, 4H), 2.90 (t, *J* = 7.5 Hz, 2H), 4.22 (t, *J* = 6.0 Hz, 2H), 4.31 (t, *J* = 6.0 Hz, 2H), 4.82 (dd, *J* = 6.0, 9.6 Hz, 1H), 6.47 (d, *J* = 3.0 Hz, 1H), 6.85 (dd, *J* = 2.4, 9.0 Hz, 1H), 7.06−7.10 (m, 2H), 7.18−7.21 (m, 2H), 7.53 (d, *J* = 8.7 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{26}H_{27}O_5N_2F_3$ 504.1872, found 504.1885.

2-{5-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}acetonitrile (27a). A mixture of compound **10d** (0.030 g, 0.07 mmol), chloroacetonitrile (0.022 g, 0.29 mmol, 0.02 mL), potassium *tert*-butoxide (0.016 g, 0.14 mmol), and potassium iodide (0.003 g, 0.02 mmol) in 10 mL of acetonitrile was heated at reflux for 12 h. The mixture was cooled to room temperature and filtered to remove suspended salts. The solvent was removed in vacuo, and the residue was partitioned between dichloromethane and water. The organic layer was washed with water (2 × 20 mL) followed by brine (2 × 20 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed, and the residue was chromatographed over silica gel eluting with *n*-hexane/ethyl acetate (95:5) to give the desired methyl ester **27a** (20 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, *J* = 7.5 Hz, 3H), 1.60−1.68 (m, 2H), 2.24−2.32 (p, *J* = 6.0 Hz, 2H), 2.89 (t, *J* = 7.5 Hz, 2H), 4.20 (t, *J* = 6.0 Hz, 2H), 4.25 (t, *J* = 6.0 Hz, 2H), 4.98 (s, 2H), 6.80 (dd, *J* = 2.4, 9.0 Hz, 1H), 7.03 (d, *J* = 2.1, 9.0 Hz, 1H), 7.18−7.25 (m, 4H), 7.56 (d, *J* = 8.4 Hz, 1H). MS (ESI *m/z*) 457.2 (M + H)$^+$.

{5-[3-(7-Propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}(2*H*-tetrazol-5-yl)methane (27). To a suspension of **27a** (0.020 g, 0.04 mmol) in toluene (4 mL) were added trimethylsilyl azide (0.02 mL, 0.15 mmol) and dibutyltin oxide (0.002 g, 0.01 mmol). The mixture was stirred under nitrogen at 110 °C for 18h. When the solution had cooled, the volatiles were evaporated under reduced pressure. The residue was purified by column chromatography over silica gel (CH_2Cl_2/MeOH/25% NH_3(aq) = 90:9:1) to afford **27** (9 mg, 41%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.88 (t, *J* = 7.5 Hz, 3H), 1.59−1.68 (m, 2H), 2.25−2.31 (m, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 4.19 (t, *J* = 6.0 Hz, 2H), 4.34 (t, *J* = 6.0 Hz, 2H), 5.57 (s, 2H), 6.78 (dd, *J* = 2.4, 9.0 Hz, 1H), 7.04 (d, *J* = 2.1 Hz, 1H), 7.21−7.29 (m, 4H), 7.60 (d, *J* = 8.4 Hz, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{24}H_{23}O_3N_6F_3$ 500.1784, found 500.1787.

4-[3-(7-Propyl-3-phenylbenzo[*d*]isoxazol-6-yloxy)propoxy]-1*H*-indole (28a). This compound was prepared as described in the case of **10d**, starting from **9c** and 7-propyl-3-phenylbenzo[*d*]isoxazol-6-ol,[22] giving an 85% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.02 (t, *J* = 7.2 Hz, 3H), 1.76−1.80 (m, 2H), 2.43 (quintet, *J* = 6.0, 12.0 Hz, 2H), 3.00 (quintet, *J* = 6.6, 7.2 Hz, 2H), 4.35 (t, *J* = 6.0 Hz, 2H), 4.39 (t, *J* = 6.0 Hz, 2H), 6.60 (d, *J* = 7.8 Hz, 1H), 6.70−6.71 (m, 1H), 7.02 (d, *J* = 3.6 Hz, 1H), 7.03 (d, *J* = 3.0 Hz, 1H), 7.04−7.15 (m, 2H), 7.14 (t, *J* = 7.8 Hz, 1H), 7.35−7.57 (m, 3H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.98 (dd, *J* = 1.8, 8.4 Hz, 2H), 8.09 (br s, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{27}H_{26}N_2O_3$ 426.1943, found 426.1929.

5-[3-(7-Propyl-3-phenylbenzo[*d*]isoxazol-6-yloxy)propoxy]-1*H*-indole (29a). This compound was prepared as described in the case of **10d**, starting from **9d** and 7-propyl-3-phenylbenzo[*d*]isoxazol-6-ol,[22] giving an 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.5 Hz, 3H), 1.52−1.69 (m, 2H), 2.26 (quintet, *J* = 6.0 Hz, 2H), 2.86 (t, *J* = 7.5 Hz, 2H), 4.13−4.30 (m, 4H), 6.37−6.38 (m, 1H), 6.79 (dd, *J* = 2.4, 8.7 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 1H), 7.01−7.09 (m, 2H), 7.18 (d, *J* = 8.7 Hz, 1H), 7.37−7.45 (m, 3H), 7.55 (d, *J* = 8.7 Hz, 1H), 7.82−7.86 (m, 2H), 8.05 (br s, 1H). MS (ESI *m/z*) 427.1 (M + H)$^+$.

5-[3-(7-Propyl-1*H*-indol-6-yloxy)propoxy]-1*H*-indole (30a). This compound was prepared as described in the case of **10d**, starting from **9d** and 7-propyl-1*H*-indol-6-ol, giving a 60% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.5 Hz, 3H), 1.51−1.65 (m, 2H), 2.25 (quintet, *J* = 6.0 Hz, 2H), 2.72 (t, *J* = 7.5 Hz, 2H), 4.13−4.19 (m, 4H), 6.32−6.39 (m, 2H), 6.76−6.82 (m, 2H), 6.99−7.05 (m, 4H), 7.16 (d, *J* = 9.6 Hz, 1H), 7.84 (br s, 1H), 7.94 (br s, 1H). MS (ESI *m/z*) 349.2 (M + H)$^+$.

