Design and Synthesis of a Novel Series of *N*-Alkyl Isatin Acylhydrazone Derivatives that Act as Selective Cannabinoid Receptor 2 Agonists for the Treatment of Neuropathic Pain

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There is growing interest in using cannabinoid receptor 2 (CB2) agonists for the treatment of neuropathic pain. We have synthesized a novel series of *N*-alkyl isatin acylhydrazone derivatives and have identified and characterized several of them as novel analogues with high functional activity and selectivity at human CB2 receptors using [^{35}S]GTP-γ-S assays. Binding affinities at human CB2 and CB1 were determined for compounds **28**, **33**, **40**, **48**, and **58**. Structure−activity relationship studies of this novel series led to optimization of our lead compound, compound **33** (MDA19). Compound **33** possessed potent antiallodynic effects in a rat model of neuropathic pain but did not affect rat locomotor activity. More potent and more CB2-receptor−selective compounds, including compounds **37**, **40**, and **48**, were also discovered.

Introduction

Neuropathic pain is caused by lesions in the central (brain and spinal cord) or peripheral nervous system. It is not a single disease entity and may result from a wide range of heterogeneous conditions that differ in etiology. Neuropathic pain is triggered by conditions such as diabetic neuropathy, AIDS-related neuropathy, postherpetic neuralgia, degenerative spinal disease, chemotherapy, radiotherapy, complex regional pain syndrome, phantom limb pain, trigeminal neuralgia, and multiple sclerosis. Allodynia (touch-evoked pain) and hyperalgesia are common clinical characteristics of neuropathic pain.

The prevalence of neuropathic pain is estimated to be about 8% worldwide.[1] In the United States, the annual health care cost attributable to neuropathic pain is almost $40 billion.[2] Currently, treatment effectiveness for neuropathic pain is limited.[3] Recently, however, cannabinoid receptor 2 (CB2) [a] agonists emerged as new agents for the treatment of this pain.[4–7]

Two CB receptors, CB1 and CB2, have been characterized and cloned.[8,9] The CB1 receptor is found predominantly in the brain, with highest densities in the hippocampus, cerebellum, and striatum.[10] Impairment of cognitive functions (and psychoactivity) induced by Δ^9-tetrahydrocannabinol (Δ^9-THC) is mediated by CB1 receptors in the isocortex and allocortex (i.e., hippocampus).[11] CB1 agonists have shown promise for pain relief, but the altered psychological state and motor impairment associated with their use have affected their pharmaceutical development and use.

CB2 receptors are expressed mainly on immune cells (monocytes, B and T lymphocytes) and tissues (spleen, tonsils).[9,12] However, CB2 gene transcripts and receptors have been discovered in the central nervous system (CNS). The expression of CB2 receptors in the brain suggests that they may play broader roles in the CNS than previously appreciated.[13]

It is believed that selective CB2 agonists may be devoid of pyschoactivity. CB2-receptor mRNA and proteins are increased in the dorsal root ganglia and spinal cord in animals after spinal nerve ligation,[5,14] sciatic nerve injury,[15] or saphenous nerve ligation.[16,17]

Several CB2-selective agonists have been described previously.[18–28] Some of these compounds either failed to exhibit potent agonist efficacy or were not been fully characterized in functional assays. Recently, however, functional studies for various CB2 agonists were described.[28] The chemical structures of a number of CB2 agonists are shown in Figure 1. Compounds **1**[29] and **2**[30] (Figure 1) have condition-dependent CB2 functional activities and are described as protean agonists.[31,32] The mechanisms of action of compound **1** in pain relief[5,6,33] are complex, and this compound interacted with the opioid system.[34] A recently published study disclosed that another CB2-selective agonist, compound **4**,[19] was chosen as a clinical candidate for the treatment of inflammatory pain.[19] Compound **5**,[35] a well-characterized CB2 agonist[35] structurally based on Δ^9-THC, inhibited neuropathic hyperalgesia through a CB2-selective mechanism.[36] Compound **3**,[37] another well-characterized selective CB2 agonist, showed efficacy in models of inflammatory, postoperative, neuropathic, and osteoarthritic pain,[28] all of which were selectively blocked by CB2 but not by CB1 or μ-opioid receptor-selective antagonists. Recently, a nonselective CB1/CB2 dual agonist with limited brain penetration was shown to reverse neuropathic mechanical hyperalgesia in a rat model of neuropathic pain.[38]

In our efforts to understand and improve the treatment of neuropathic pain, we sought to design and synthesize a series of proprietary CB2 agonists. The library design was based on compound **8**[39] (Figure 2), a nonspecific cannabinoid agonist showing a high affinity for the *h*CB2 receptor.[40] The indole ring of compound **8** was replaced by the isatin ring (compound **9**). An acylhydrazone moiety was used in order to replace the 1-naphthoyl moiety. In compound **9**, either the imino or the carbonyl moiety can be located in the same region as the carbonyl moiety of compound **8**.

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[a] Abbreviations: BSA, bovine serum albumin; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CI, confidence interval; CNS, central nervous system; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; EC_{50}, half-maximal effective concentration; ED_{50}, effective dose in 50% of the animal population; ED_{90}, effective dose in 90% of the animal population; EDTA, ethylenediaminetetraacetic acid; GTP, guanosine-5′-triphosphate; *h*CB1, human CB1; *h*CB2, human CB2; HPLC, high-performance liquid chromatography; IC_{50}, median inhibition concentration; NMR, nuclear magnetic resonance; ROESY, rotational nuclear Overhauser effect spectroscopy; Δ^9-THC, Δ^9-tetrahydrocannabinol; THF, tetrahydrofuran; HRMS, high resolution mass spectroscopy.