5-[3-(3-Cyano-7-propyl-1*H*-indol-6-yloxy)propoxy]-1*H*-indole (31a). This compound was prepared as described in the case of **10d**, starting from **9d** and 3-cyano-7-propyl-1*H*-indol-6-ol, giving a 50% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.5 Hz, 3H), 1.47−1.63 (m, 2H), 2.22 (quintet, *J* = 6.0 Hz, 2H), 2.71 (t, *J* = 7.5 Hz, 2H), 4.01−4.08 (m, 4H), 6.36−6.38 (m, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.78 (dd, *J* = 2.4, 9.0 Hz, 1H), 7.05−7.08 (m, 2H), 7.18 (d, *J* = 8.7 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 2.7 Hz, 1H), 8.09 (br s, 1H), 8.79 (br s, 1H). MS (ESI *m/z*) 374.1 (M + H)$^+$.

5-{3-[3-(2,2,2-Trifluoroethyl-1-one)-7-propyl-1*H*-indol-6-yloxy]propoxy}-1*H*-indole (32a). This compound was prepared as described in the case of **10d**, starting from **9d** and 3-(2,2,2-trifluoroethyl-1-one)-7-propyl-1*H*-indol-6-ol, giving a 54% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (t, *J* = 7.5 Hz, 3H), 1.45−1.61 (m, 2H), 2.25 (quintet, *J* = 6.0 Hz, 2H), 3.02 (t, *J* = 7.8 Hz, 2H), 3.93 (t, *J* = 5.4 Hz, 2H), 4.50 (t, *J* = 6.9 Hz, 2H), 6.37−6.38 (m, 1H), 6.76−6.81 (m, 2H), 6.99 (d, *J* = 2.4 Hz, 1H), 7.10 (t, *J* = 2.7 Hz, 1H), 7.21 (d, *J* = 8.7 Hz, 1H), 7.82 (d, *J* = 1.5 Hz, 1H), 8.06 (br s, 1H), 8.15 (d, *J* = 9.0 Hz, 1H), 9.12 (br s, 1H). MS (ESI *m/z*) 445.2 (M + H)$^+$.

4-[3-(2-Phenyl-7-propyl-1*H*-indol-6-yloxy)propoxy]-1*H*-indole (33a). This compound was prepared as described in the case of **10d**, starting from **9c** and 2-phenyl-7-propyl-1*H*-indol-6-ol, giving a 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (t, *J* = 7.2 Hz, 3H), 1.70−1.74 (m, 2H), 2.38−2.41 (m, 2H), 2.85−2.88 (m, 2H), 4.28−4.30 (m, 2H), 4.39 (t, *J* = 4.0 Hz, 2H), 6.61 (d, *J* = 5.2 Hz, 1H), 6.65−6.67 (m, 1H), 6.76 (d, *J* = 1.6 Hz, 1H), 6.88 (dd, *J* = 1.2, 5.6 Hz, 2H), 7.04 (d, *J* = 2.0 Hz, 1H), 7.15 (t, *J* = 5.2 Hz, 1H), 7.28−7.31 (m, 2H), 7.39−7.44 (m, 2H), 7.65 (dd, *J* = 0.8, 5.6 Hz, 2H), 8.06 (br s, 1H), 8.09 (br s, 1H).

4-[3-(3-Phenyl-7-propylbenzo[*b*]furan-6-yloxy)propoxy]-1*H*-indole (34a). This compound was prepared as described in the case of **10d**, starting from **9c** and 3-phenyl-7-propyl-benzo[*b*]furan-6-ol, giving a 92% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.97 (t, *J* = 4.8 Hz, 3H), 1.69−1.72 (m, 2H), 2.39−2.42 (m, 2H), 2.92 (t, *J* = 4.8 Hz, 2H), 4.30 (t, *J* = 4.0 Hz, 2H), 4.38 (t, *J* = 4.0 Hz, 2H), 6.58 (d, *J* = 5.2 Hz, 1H), 6.67 (d, *J* = 3.2 Hz, 1H), 6.98−7.13 (m, 4H), 7.33−7.47 (m, 4H), 7.56 (d, *J* = 8.8 Hz, 1H), 7.62−7.64 (m, 1H), 7.71 (s, 1H), 8.14 (br s, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{28}H_{27}NO_3$ 425.1991, found 425.1973.

4-[3-(2-Phenyl-7-propylbenzo[*b*]furan-6-yloxy)propoxy]-1*H*-indole (35a). This compound was prepared as described in the case of **10d**, starting from **9c** and 2-phenyl-7-propyl-benzo[*b*]furan-6-ol, giving an 85% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, *J* = 7.6 Hz, 3H), 1.69−1.75 (m, 2H), 2.38 (quintet, *J* = 2.4, 6.0 Hz, 2H), 2.92 (t, *J* = 7.6 Hz, 2H), 4.25 (t, *J* = 6.4 Hz, 2H), 4.35 (t, *J* = 6.0 Hz, 2H), 6.58 (d, *J* = 7.6 Hz, 1H), 6.62−6.64 (m, 1H), 6.87 (t, *J* = 8.0 Hz, 2H), 6.93 (s, 1H), 7.02 (d, *J* = 3.2 Hz, 1H), 7.12 (t, *J* = 8.0 Hz, 1H), 7.28−7.31 (m, 2H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.81 (dd, *J* = 1.2, 8.4 Hz, 2H), 8.07 (br s, 1H).