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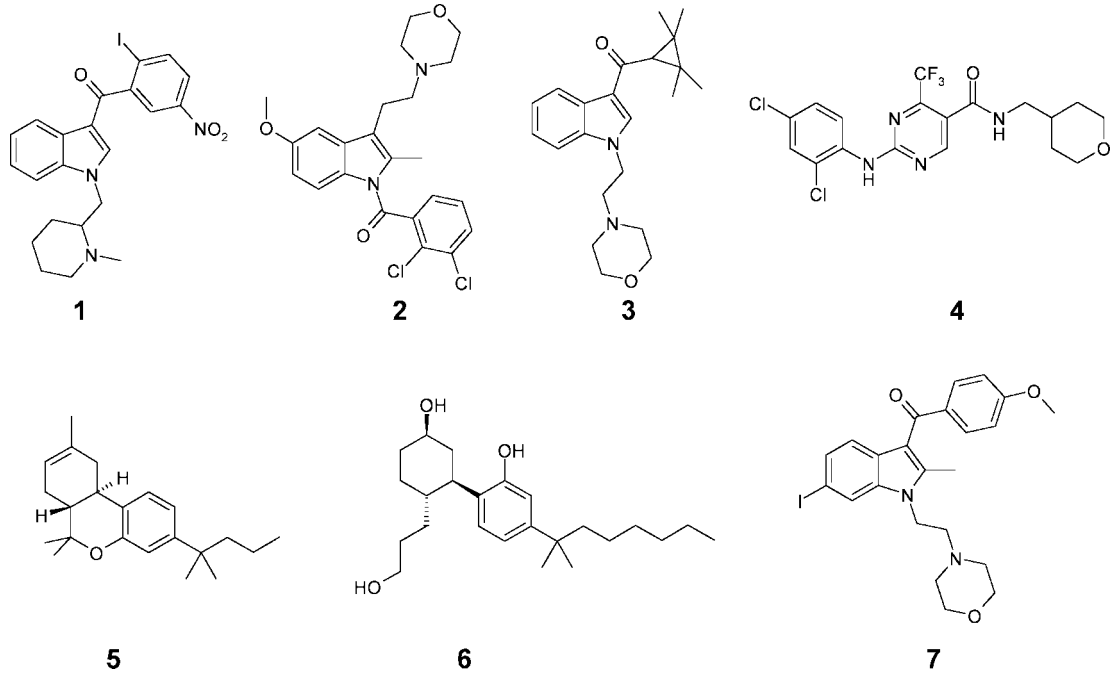


Figure 1. Chemical structures of CB2-selective agonists; **1** (AM1241),[29] **2** (GW405833/L768242),[30] **3** (A-796260),[37] **4** (GW842166X),[19] **5** (JWH133),[35] nonselective agonist **6** (CP55940),[44] and CB2 antagonist **7** (AM630).[52]

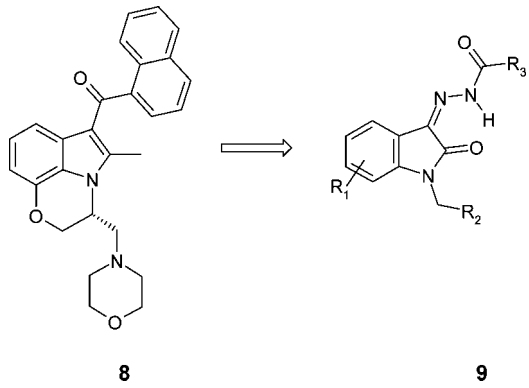


Figure 2. Rational design of the isatin series.

First, we assessed the effect on activity of replacing the carbonyl of the acylhydrazone moiety. On the basis of our hypothesis, we expected the isatin ring to occupy the same pocket in the CB2 receptor as the oxazinoindole ring occupied in compound **8**. Substituents bore by the acylhydrazone moiety were expected to occupy the same pocket as the naphthyl ring of compound **8**. Prediction of the optimal moiety for R_3 in compound **9** was difficult because the angle and the length between R_2 and R_3, compared with the naphthyl and morpholinyl moieties in compound **8**, are very different. To determine the extent to which the structure−activity relationship (SAR) of this new series resembled that of (aminoalkyl)indole, the effect on biological activity of varying the nature of substitution at either the acylhydrazone moiety and at the nitrogen of the isatin ring was evaluated by using parallel synthesis. To mimic the oxazinyl moiety of compound **8**, substitution of the isatin scaffold was used (R_1 in Figure 2).

After the design of our library, a patent was published for use of a CB2 agonist based on the isatin scaffold for hair and/or scalp care[41] with affinities in the milimolar range. The approach used in the patent was based on screening of commercially available compounds. None of the compounds described in this article were mentioned in the patent.

Methods

Chemistry. The synthesis outlined in Scheme 1 proceeded in two chemical steps, beginning with commercially available isatin.

N-Alkylation of the isatin with commercially available alkyl halide using sodium hydride at room temperature or cesium carbonate with microwave irradiation in *N,N*-dimethylformamide (DMF) afforded the desired products in moderate to good yields (Table 1).

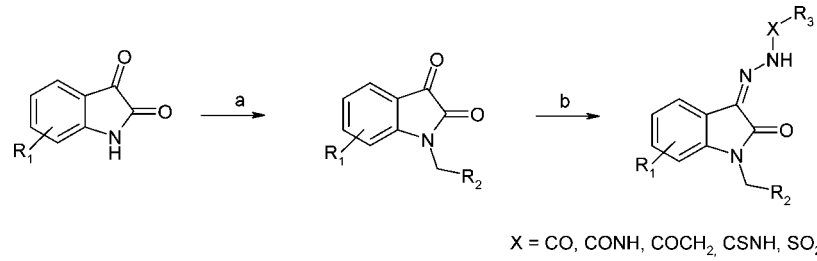
Condensation of the resulting N-substituted isatin with hydrazine derivatives afforded the desired hydrazone in good to moderate yields depending on the rate of crystallization in the final step. For hydrazone derivatives **29** and **34**, described below, the ^{1}H nuclear magnetic resonance (NMR) spectra indicated the presence of two rotamers, as previously described.[42]

Synthesis of *tert*-butyl 2-(1-hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazinecarboxylate afforded two isomers (compounds **40** and **41**), which were isolated by column chromatography and studied by use of two-dimensional (2D) rotational nuclear Overhauser effect spectroscopy (ROESY) NMR experiments (see the 2D ROESY NMR Analyses section below).

Crystallographic Analyses. The structure of compound **33** was confirmed by X-ray diffraction. Compound **33** yielded crystals of suitable quality for X-ray diffraction by slow evaporation of an ethyl acetate solution. The two structures depicted in Figure 3 merely reflect the fact that there is a certain degree of flexibility in the hexyl side chain. As expected, the structure obtained is the one in which the hydrogen borne by nitrogen atom N12 might form a hydrogen bond with the oxygen atom O10.

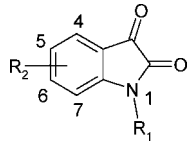
2D ROESY NMR Analyses. The 2D NMR spectra were recorded for compounds **33**, **40**, and **41** to reveal information about short interproton distances (off-resonance Overhauser enhancement spectroscopy ROESY). ROESY 2D NMR estab-

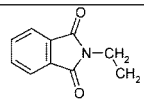
Scheme 1. Synthetic Route[a]



[a] Reagents and conditions: (a) NaH, DMF, room temperature, 2 h, followed by R_2CH_2Br, room temperature, 12 h, or $CsCO_3$, DMF, R_2CH_2Br, microwave irradiation, 140°C, 10 min; (b) H_2N-HN-X-R_3, AcOH, EtOH, THF, room temperature, 12 h.

Table 1. Chemical Yields for Isatin Synthesis



Compound	R1	R2	R2 Position
10	2-cyclohexylethyl	H	-
11	$CH_3(CH_2)_5$	H	-
12	$CH_3(CH_2)_2$	H	-
13	$CH_3(CH_2)_3$	H	-
14	$CH_3(CH_2)_4$	H	-
15	cyclohexylmethyl	H	-
16	Benzyl	H	-
17		H	-
18	$CH_3(CH_2)_5$	Me	7
19	$CH_3(CH_2)_5$	Cl	7
20	$CH_3(CH_2)_5$	I	7
21	Benzyl	I	7
22	$CH_3(CH_2)_5$	Me	5
23	$CH_3(CH_2)_5$	MeO	5
24	$CH_3(CH_2)_5$	F	5
25	$CH_3(CH_2)_5$	Cl	5
26	$CH_3(CH_2)_4$	I	5

lished the E-geometry of the imino double bond for compound **41**. A cross-peak for N−H and the aromatic C−H(4), characterizing a through space interaction of the hydrazino hydrogen and the aromatic C−H(4), established the E-geometry for compound **41**. This interaction was absent for compound **40** and compound **33**.

Pharmacologic Analysis: Binding Assays. All of the compounds synthesized were screened at one concentration (1 or 10 μM) in a competitive binding experiment using membranes of Chinese hamster ovarian cells selectively expressing either the human CB1 (*h*CB1) receptor or the human CB2 (*h*CB2) receptor. [^{3}H]**6** at a concentration of 0.5 nM and [^{3}H]**8** at a concentration of 0.8 nM were used as radioligands for *h*CB1

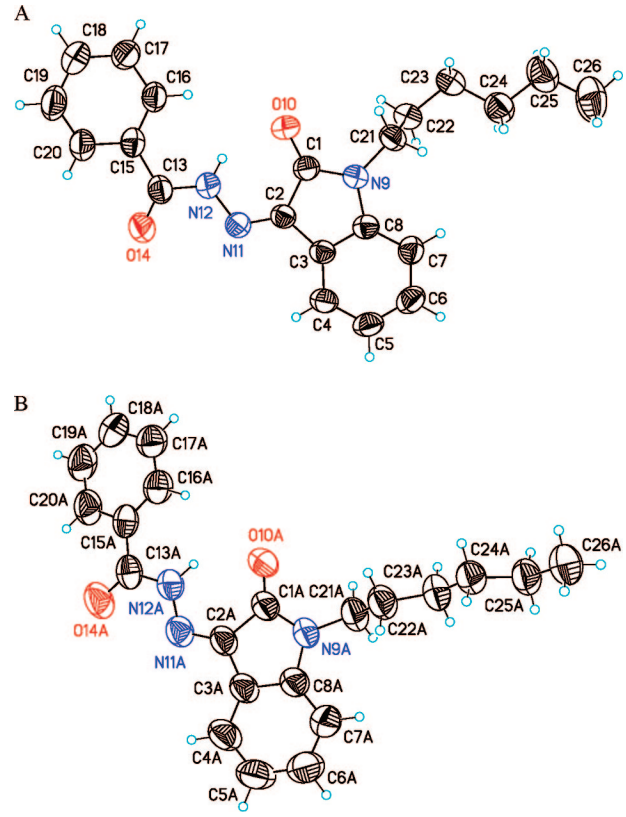


Figure 3. (A) View of molecule **33a** showing the atom labeling scheme. Displacement ellipsoids are scaled to the 50% probability level. (B) View of molecule **33b** showing the atom-labeling scheme. Displacement ellipsoids are scaled to the 50% probability level.

and *h*CB2 assays, respectively. The results are expressed as percentage inhibition of control specific binding. Compounds that displaced the radioligand by more than 50% for either *h*CB1 or *h*CB2 receptors were chosen for functional activity determination.