Ethyl 2-{4-[3-(3-Phenyl-7-propylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoate (28b). This compound was prepared as described in the case of **14a**, by reacting **28a** with ethyl-2-bromoacetate, giving an 85% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.96 (t, *J* = 7.2 Hz, 3H), 1.23 (t, *J* = 7.2 Hz, 3H), 1.70−1.75 (m, 2H), 2.38−2.42 (m, 2H), 4.19 (quintet, *J* = 6.6 Hz, 2H), 4.32−4.37 (m, 4H), 4.80 (s, 2H), 6.57 (d, *J* = 5.2 Hz, 1H), 6.66 (dd, *J* = 0.6, 2.4 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.99 (d, *J* = 3.0 Hz, 1H), 7.02 (d, *J* = 6.0 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 7.48−7.54 (m, 3H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.92−7.93 (m, 2H). HRMS (EI$^+$ *m/z*) calcd for $C_{31}H_{32}N_2O_5$ 512.2311, found 512.2296.

Methyl 2-{5-[3-(3-Phenyl-7-propylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoate (29b). This compound was prepared as described in the case of **14a**, starting from **29a**, giving a 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.5 Hz, 3H), 1.54−1.69 (m, 2H), 2.27 (quintet, *J* = 6.0 Hz, 2H), 2.86 (t, *J* = 7.5 Hz, 2H), 3.65 (s, 3H), 4.16−4.25 (m, 4H), 4.74 (s, 2H), 6.39 (d, J = 3.0 Hz, 1H), 6.81 (dd, J = 2.4, 8.7 Hz, 1H), 6.94 (d, J = 8.7 Hz, 1H), 6.97 (d, J = 3.3 Hz, 1H), 7.01−7.06 (m, 2H), 7.40−7.46 (m, 3H), 7.56 (d, J = 8.7 Hz, 1H), 7.83−7.86 (m, 2H). MS (ESI *m/z*) 499.1 $(M + H)^+$.

Methyl 2-{5-[3-(7-Propyl-1*H*-indol-6-yloxy)propoxy]indol-1-yl}ethanoate (30b). This compound was prepared as described in the case of **14a**, starting from **30a**, giving a 62% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J = 7.5 Hz, 3H), 1.51−1.65 (m, 2H), 2.23 (quintet, J = 6.0 Hz, 2H), 2.73 (t, J = 7.5 Hz, 2H), 3.60 (s, 3H), 4.13−4.17 (m, 4H), 4.74 (s, 2H), 6.38−6.39 (m, 2H), 6.76−6.83 (m, 2H), 6.96−7.04 (m, 4H), 7.32 (d, J = 8.4 Hz, 1H), 7.92 (br s, 1H). MS (ESI *m/z*) 421.2 $(M + H)^+$.

Methyl 2-{5-[3-(3-Cyano-7-propyl-1*H*-indol-6-yloxy)propoxy]indol-1-yl}ethanoate (31b). This compound was prepared as described in the case of **14a**, starting from **31a**, giving a 62% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.40−1.55 (m, 2H), 2.48 (quintet, J = 6.0 Hz, 2H), 2.69−2.74 (m, 2H), 3.69 (s, 3H), 4.14−4.20 (m, 4H), 4.88 (s, 2H), 6.38−6.39 (m, 1H), 6.78 (dd, J = 2.4, 8.7 Hz, 1H), 6.93 (d, J = 8.7 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 7.10 (t, J = 3.0 Hz, 1H), 7.17 (s, 1H), 7.20 (d, J = 8.7 Hz, 1H), 7.44 (d, J = 8.7 Hz, 1H), 7.99 (br s, 1H). MS (ESI *m/z*) 446.1 $(M + H)^+$.

Methyl 2-(5-{3-[3-(2,2,2-Trifluoroethyl-1-one)-7-propyl-1*H*-indol-6-yloxy]propoxy}indol-1-yl)ethanoate (32b). This compound was prepared as described in the case of **14a**, starting from **32a**, giving a 62% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (t, J = 7.5 Hz, 3H), 1.42−1.63 (m, 2H), 2.25 (quintet, J = 6.0 Hz, 2H), 3.02 (t, J = 7.8 Hz, 2H), 3.73 (s, 3H), 3.93 (t, J = 5.4 Hz, 2H), 4.50 (t, J = 6.9 Hz, 2H), 4.65 (s, 2H), 6.37−6.39 (m, 1H), 6.77−6.82 (m, 2H), 7.00 (d, J = 2.4 Hz, 1H), 7.10 (t, J = 2.7 Hz, 1H), 7.21 (d, J = 8.7 Hz, 1H), 7.81 (d, J = 1.5 Hz, 1H), 8.06 (br s, 1H), 8.15 (d, J = 9.0 Hz, 1H). MS (ESI *m/z*) 517.2 $(M + H)^+$.

Ethyl 2-{4-[3-(2-phenyl-7-propyl-1*H*-indol-6-yloxy)propoxy]indol-1-yl}ethanoate (33b). This compound was prepared as described in the case of **14a**, by reacting **33a** with ethyl-2-bromoacetate, giving a 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (t, J = 7.2 Hz, 3H), 1.24 (t, J = 7.6 Hz, 3H), 1.70−1.74 (m, 2H), 2.38−2.41 (m, 2H), 2.85−2.88 (m, 2H), 4.20 (q, J = 4.8, 9.6 Hz, 2H), 4.28−4.30 (m, 2H), 4.39 (t, J = 4.0 Hz, 2H), 4.80 (s, 2H), 6.61 (d, J = 5.2 Hz, 1H), 6.70 (dd, J = 0.4, 2.0 Hz, 1H), 6.76 (d, J = 1.6 Hz, 1H), 6.88 (dd, J = 1.2, 5.6 Hz, 2H), 6.99 (d, J = 2.0 Hz, 1H), 7.15 (t, J = 5.2 Hz, 1H), 7.28−7.31 (m, 2H), 7.39−7.44 (m, 2H), 7.65 (dd, J = 0.8, 5.6 Hz, 2H), 8.09 (br s, 1H). HRMS (EI^+ *m/z*) calcd for $C_{32}H_{34}N_2O_4$: 510.2519, found 510.2522.