Several compounds were also screened in a competitive binding experiment by using membranes of CHO-K1 cells selectively expressing the *h*CB2 receptor at different concentrations in duplicate.[43] The competitive binding experiment was performed in 96-well plates (Masterblock) containing binding buffer (50 mM Tris, pH 7.4, 2.5 mM ethylenediaminetetraacetic acid [EDTA], 0.5% protease-free bovine serum albumin [BSA]), recombinant membrane extracts (0.25 μg protein/well), and 1 nM [^{3}H]**6** (Perkin-Elmer, NEX-1051, 161 Ci/mmol, diluted in binding buffer). Nonspecific binding was determined in the presence of 10 μM of compound **6** (Tocris Bioscience).[44] The sample was incubated in a final volume of 0.1 mL for 60 min at 30 °C and then filtered on a GF/B UniFilter microplate (Perkin-Elmer, catalogue no. 6005177) presoaked in 0.5%

polyethyleneimine for 2 h at room temperature. Filters were washed six times with 4 mL of cold binding buffer (50 mM Tris, pH 7.4, 2.5 mM EDTA, 0.5% protease-free BSA), and bound [^{3}H]**6** was determined by liquid scintillation counting. The median inhibition concentration (IC_{50}) was determined by nonlinear regression using one-site competition equation. The inhibition constants (K_i) were calculated by using the Cheng−Prusoff equation ($K_i = IC_{50}/(1 + (L/K_D))$, where L = concentration of radioligand in the assay and K_D = affinity of the radioligand for the receptor.

Pharmacologic Analysis of Cannabinoid Receptor-Mediated Functional Activity. Functional activity for acyl hydrazone was evaluated by using a [^{35}S] guanosine-5′-triphosphate (GTP)-γ-S assay in Chinese hamster ovarian cell membrane extracts expressing recombinant *h*CB1 or *h*CB2 receptor. The assay relies on the binding of [^{35}S]GTP-γ-S, a radiolabeled nonhydrolyzable GTP analogue, to the G protein upon binding of an agonist of the G-protein-coupled receptor. In this system, agonists stimulate [^{35}S]GTP-γ-S binding, whereas antagonists have no effect and inverse agonists decrease [^{35}S]GTP-γ-S basal binding. CB1 and CB2 assay data are presented as the mean of two determinations. Assay reproducibility was monitored by the use of a reference compound **6**. For replicate determinations, the maximum variability tolerated in the test was ± 20% of the average of the replicates. Efficacies (E_{max}) for CB1 or CB2 were expressed as a percentage of the efficacy of compound **6**.

In Vivo Evaluation. Animals. Adult male Sprague−Dawley rats (Harlan Sprague−Dawley, Indianapolis, IN) weighing 120−150 g were used in experimental procedures approved by the Animal Care and Use Committee of The University of Texas M. D. Anderson Cancer Center. Animals were housed three per cage on a 12 h light/12 h dark cycle with water and food pellets available ad libitum.

Lumbar 5/6 Spinal Nerve Ligation Pain Model. All surgical procedures were performed under deep isoflurane anesthesia in 100% O_2. The spinal nerve ligation was performed as described previously.[45] Briefly, a midline incision was made above the lumbar spine to expose the left L6 transverse process. The process was then removed, the left L5 and L6 spinal nerves were isolated, and both nerves were tightly ligated with 6−0 silk. A prophylactic antibiotic (5 mg/kg of norfloxacin subcutaneously) and a prophylactic analgesic (2.5 mg/kg of morphine subcutaneously) were administered once daily for 3 days. The rats were allowed to recover for 5−6 days before being used for behavioral testing. All of the experiments were conducted 10−14 days after spinal nerve ligation.

Assessment of Mechanical Withdrawal Thresholds. For assessment of antiallodynic effect, rats were placed in a compartment with a wire mesh bottom and allowed to acclimate for at least 30 min before testing. Mechanical sensitivity was assessed by using a series of Von Frey filaments with logarithmic incremental stiffness (0.41, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.1 g) (Stoelting, Wood Dale, IL), as previously described,[46] and 50% probability withdrawal thresholds were calculated with the up−down method.[47] In brief, beginning with the 2.00 g probe, filaments were applied one by one to the plantar surface of a hind paw for 6−8 s. If no withdrawal response was observed, the next stiffer filament was applied; if there was a withdrawal response, the next less stiff filament was applied. Six consecutive responses from the first change in the response were used to calculate the withdrawal threshold (in grams). When the response thresholds fell outside the range of detection, 15.00 g was assigned for absence of response to all tested fibers, and 0.25 g was assigned for withdrawal response to all tested fibers. The percentage maximal possible effect was calculated as ([postdrug threshold − baseline threshold]/[cutoff threshold (15 g) − baseline threshold]) × 100.

Open-Field Chamber Testing. For assessment of potential CNS effects, compound **33** and the vehicle were tested in an automated open-field chamber (Med Associates ENV-515 Test Environment, St. Albans, VT), 43.2 cm × 43.2 cm × 30.5 cm (L × W × H), equipped with three pairs of 16 infrared arrays that continually monitored the animal's movement. Rats were individually tested 15 min after intraperitoneal drug administration. The infrared beams were set 2.5 cm apart horizontally and 3 cm above the floor, and the rearing array was set 12 cm above the floor. The area in the box was divided into four equal zones, with data collected within each zone and across zones. *Ambulatory movement* was defined as movement of at least 5 cm and was coded by quadrant. *Vertical movement* was defined as movement of at least 12 cm from the floor. A *zone entry* was defined as an ambulatory movement during which the rat moved far enough into a new zone to break two sets of photoelectric beams in the new zone.