Ethyl 2-{4-[3-(3-phenyl-7-propylbenzo[*b*]furan-6-yloxy)propoxy]indol-1-yl}ethanoate (34b). This compound was prepared as described in the case of **14a**, by reacting **34a** with ethyl-2-bromoacetate, giving a 92% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.95 (t, J = 7.2 Hz, 3H), 1.23 (t, J = 7.6 Hz, 3H), 1.67−1.71 (m, 2H), 2.37−2.40 (m, 2H), 2.89−2.91 (m, 2H), 4.20 (q, J = 4.8, 9.6 Hz, 2H), 4.27 (t, J = 6.0 Hz, 2H), 4.35 (t, J = 6.0 Hz, 2H), 4.83 (s, 2H), 6.58 (d, J = 7.8 Hz, 1H), 6.67 (dd, J = 0.6, 3.0 Hz, 1H), 6.85 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.95 (d, J = 3.0 Hz, 1H), 7.13 (t, J = 8.4 Hz, 1H), 7.32−7.35 (m, 1H), 7.43−7.46 (m, 2H), 7.55 (d, J = 8.4 Hz, 1H), 7.61−7.62 (m, 2H), 7.70 (s, 1H).

Ethyl 2-{4-[3-(2-phenyl-7-propylbenzo[*b*]furan-6-yloxy)propoxy]indol-1-yl}ethanoate (35b). This compound was prepared as described in the case of **14a**, by reacting **35a** with ethyl-2-bromoacetate, giving a 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (t, J = 7.6 Hz, 3H), 1.23 (t, J = 7.2 Hz, 3H), 1.70−1.76 (m, 2H), 2.38 (quintet, J = 2.4, 6.0 Hz, 2H), 2.94 (t, J = 7.6 Hz, 2H), 4.19 (q, J = 7.2 Hz, 2H), 4.27 (t, J = 6.4 Hz, 2H), 4.36 (t, J = 6.0 Hz, 2H), 4.80 (s, 2H), 6.58 (d, J = 7.6 Hz, 1H), 6.66 (d, J = 3.2 Hz, 1H), 6.87 (t, J = 8.0 Hz, 2H), 6.92 (s, 1H), 6.98 (d, J = 3.2 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 7.28−7.31 (m, 2H), 7.41 (t, J = 8.0 Hz, 2H), 7.81 (dd, J = 1.2, 8.4 Hz, 2H). HRMS (EI^+ *m/z*) calcd for $C_{32}H_{33}NO_5$ 511.2359, found 511.2364.

2-{4-[3-(3-Phenyl-7-propylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoic Acid (28). This compound was prepared as described in the case of **14**, starting from **28b**, giving a 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (t, J = 7.2 Hz, 3H), 1.71−1.77 (m, 2H), 2.42−2.45 (m, 2H), 2.93 (t, J = 7.2 Hz, 2H), 4.34 (q, J = 4.8 Hz, 4H), 4.88 (s, 2H), 6.60 (d, J = 7.6 Hz, 1H), 6.68 (d, J = 2.8 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 2.8 Hz, 1H), 7.03 (d, J = 8.8 Hz, 1H), 7.16 (t, J = 8.0 Hz, 2H), 7.51−7.56 (m, 2H), 7.65 (d, J = 8.8 Hz, 1H), 7.93 (dd, J = 1.2, 7.2 Hz, 2H). HRMS (EI^+ *m/z*) calcd for $C_{29}H_{28}N_2O_5$ 484.1998, found 484.1987.

2-{5-[3-(3-Phenyl-7-propylbenzo[*d*]isoxazol-6-yloxy)propoxy]indol-1-yl}ethanoic Acid (29). This compound was prepared as described in the case of **14**, starting from **29b**, giving a 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, J = 7.2 Hz, 3H), 1.66−1.79 (m, 2H), 2.34 (quintet, J = 6.0 Hz, 2H), 2.93 (t, J = 7.5 Hz, 2H), 4.24 (t, J = 6.0 Hz, 2H), 4.31 (t, J = 6.0 Hz, 2H), 4.76 (s, 2H), 6.39 (d, J = 3.0 Hz, 1H), 6.88 (dd, J = 2.4, 8.7 Hz, 1H), 7.02−7.06 (m, 2H), 7.12−7.16 (m, 2H), 7.51−7.67 (m, 3H), 7.65 (d, J = 8.7 Hz, 1H), 7.91−7.94 (m, 2H). HRMS (EI^+ *m/z*) calcd for $C_{29}H_{28}O_5N_2$ 484.1998, found 484.2012.

2-{5-[3-(7-Propyl-1*H*-indol-6-yloxy)propoxy]indol-1-yl}ethanoic Acid (30). This compound was prepared as described in the case of **14**, starting from **30b**, giving a 94% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J = 7.5 Hz, 3H), 1.51−1.65 (m, 2H), 2.21 (quintet, J = 6.0 Hz, 2H), 2.73 (t, J = 7.5 Hz, 2H), 3.60 (s, 3H), 4.08−4.19 (m, 4H), 4.76 (s, 2H), 6.39−6.40 (m, 2H), 6.78 (d, J = 8.7 Hz, 1H), 6.82 (dd, J = 2.4, 9.0 Hz, 1H), 6.95 (d, J = 3.0 Hz, 1H), 7.03−7.05 (m, 3H), 7.32 (d, J = 8.7 Hz, 1H), 7.87 (br s, 1H). HRMS (EI^+ *m/z*) calcd for $C_{24}H_{26}O_4N_2$ 406.1893, found 406.1888.

2-{5-[3-(3-Cyano-7-propyl-1*H*-indol-6-yloxy)propoxy]indol-1-yl}ethanoic Acid (31). This compound was prepared as described in the case of **14**, starting from **31b**, giving a 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.5 Hz, 3H), 1.36−1.51 (m, 2H), 2.40 (quintet, J = 6.0 Hz, 2H), 2.73 (t, J = 7.8 Hz, 2H), 4.14−4.18 (m, 4H), 4.89 (s, 2H), 6.37−6.39 (m, 1H), 6.80 (dd, J = 2.4, 8.7 Hz, 1H), 6.93 (d, J = 8.7 Hz, 1H), 7.04 (d, J = 2.4 Hz, 1H), 7.10 (t, J = 2.7 Hz, 1H), 7.19 (d, J = 8.7 Hz, 1H), 7.29 (s, 1H), 7.43 (d, J = 8.7 Hz, 1H), 8.02 (br s, 1H). HRMS (EI^+ *m/z*) calcd for $C_{25}H_{25}O_4N_3$ 431.1845, found 431.1832.