Data Analyses. Statistical analyses were carried out by using BMDP 2007 (Statistical Solutions, Saugus, MA). Data were analyzed using one-way analysis of variance (ANOVA) or *t* test, where appropriate. If findings on ANOVA were significant, Tukey−Kramer post hoc analysis was used for multiple group comparison. The area under the curve was calculated by using the trapezoidal rule. The results were presented as mean ± SEM and were considered significant at $P < 0.05$. Analyses of the dose−response curves and statistics were obtained by using the pharmacological software programs of Tallarida and Murray[48] and included the calculation of the effective dose in 50% of the animal population (ED_{50}) values and their 95% confidence intervals (CI).

Results and Discussion

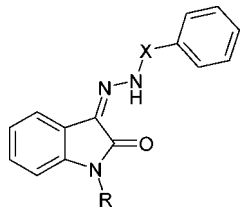
Our first objective was to study the effects on affinity for cannabinoid receptors of a hydrogen bond acceptor borne by the hydrazine moiety. By analogy with (aminoalkyl)indole derivatives such as compounds **1**, **2**, **3**, or **8**, isatin was alkylated with 2-cyclohexylethylbromide to mimic the piperidine or the morpholine ring. A hexyl chain was used by analogy with classical cannabinoids such as compound **5** or **6**. None of the sulfone derivatives (**31** and **36**), urea derivatives (**27** and **32**), or thiourea derivatives (**30** and **35**) displayed significant binding at 10 μM for either CB1 or CB2 (Table 2). The acyl hydrazone derivatives **28**, **29**, **33**, and **34** displaced a significant percentage of the radiolabeled ligands for both CB1 and CB2 receptors.

The inactivity of compounds **27** and **32** compared with the activity of compounds **29** and **34** might be explained by the unfavorable interaction between the receptor and the polar N−H functionality or by a difference in the positioning of the phenyl ring. In the case of compounds **27** and **32**, a hydrogen bond between the hydrogen of the aniline moiety and the =N is expected to favor conformation A (Scheme 2) compared with compounds **29** and **34** (see conformation A compared with conformations B and C in Scheme 2).

Considering the small increase in affinity of compound **35** compared with compound **32** (Table 2), the hydrogen atom of a thiol tautomer form might be involved in a hydrogen bond interaction with the =N, yielding conformation similar to conformation C.

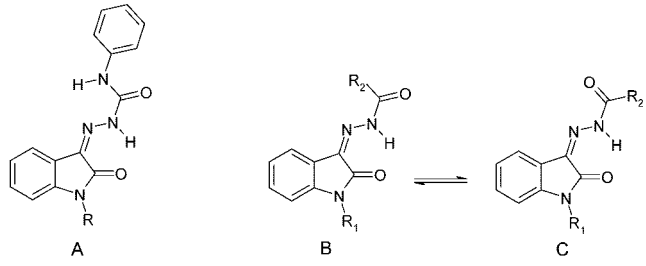
As previously mentioned, compounds that exhibited percentage inhibition of control specific binding greater than 50% for either *h*CB1 or *h*CB2 receptors were chosen for functional

Table 2. Percentage Inhibition of Specific Binding of [^{3}H]**6** on *h*CB1 Receptor and of [^{3}H]**8** on *h*CB2 Receptor by Compounds **27**−**36** at 10 μM



			% inhibition	
compd	R	X	*h***CB$_1$**receptor	*h***CB$_2$**receptor
27	2-cyclohexylethyl	(C═O)NH	<40	<40
28	2-cyclohexylethyl	C═O	96 ± 3.7	79.8 ± 6.3
29	2-cyclohexylethyl	(C═O)CH_2	88.8 ± 0.6	47.2 ± 7.7
30	2-cyclohexylethyl	(C═S)NH-	<40	<40
31	2-cyclohexylethyl	SO_2	<40	<40
32	$CH_3(CH_2)_5$	(C═O)NH	<40	<40
33	$CH_3(CH_2)_5$	C═O	95.5 ± 0	92.8 ± 8.7
34	$CH_3(CH_2)_5$	(C═O)CH_2	98.2 ± 0.8	91.5 ± 0.1
35	$CH_3(CH_2)_5$	(C═S)NH	49 ± 5.2	37.7 ± 2.5
36	$CH_3(CH_2)_5$	SO_2	<40	<40

Scheme 2. Possible Isomers for Acyl Hydrazone Derivatives of Isatin



27 R = Cyclohexyl$(CH_2)_2$
32 R = $CH_3(CH_2)_5$

28 R_1 = Cyclohexyl$(CH_2)_2$, R_2 = Phenyl
29 R_1 = Cyclohexyl$(CH_2)_2$, R_2 = Benzyl
33 R = $CH_3(CH_2)_5$, R_2 = Phenyl
34 R = $CH_3(CH_2)_5$, R_2 = Benzyl

activity determination (Table 3). As acyl hydrazone derivatives **28**, **29**, **33,** and **34** displaced the radioligand by more than 50% in the binding assay, the functional activities of these compounds were evaluated. All four of these compounds exhibited agonist activity for CB1 with moderate potency (EC_{50}): the highest EC_{50} obtained was 459 nM. The corresponding efficacies were also moderate to good, ranging from 56.8% to 75.0%, compared with the reference compound **6**. The potencies for the CB2 receptor were 2−14 times higher than those for the CB1 receptor. Compared with compounds **28**, **29**, and **34**, the benzoyl hydrazone **33** exhibited better functional activity, being seven times less potent than the reference compound **6**. Moreover, compound **33** showed a CB2 receptor selectivity, with EC_{50}(CB1)/EC_{50}(CB2) = 14 and moderate potency against CB1. The *N*-hexyl moiety was better tolerated by CB1 or CB2 than was the *N*-cyclohexylethyl moiety because compounds **33** and **34** were more potent and efficient than were compounds **28** and **29** for both CB1 and CB2 receptors. An additional methylene moiety between the carbonyl and the phenyl ring, as in the phenylacetyl derivative **34**, resulted in a slight increase of CB1 potency and efficacy and a decrease of CB2 potency and efficacy.

Because our goal was to design CB2-selective compounds, we decided to modify the benzoyl moiety of compound **33** to improve its potency and selectivity (Table 4). Bulky aromatic moieties such as 2-naphthyl (compound **42**) and benzothiophene (compound **44**) were not tolerated by either CB1 or CB2 receptors, but compound **45**, bearing a 1-naphthyl group, exhibited affinity for both CB1 and CB2 receptors as expected and as previously shown for classical aminoalkylindoles.[49] Surprisingly, the adamantyl derivative **46** at a concentration of 1 μM was able to displace more than 85% of the [^{3}H]**6** from both CB1 and CB2 receptors. The effect of the intracyclic nitrogen substitution was studied. Length variation from four to six carbon atoms (compounds **33**, **48**, and **49**) had no effect on the percentage of displacement of the radiolabeled ligands for either CB1 or CB2 receptors. The propyl derivative **47** exhibited weak affinity. On the other hand, the dodecyl derivative **50**, the rigid phenyl derivative **51**, and the phthalimide derivative **55** were not able to displace the ligand from either CB1 or CB2 receptors. Less rigid aryl or cycloalkyl rings such as those in compounds **52**, **53**, and **54** showed good affinity. Isatin substitution in position 5 either by electron donor or withdrawing groups (compounds **59**−**63**) resulted in a loss of affinity for either CB1 or CB2 receptors compared with compound **33**. Isatin substitution in position 7 had a less drastic effect in terms of loss of affinity. Compounds **56**−**58** showed less affinity than did compound **33**.

Functional activity was determined for compounds that displaced the radioligand by more than 50% for either *h*CB1 or *h*CB2 receptors (Table 5). According to previous studies,[50] in the cannabimimetic aminoalkylindole series, aromatic stacking interactions are the primary interactions for CB1 receptor binding. As expected, replacement of the compound **33** phenyl ring by a cyclohexyl ring (compound **37**) resulted in a decrease of CB1 activity. CB2 activities for both compounds are in the same range and did not experience this potential loss of aromatic stacking interactions. The same effect on CB1 functional activity was obtained using the bulky *tert*-butoxyl radical in compound **40**. Furthermore, in this case, CB2 functional activity potency improved. The corresponding *E* conformer, compound **41**, failed to exhibit functional activity on both CB1 and CB2 receptors. Compound **43**, which results from substitution of a methoxy in the para position of the phenyl hydrazoic moiety of compound **33**, showed decreased potency and efficacy for the CB2 receptor but unchanged activity for CB1 receptor. Substitution at this position seems to be critical for CB2, as confirmed by the loss of affinity of compound **39**. Compounds **45** and **46** showed more than 80% and 69% displacement of [^{3}H]**6** in the primary CB1 and CB2 screening, respectively, but exhibited moderate activity in the functional assays compared with that exhibited by compound **33**. Therefore, as mentioned above, positioning of the naphthyl bore by the acylhydrazone moiety compared with the one bore by the acyl moiety in compound **8** is clearly different.

Length variation of the aliphatic chain borne by nitrogen, from three to six carbon atoms (compounds **33**, **47**, **48**, and **49**), had a dramatic effect on CB1 functional activity. The propyl derivative **47** experienced a small decrease in terms of CB2 functional activity compared with the hexyl derivative **33** or the butyl derivative **48**. On the other hand, despite modest CB2 activity, compound **47** showed CB2 selectivity. This selectivity was almost maintained with compound **48**, which had increased CB2 functional activity compared with compound **33**. Surprisingly, functional activity for the pentyl analogue **49** was lower for CB2 and higher for CB1. Aliphatic chain replacement by a cyclohexylmethyl moiety (compound **52**) resulted in a large increase in both CB1 and CB2 functional activity compared with that of compound **33**. The cyclohexylmethyl moiety of compound **52** might occupy the same pocket as the six-membered ring borne by the nitrogen of the indole ring for the

Table 3. Determination of Potency (EC_{50}) and Maximal Stimulation (E_{max}) on *h*CB1 and *h*CB2 Receptors of Compounds **28**, **29**, **33**, and **34**[a]

			[^{35}S]GTP-γ-S(*h*CB1)		[^{35}S]GTP-γ-S (*h*CB2)	
compd	R	X	**EC_{50}** (nM ± SEM)	**E_{max}**(%)	EC_{50} (nM ± SEM)	E_{max}(%)
6			10.3 ± 1	100	8.66 ± 1	100
28	2-cyclohexylethyl	C=O	ND	56.8	103.4 ± 1.6	55.6
29	2-cyclohexylethyl	(C=O)CH_2	1238.8 ± 1.2	75	ND	21
33	$CH_3(CH_2)_5$	CO	867.0 ± 1.1	59.7	63.4 ± 1.3	68.8
34	$CH_3(CH_2)_5$	(C=O)CH_2	459.0 ± 1.1	71.9	188.7 ± 1.3	45.8

[a] ND = not determined (plateau was not reached at 10 μM dose).

(aminoalkyl)indole derivatives. Compound **52** was the most potent of the series in terms of CB2 functional activity. The benzyl derivative **53** had the same CB2 functional activity as compound **33** but with a loss of CB1 functional activity. Substitution by a chlorine atom in the para position of the benzyl ring (compound **54**) resulted in a decrease in both selectivity and CB2 activity. Despite good CB2 potency, compound **56** exhibited a low efficacy for CB2. Increasing the bulkiness of the substituent in compounds **57** and **58** resulted in a loss of CB2 functional activity. Substitution in the position 7 of the isatine ring resulted in a loss of CB1 activity. Compound **61** showed a weak CB2 activity.