2-(5-{3-[3-(2,2,2-Trifluoroethyl-1-one)-7-propyl-1*H*-indol-6-yloxy]propoxy}indol-1-yl)ethanoic acid (32). This compound was prepared as described in the case of **14**, starting from **32b**, giving a 92% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (t, J = 7.5 Hz, 3H), 1.56−1.64 (m, 2H), 2.25 (quintet, J = 6.0 Hz, 2H), 3.01 (t, J = 7.8 Hz, 2H), 3.95 (t, J = 5.4 Hz, 2H), 4.52 (t, J = 6.9 Hz, 2H), 4.68 (s, 2H), 6.37−6.39 (m, 1H), 6.79 (dd, J = 2.1, 8.7 Hz, 1H), 6.85 (d, J = 8.7 Hz, 1H), 7.00 (d, J = 2.4 Hz, 1H), 7.13 (t, J = 2.7 Hz, 1H), 7.23 (d, J = 8.7 Hz, 1H), 7.82 (d, J = 1.5 Hz, 1H), 8.05 (br s, 1H), 8.17 (d, J = 8.7 Hz, 1H). HRMS (EI^+ *m/z*) calcd for $C_{26}H_{25}O_5N_2F_3$ 502.1716, found 502.1711.

2-{4-[3-(2-Phenyl-7-propyl-1*H*-indol-6-yloxy)propoxy]indol-1-yl}ethanoic Acid (33). This compound was prepared as described in the case of **14**, starting from **33b**, giving an 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (t, J = 7.2 Hz, 3H), 1.65−1.71 (m, 2H), 2.33−2.39 (m, 2H), 2.81−2.86 (m, 2H), 4.27 (q, J = 6.0, 12.8 Hz, 2H), 4.36 (q, J = 6.0, 12.4 Hz, 2H), 4.83 (s, 2H), 6.57 (t, J = 7.2 Hz, 1H), 6.67 (d, J = 3.2 Hz, 1H), 6.73 (s, 1H), 6.83−6.86 (m, 2H), 7.01−7.15 (m, 2H), 7.28 (t, J = 8.0 Hz, 1H), 7.36−7.43 (m, 3H), 7.63 (d, J = 7.2 Hz, 2H), 8.02 (br s, 1H). HRMS (EI^+ *m/z*) calcd for $C_{30}H_{30}N_2O_4$ 482.2206, found 482.2198.

2-{4-[3-(3-Phenyl-7-propylbenzo[*b*]furan-6-yloxy)propoxy]indol-1-yl}ethanoic Acid (34). This compound was prepared as described in the case of **14**, starting from **34b**, giving a 92% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.95 (t, J = 7.2 Hz, 3H), 1.67−1.71 (m, 2H), 2.37−2.40 (m, 2H), 2.89−2.91 (m, 2H), 4.27 (t, J = 6.0 Hz, 2H), 4.35 (t, J = 6.0 Hz, 2H), 4.83 (s, 2H), 6.58 (d, J = 7.8 Hz, 1H), 6.67 (dd, J = 0.6, 3.0 Hz, 1H), 6.85 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.95 (d, *J* = 3.0 Hz, 1H), 7.13 (t, *J* = 8.4 Hz, 1H), 7.32−7.35 (m, 1H), 7.43−7.46 (m, 2H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.61−7.62 (m, 2H), 7.70 (s, 1H). HRMS (EI$^+$ *m/z*) calcd for $C_{30}H_{29}NO_5$ 483.2046, found 483.2046.

2-{4-[3-(2-Phenyl-7-propylbenzo[*b*]furan-6-yloxy)propoxy]indol-1-yl}ethanoic acid (35). This compound was prepared as described in the case of **14**, starting from **35b**, giving an 89% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, *J* = 7.2 Hz, 3H), 1.71−1.79 (m, 2H), 2.37 (t, *J* = 6.4 Hz, 2H), 2.93 (t, *J* = 7.2 Hz, 2H), 4.26 (t, *J* = 6.0 Hz, 2H), 4.35 (t, *J* = 6.0 Hz, 2H), 4.86 (s, 2H), 6.59 (d, *J* = 8.0 Hz, 1H), 6.68 (d, *J* = 3.6 Hz, 1H), 6.86 (dd, *J* = 5.2, 8.0 Hz, 1H), 6.91 (s, 1H), 6.97 (d, *J* = 3.6 Hz, 1H), 7.12−7.33 (m, 4H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.81 (d, *J* = 7.2 Hz, 2H). HRMS (EI$^+$ *m/z*) calcd for $C_{30}H_{29}NO_5$ 483.2046, found 483.2046.

Biology. Procedures for In Vitro Biological Assays. Ligand Binding Assay. To determine the binding affinity of synthesized compounds to PPARγ, scintillation proximity assay (SPA)[27] was conducted based on the published procedure with some modification. Briefly, the ligand binding domains of hPPARγ were expressed in *Escherichia coli* as glutathione S-transferase (GST) fusion proteins. Recombinant proteins were isolated by affinity purification using glutathione−sepharose following the supplier's recommendation (Amersham Biosciences, Piscataway, NJ). SPA binding assays were performed in binding buffer: 10 mM Tris-Cl, pH 7.2, 1 mM EDTA, 10% (w/v) glycerol, 10 mM sodium molybdate, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/mL benzamidine, and 0.1 mg/mL BSA. [^{3}H]-Rosiglitazone (60 Ci/mmol; purchased from American Radiolabeled Chemicals, St. Louis, MO) was dissolved in ethanol and diluted to a final concentration of 10 nM under reaction. The recombinant GST-hPPARγ was added to the SPA binding buffer to a final concentration of 10 nM. Goat anti-GST antibodies were obtained from Amersham Pharmacia Biotech (catalog number 27-4577-01) and were used at 400-fold dilution. Test compound (20 μL) was added so as to keep the final concentration of DMSO less than 2%. Protein A−yttrium silicate SPA beads were diluted as per the supplier's recommendations (Amersham Biosciences, Piscataway, NJ). The GST-hPPARγ, goat anti-GST antibodies, and test compound SPA beads were diluted in assay buffer and combined in a total volume of 80 μL in the microtiter plate. Following the addition of 20 μL of [^{3}H]-rosiglitazone to each well, the plate was incubated at 15 °C for 24 h with shaking. Radioactivity was quantified in a Packard Topcount scintillation counter.

Cell Culture and PPAR Transactivation Assay (TA). Huh-7 cells were seeded at 1 × 10^5 cells/well in 24-well cell culture plates in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (Gemini Bio-Products), 100 units/mL penicillin G, and 100 mg/mL streptomycin sulfate, and cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2. After 24 h, transfections were performed with transfection reagent (Roche, Indianapolis, IN, 11 988 387) according to the instructions provided by the manufacturer. Briefly, transfection mixtures for each well contained 0.48 μL of transfection reagent, 40 ng of pcDNA3-GAL4/PPAR expression vector, 137 ng of pUAS(5×)-tk-luc reporter vector, and 0.245 ng of SV40−Ren as an internal control for transfection efficiency. Cells were incubated in the transfection mixture for 6 h at 37 °C in an atmosphere of 5% CO_2. The cells were then incubated for another 24 h in fresh culture medium with various concentrations of test compounds. Since the compound stock solutions were prepared in DMSO, control cells were incubated with equivalent concentrations of DMSO. Final DMSO concentration up to 0.1% was shown not to affect the cell-based transactivation activity. Cell lysates were produced using Reporter Lysis Buffer (Promega, Madison, WI) according to the manufacturer's instructions. Luciferase activity in cell extracts was determined using Luciferase Assay kit (Promega, Madison, WI) in a SIRIUS-0 luminometer (Berthold detection systems, Pforzheim, Germany).

Measurement of aP2 mRNA. Confluent 3T3-L1 cells were incubated in DMEM with 10% fetal calf serum, 100 units/mL penicillin G, 10 μg/mL streptomycin sulfate, 1 μM dexamethasone, and 150 nM insulin in the absence or presence of increasing concentrations of test compound for 3 days at 37 °C in 5% CO_2. Total RNA was prepared from cells using RNA isolation kit (Invitrogen, 15596-018), and RNA concentration was estimated from absorbency at 260 nM. The aP2 mRNA was quantified by quantitative PCR (qPCR) method with aP2 specific primers using the Roche LightCycler instrument (2 011 468). Statistical significance was evaluated by using Student's test by comparison of untreated samples with different treatment conditions. To compensate for multiple *t* test, $P < 0.01$ was set as the level of a significant difference.

Preadipocyte Differentiation Assay. Adipocyte differentiation was detected by staining the lipids with Oil Red-O (Sigma) as described previously.[28] In short, cells were fixed in 10% formalin for at least 1 h, stained by immersion in Oil Red-O for 2 h, and exhaustively rinsed with water. Excess water was evaporated by placing the stained samples at 32 °C.

[^{3}H]-2-Deoxy-D-Glucose Uptake Assay in Differentiated 3T3-L1 Adipocytes. The assay described by Mukherjee et al. was used.[29] For the glucose uptake assay, 3T3-L1 cells were grown in 12-well tissue culture plates (Corning, Inc. Costar, Corning, NY) and differentiated into adipocytes with 0.25 mM 1-methyl-3-isobutyl xanthine, 1 μM dexamethasone, and 2 μM insulin. Seven days after induction of differentiation, test compounds were added for an additional 2 days. After two rinses with serum-free DMEM, cells were incubated for 3 h in serum-free DMEM and rinsed at room temperature four times with freshly prepared PBS. The buffer was removed, and the cells were incubated with or without 100 nM insulin in PBS buffer at 37 °C for 20 min. The buffer was replaced with 1 μCi/well of [^{3}H]-2-deoxy-D-glucose (NEN Life Science Products, Boston, MA) in PBS buffer supplemented with 100 μM 2-deoxy-D-glucose (Aldrich, Milwaukee, WI) with incubation for 15 min at room temperature. The supernatant was removed, and plates were rinsed carefully four times with cold PBS. Plates were drained briefly, and cells were lysed overnight in 0.7 mL/well of 0.1% Tritone X-100. Four hundred microliters of lysate was added to a scintillation vial, and 4 mL of Ecoscint A scintillation fluid (National Diagnostics; Atlanta, GA) was added. The vials were mixed and counted.

In Vivo Studies in KKA^y Mice. Adult male KKA^y/TaJcl mice were purchased from Clea Japan (Tokyo), kept on a 12 h light−dark cycle, and provided with food and water ad libitum. Blood samples were collected from the tail vein, and blood glucose was measured using ACCU−CHEK from Roche (Mannheim, Germany). The mice were monitored for blood glucose and divided into groups of seven animals in which the average blood glucose levels among animal groups were similar. Compound **14** was given orally at doses of 1, 10, and 30 mg/kg according to the indicated dosing regimens. Animals of the vehicle control group were orally gavaged with 0.5% methyl cellulose (Sigma, St. Louis, MO). Blood glucose levels of the treated mice were monitored at the times indicated before, during, and after the treatments.

Crystallography. Crystallization and Structure Determination. Crystals of **14**/PPARγ LBD were obtained by the hanging drop method. Typically, 25 μL of PPARγ (8.0 mg/mL in a buffer of 20 mM Tris-Cl (pH 8.0), 5 mM DTT, 100 mM NaCl, and 0.5 mM EDTA) was mixed with 0.5 μL of **14** (10 mM in a buffer of 20 mM Tris-Cl (pH 8.0), 5 mM DTT, 100 mM NaCl, and 0.5 mM EDTA) and equilibrated for 1 h on ice. The complex solution was then centrifuged for 1 min at 4 °C. The supernatant solution was withdrawn carefully by pipet and used for crystallization trials. In the crystallization trails, 1.5 μL of the complex solution was added to 1.5 μL of well solution. The well solution contained 20% PEG3350. The complex crystals were obtained after 3−7 days at 18 °C.