The competitive binding assays were also performed in membranes of CHO-K1 cells selectively expressing the *h*CB2 receptor for the most active compounds (**28**, **33**, **40**, **48**, and **53**) in the functional assays. As expected, these compounds showed high affinity for CB2 receptors (Table 6). For instance, compound **40** has the highest affinity (K_i of 13.9 nM) and functional activity (EC_{50} of 22 nM) at CB2 receptors. Compound **40** is approximately 8 times less potent than is compound **6**, one of the currently most active CB2 ligands.

To summarize, replacing the phenyl ring of compound **33** by an aliphatic ring and shortening its *n*-hexyl chain from six to four carbon atoms resulted in a decrease in terms of CB1 functional activity and maintained or improved CB2 functional activity. Substitution in positions 5 or 7 of the oxindole ring led to a loss of CB1 functional activity and a decrease of CB2 functional activity. The structure−activity relationships are summarized in Figure 4.

In rats, spinal nerve ligation produced tactile allodynia 1 week after surgery, as demonstrated by a reduction in paw withdrawal threshold to mechanical stimulation to 1.6 ± 0.9 g using Von Frey filaments. Compound **33** treatment administered intraperitoneally attenuated tactile allodynia in a dose-related manner, with an ED_{50} of 5.9 mg/kg (95% CI, 4.5−7.9 mg/kg) and an ED_{90} of 12 mg/kg (95% CI, 9.6−15.5 mg/kg). The higher doses (10 mg/kg and 15 mg/kg) produced a significantly greater antiallodynic effect ($P < 0.01$) than that noted with the 5 mg/kg dose of compound **33** (Figure 5). The antiallodynic effects of compound **1** (15 mg/kg intraperitoneally), a CB2 ligand,[33] were significantly less ($P < 0.001$) than were those observed with 10 or 15 mg/kg of compound **33** (Figure 5). Intraperitoneal administration of 5 mg/kg of a selective CB2 antagonist, compound **7**,[51,52] before administration of compound **33** antagonized the effects of compound **33** (Figure 5). Intraperitoneal administration of 15 mg/kg of compound **33** did not decrease the exploratory behavior in rats (Figure 6).

In summary, we have discovered a series of novel CB2 receptor agonists that are potent and selective. A major focus of the optimization effort was to increase selectivity to avoid the potential CB1 receptor CNS adverse effects of this novel series. Compound **33** was active in models of neuropathic pain without producing any CNS adverse effects as measured by the open-field model. More potent and more CB2 receptor-selective compounds, such as compounds **40**, **48**, and **53**, were also discovered and will be evaluated for their in vivo activities. Absorption, distribution, excretion, metabolism, toxicity (ADMET) studies of compound **33** (also called MDA19)[53] are ongoing to assess its ability to cross the blood−brain barrier and to address potential issues of this novel series.

Experimental Section

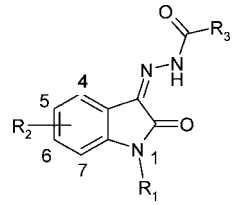
All chemicals were purchased from Sigma-Aldrich or Acros. Microwave reactions were conducted using an Initiator EXP Microwave System (Biotage, Charlottesville, VA). Thin-layer chromatographic analyses were performed on Sigma-Aldrich 60 F254 thin-layer chromatographic plates. All air-sensitive reactions were carried out under a nitrogen atmosphere. Column chromatography was performed with silica gel 230−400 mesh. The high resolution mass spectroscopy (HRMS) studies were performed using a mass spectrometer (9.4 T) FT-ICR-MS from Varian. Ionization technique was electrospray ionization. ^{1}H NMR spectra were recorded on a Bruker 300 MHz DPX NMR spectrometer. ^{13}C NMR spectra were recorded on a Bruker 500 MHz DRX NMR spectrometer. Chemical shifts in ppm are reported relative to either residual dimethyl sulfoxide (DMSO) (3.35 ppm) or $CHCl_3$ (7.24 ppm) as internal standards. Signals were abbreviated as follows: s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. Coupling constants (J) are expressed in hertz.

X-Ray Diffraction of Compound 33. X-ray experimental data for $C_{21}H_{23}N_3O_2$: Crystals grew as large, yellow plates by slow evaporation of an ethyl acetate solution. The data crystal was cut from a larger crystal and had approximate dimensions 0.27 mm × 0.25 mm × 0.10 mm. The data were collected at room temperature on a Nonius Kappa CCD diffractometer using a graphite monochromator with Mo Kα radiation ($\lambda = 0.71073$ Å). A total of 383 frames of data were collected using ω-scans with a scan range of 1.1° and a counting time of 136 s per frame. The data were collected at 153 K using an Oxford Cryostream low-temperature device. Details of crystal data, data collection, and structure refinement are listed in Table 1. Data reduction was performed using DENZO-SMN.[54] The structure was solved by direct methods using SIR97[55] and refined by full-matrix least-squares on F^2 with anisotropic displacement parameters for the non-H atoms using SHELXL-97.[56] The hydrogen atoms on carbon were calculated in ideal positions with isotropic displacement parameters set to 1.2 × Ueq of the attached atom (1.5 × Ueq for methyl hydrogen atoms). The hydrogen atoms bound to nitrogen were observed in a ΔF map and refined with isotropic displacement parameters. The function $\sum(|F_o|^2 - |F_c|^2)^2$ was minimized, where $w = 1/[(\sigma(F_o))^2 + (0.0567P)^2 + (0.2659P)]$ and $P = (|F_o|^2 + 2|F_c|^2)/3$. $R_w(F^2)$ refined to 0.144, with $R(F)$ equal to 0.0504 and a goodness of fit, S, = 1.03. Definitions used for calculating $R(F)$, $R_w(F^2)$, and the goodness of fit, S, are given below.