A crystal of about 0.2 mm in length was mounted in a 0.1−0.2 mm Cryoloop (Hampton Research, Inc.). The crystal was immersed briefly in a cryoprotectant containing 20% glycerol and then flash-frozen in liquid nitrogen. Diffraction data were collected at 100 K at the NSRRC synchrotron facility on station BL17B2 with an ADSC Quantum4. The data were processed by DENZO[30] and reduced with SCALEPACK. The structure was solved by molecular replacement by MOLREP[31] using a monomer of the published PPARγ LBD structure (PDB code 2PRG) as the search model. The programs CNS[32] and REFMAC[33] were used for structural refinement and the addition of water molecules. Several rounds of refinement and model building were carried out with the program O.[34] The coordinates of the PPARγ−**14** structure have been deposited in the Protein Data Bank, ID 2ATH.

Acknowledgment. The authors thank Ms. Hsiao-Wen Edith Chu, Ms. Huai-Tzu Chang, Ms. Pey-Yea Yang, and Ms. Chi-Wen Chen for their administrative support, the staff at beamline 17B2 at NSRRC for technical assistance, National Health Research Institutes, Taiwan (Grant Nos. BP-092-PP-05, BP-093-PP-02, BP-093-PP-06, BP-094-PP-02, and BP-094-PP-06), and National Science Council of the Republic of China (Grant Nos. NSC 92-2323-B-007-001 and NSC 93-2323-B-007-001) for financial support.

Supporting Information Available: Purity data, syntheses of 7-propyl-3-trifluoromethylbenzo[*d*]isoxazol-6-ol, 3-phenyl-7-propylbenzo[*d*]isoxazol-6-ol, 7-propyl-1*H*-indol-6-ol, 3-cyano-7-propyl-1*H*-indol-6-ol, 3-(2,2,2-trifluoroethyl-1-one)-7-propyl-1*H*-indol-6-ol, 2-phenyl-7-propyl-1*H*-indol-6-ol, 3-phenyl-7-propylbenzo[*b*]furan-6-ol, and 2-phenyl-7-propylbenzo[*b*]-furan-6-ol, experimental details of the pharmacokinetic studies, expression and purification of PPARγ-LBD, the X-ray data collection and refinement statistics of the PPARγ−**14** complex, and protein to ligand distances of **14**, **1**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Diabetes: The Cost of Diabetes. http://www.who.int/mediacentre/factsheets/fs236/en/ (accessed May 2005).
(2) Diabetes Facts and Figures. http://www.idf.org/home/index.cfm?unode=3B96906B−C026−2FD3−87B73F80BC22682A (accessed May 2005).
(3) Moller, D. E. New Drug Targets for Type 2 Diabetes and the Metabolic Syndrome. *Nature* **2001**, *414*, 821−827.
(4) Nuclear Receptors Nomenclature Committee. A unified nomenclature system for the nuclear receptor superfamily. *Cell* **1999**, *97*, 161−163.
(5) Wysowski, D. K.; Armstrong, G.; Governale, L. Rapid Increase in the Use of Oral Antidiabetic Drugs in the United States, 1990−2001. *Diabetes Care* **2003**, *26*, 1852−1855.
(6) BioPortfolio Diabetes: Time for a New Regime? http://www.bioportfolio.com/news/datamonitor_39.htm (accessed May 2005).
(7) Tugwood, J. D.; Montague, C. T. Biology and Toxicology of PPARγ Ligands. *Hum. Exp. Toxicol.* **2002**, *21*, 429−437.
(8) Pickavance, L. C.; Tadayyon, M.; Widdowson, P. S.; Buckingham, R. E.; Wilding, J. P. Therapeutic Index for Rosiglitazone in Dietary Obese Rats: Separation of Efficacy and Haemodilution. *Br. J. Pharmacol.* **1999**, *128*, 1570−1576.
(9) Murakami, K.; Tobe, K.; Ide, T.; Mochizuki, T.; Ohashi, M.; Akanuma, Y.; Yazaki, Y.; Kadowaki, T. A Novel Insulin Sensitizer Acts as a Coligand for Peroxisome Proliferator-Activated Receptor-α (PPARα) and PPARγ: Effect of PPARα Activation on Abnormal Lipid Metabolism in Liver of Zucker Fatty Rats. *Diabetes* **1998**, *47*, 1841−1847.
(10) Shearer, B. G.; Hoekstra, W. J. Recent Advances in Peroxisome Proliferator-Activated Receptor Science. *Curr. Med. Chem.* **2003**, *10*, 267−280.
(11) Henke, B. R. Peroxisome Proliferator-Activated Receptor α/γ Dual Agonists for the Treatment of Type 2 Diabetes. *J. Med. Chem.* **2004**, *47*, 4118−4127.
(12) Gershell, L. Type 2 Diabetes Market. *Nat. Rev. Drug. Discovery* **2005**, *4*, 367−368.
(13) Barlocco, D. Muraglitazar (Bristol-Myers Squibb/Merck). *Curr. Opin. Invest. Drugs* **2005**, *6*, 427−434.
(14) Evans, R. M.; Barish, G. D.; Wang, Y. X. PPARs and the Complex Journey to Obesity. *Nat. Med.* **2004**, *10*, 355−361.
(15) Mogensen, J. P.; Jeppesen, L.; Bury, P. S.; Pettersson, I.; Fleckner, J.; Nehlin, J.; Frederiksen, K. S.; Albrektsen, T.; Din, N.; Mortensen, S. B.; Svensson, L. A.; Wassermann, K.; Wulff, E. M.; Ynddal, L.; Sauerberg, P. Design and Synthesis of Novel PPARα/γ/δ Triple Activators Using a Known PPARα/γ Dual Activator as Structural Template. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 257−260.
(16) Santini, C.; Berger, G. D.; Han, W.; Mosley, R.; MacNaul, K.; Berger, J.; Doebber, T.; Wu, M.; Moller, D. E.; Tolman, R. L.; Sahoo, S. P. Phenylacetic Acid Derivatives as hPPAR Agonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1277−1280.
(17) Adams, A. D.; Yuen, W.; Hu, Z.; Santini, C.; Jones, A. B.; MacNaul, K. L.; Berger, J. P.; Doebber, T. W.; Moller, D. E. Amphipathic 3-Phenyl-7-propylbenzisoxazoles; Human PPAR γ, δ and α Agonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 931−935.
(18) GlaxoSmithKline Product Development Pipeline − February 2005. http://science.gsk.com/pipeline/pipeline-march2005.pdf (accessed May 2005).
(19) Plexxikon PPAR Pan-agonist Fact Sheet. http://www.plexxikon.com/plx-ppar.pdf (accessed May 2005).
(20) Henke, B. R.; Adkison, K. K.; Blanchard, S. G.; Leesnitzer, L. M.; Mook, R. A., Jr.; Plunket, K. D.; Ray, J. A.; Roberson, C.; Unwalla, R.; Willson, T. M. Synthesis and Biological Activity of a Novel Series of Indole-Derived PPARγ Agonists. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3329−3334.
(21) Jones, A. B. Peroxisome Proliferator-Activated Receptor (PPAR) Modulators: Diabetes and Beyond. *Med. Res. Rev.* **2001**, *21*, 540−552.
(22) Adams, A. D.; Berger, J. P.; Berger, G. D.; Fitch, K. J.; Graham, D. W.; Jones, A. B.; Von Langen, D.; Leibowitz, M. D.; Moller, D. E.; Patchett, A. A.; Santini, C.; Sahoo, S. P.; Tolman, R. L.; Toupence, R. B.; Walsh, T. F. Heterocyclic Derivatives as Antidiabetic and Antiobesity Agents. World Patent WO 97/28137, January 31, 1997.
(23) Ebdrup, R.; Pettersson, I.; Rasmussen, H. B.; Deussen, H. J.; Jensen, A. F.; Mortensen, S. B.; Fleckner, J.; Pridal, L.; Nygaard, L.; Sauerberg, P. Synthesis and Biological and Structural Characterization of the Dual-Acting Peroxisome Proliferator-Activated Receptor/Agonist Ragaglitazar. *J. Med. Chem.* **2003**, *46*, 1306−1317.
(24) Sauerberg, P.; Pettersson, I.; Jeppesen, L.; Bury, P. S.; Mogensen, J. P.; Wassermann, K.; Brand, C. L.; Sturis, J.; Woldike, H. F.; Fleckner, J.; Andersen, A. S.; Mortensen, S. B.; Svensson, L. A.; Rasmussen, H. B.; Lehmann, S. V.; Polivka, Z.; Sindelar, K.; Panajotova, V.; Ynddal, L.; Wulff, E. M. Novel Tricyclic-α-alkyloxyphenylpropionic Acids: Dual PPARα/γ Agonists with Hypolipidemic and Antidiabetic Activity. *J. Med. Chem.* **2002**, *45*, 789−804.
(25) Nolte, R. T.; Wisely, G. B.; Westin, S.; Cobb, J. E.; Lambert, M. H.; Kurokawa, R.; Rosenfeld, M. G.; Willson, T. M.; Glass, C. K.; Milburn, M. V. Ligand Binding and Co-activator Assembly of the Peroxisome Proliferator-Activated Receptor-γ. *Nature* **1998**, *395*, 137−143.
(26) Xu, H. E.; Lambert, M. H.; Montana, V. G.; Plunket, K. D.; Moore, L. B.; Collins, J. L.; Oplinger, J. A.; Kliewer, S. A.; Gampe, R. T., Jr.; McKee, D. D.; Moore, J. T.; Willson, T. M. Structural Determinants of Ligand Binding Selectivity Between the Peroxisome Proliferator-Activated Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13919−13924.
(27) Elbrecht, A.; Chen, Y.; Adams, A.; Berger, J.; Griffin, P.; Klatt, T.; Zhang, B.; Menke, J.; Zhou, G.; Smith, R. G.; Moller, D. E. L-764406 Is a Partial Agonist of Human Peroxisome Proliferator-Activated Receptor γ. The Role of Cys[313] in Ligand Binding. *J. Biol. Chem.* **1999**, *274*, 7913−7922.
(28) Trouba, K. J.; Wauson, E. M.; Vorce, R. L. Sodium Arsenite Inhibits Terminal Differentiation of Murine C3H 10T1/2 Preadipocytes. *Toxicol. Appl. Pharmacol.* **2000**, *168*, 25−35.
(29) Mukherjee, R.; Hoener, P. A.; Jow, L.; Bilakovics, J.; Klausing, K.; Mais, D. E.; Faulkner, A.; Croston, G. E.; Paterniti, J. R., Jr. A Selective Peroxisome Proliferator-Activated Receptor-γ (PPARγ) Modulator Blocks Adipocyte Differentiation but Stimulates Glucose Uptake in 3T3-L1 Adipocytes. *Mol. Endocrinol.* **2000**, *14*, 1425−1433.
(30) Otwinowski, Z.; Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation Mode. *Methods Enzymol.* **1997**, *276*, 307−326.
(31) Vagin, A.; Teplyakov, A. MOLREP: An Automated Program for Molecular Replacement. *J. Appl. Crystallogr.* **1997**, *30*, 1022−1025.
(32) Brunger, A. T.; Adams, P. D.; Clore, G. M.; Delano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J.-S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, G. L.; Warren, T.; Badger, J.; Berard, D.; Kumar, R. A.; Szalma, S.; Yip, P.; Griesinger, C.; Junker, J. Crystallography and NMR System: A New Software Suite for Macromolecular Structure Determination. *Acta Crystallogr.* **1998**, *D54*, 905−921.
(33) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Refinement of Macromolecular Structures by the Maximum-likelihood Method. *Acta Crystallogr.* **1997**, *D53*, 240−255.
(34) Jones, T. A.; Zou, J. Y.; Cowan, S. W.; Kjeldgaard, M. Improved Methods for Building Protein Models in Electron Density Maps and the Location of Errors in these Models. *Acta Crystallogr.* **1991**, *A47*, 110−119.

JM0506930