$R_w(F^2) = \{Sw(|F_o|^2 - |F_c|^2)^2/Sw(|F_o|)^4\}^{1/2}$, where w is the weight given each reflection. $R(F) = S(|F_o| - |F_c|)/S|F_o|\}$ for reflections with $F_o > 4(s(F_o))$. $S = [Sw(|F_o|^2 - |F_c|^2)^2/(n - p)]^{1/2}$, where n is the number of reflections and p is the number of refined parameters.

The data were corrected for secondary extinction effects. The correction takes the form: $F_{corr} = kF_c/[1 + (4.2(4) \times 10^{-5})F_c^2 \lambda^3/(\sin 2\theta)]^{0.25}$, where k is the overall scale factor. Neutral atom

Table 4. Percentage Inhibition of Specific Binding of [^{3}H]**6** on *h*CB1 Receptor and of [^{3}H]**8** on *h*CB2 Receptor by Compounds **33** at 10 μM and **37**−**63** at 1 μM



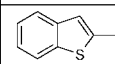
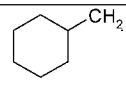
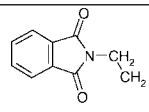
Compd	R1	R2	R2 Position	R3	% inhibition	
					*h*CB1 receptor	*h*CB2 receptor
33	$CH_3(CH_2)_5$	H	-	Phenyl	95.5±0	92.8±8.7
37	$CH_3(CH_2)_5$	H	-	Cyclohexyl	65.8±0.2	61±2.1
38	$CH_3(CH_2)_5$	H	-	$CH_3(CH_2)_4$	45.2±9.5	36.6±8.8
39	$CH_3(CH_2)_5$	H	-	4-Cl-Ph	46.6±4.3	32.7±5.3
40	$CH_3(CH_2)_5$	H	-	-O-tBu (Z-isomer)	85.1±3.3	82.3±0.8
41	$CH_3(CH_2)_5$	H	-	-O-tBu (E-isomer)	75±0.1	27±3.7
42	$CH_3(CH_2)_5$	H	-	2-Naphthyl	<30	<30
43	$CH_3(CH_2)_5$	H	-	4-MeO-Ph	81.7±1.1	56±5.7
44	$CH_3(CH_2)_5$	H	-		<30	<30
45	$CH_3(CH_2)_5$	H	-	1-Naphthyl	83.9±0.9	69.8±4
46	$CH_3(CH_2)_5$	H	-		82.6±1.2	90.7±3.2
47	$CH_3(CH_2)_2$	H	-	Phenyl	43±1.2	32±3.6
48	$CH_3(CH_2)_3$	H	-	Phenyl	86±2.4	70±2.4
49	$CH_3(CH_2)_4$	H	-	Phenyl	95±0.9	80±0.9
50	$CH_3(CH_2)_{11}$	H	-	Phenyl	23.8±4.5	24.3±4.1
51	Phenyl	H	-	Phenyl	18±1.2	8±1.1
52		H	-	Phenyl	99±1.1	100±0.4
53	Benzyl	H	-	Phenyl	87±0.4	55±0.4
54	4-chlorobenzyl	H	-	Phenyl	94.5±0.8	22±1.6
55		H	-	Phenyl	3±4.1	3±3.2
56	$CH_3(CH_2)_5$	Me	7	Phenyl	67±1.6	68±3.7
57	$CH_3(CH_2)_5$	Cl	7	Phenyl	60±1	29±4.5
58	$CH_3(CH_2)_5$	I	7	Phenyl	61±2.5	66±3.2
59	$CH_3(CH_2)_5$	Me	5	Phenyl	22±0.9	1±2.7
60	$CH_3(CH_2)_5$	MeO	5	Phenyl	26±3.1	17±1.2
61	$CH_3(CH_2)_5$	F	5	Phenyl	57±0.7	46±1.7
62	$CH_3(CH_2)_5$	Cl	5	Phenyl	9±1.4	6±1.4
63	$CH_3(CH_2)_4$	I	5	Phenyl	25±0.2	10±0.2

Table 5. Determination of Potency (EC_{50}) and Maximal Stimulation (E_{max}) on hCB_1 and hCB_2 Receptors of Compounds **33**−**61**[a]

	[^{35}S]GTP-γ-S hCB1		[^{35}S]GTP-γ-S hCB2	
compd	EC_{50} (nM ± SEM)	E_{max} (%)	EC_{50} (nM ± SEM)	E_{max}(%)
6	5.0 ± 1	100	2.7 ± 1	100
33	867.0 ± 1.1	59.7	63.4 ± 1.3	68.8
37	ND	40.9[b]	79.1 ± 1.5	58
40	937.1 ± 1.2	92	22.4 ± 1.3	43.4
41	NA	NA	ND	23.4[b]
43	852 ± 1.2	71	143.4 ± 2.6	49.5
45	ND	12.9[b]	202.2 ± 2	30.5
46	ND	34.6[b]	ND	32.12[b]
47	NA	NA	200.7 ± 1.5	61.4
48	ND	69[b]	40.1 ± 1.5	98.9
49	314.1 ± 1.6	95.1	240 ± 3.2	68.5
52	62.3 ± 1.3	99	9.8 ± 1.1	65.7
53	ND	57[b]	63.7 ± 1.7	92.7
54	895.6 ± 1.2	72	131.1 ± 1.7	39.8
56	NA	NA	84.0 ± 2.2	34.2
57	NA	NA	645.5 ± 1.3	64.8
58	NA	NA	ND	55[b]
61	NA	NA	186.9 ± 1.7	33.8

[a] CB_1 and CB_2 assay data are presented as the mean of two determinations. Assay reproducibility was monitored by the use of a reference compound **6**. For replicate determinations, the maximum variability tolerated in the test was of ±20% around the average of the replicates. Efficacies (E_{max}) for CB_1 or CB_2 are expressed as a percentage relative to the efficacy of compound **6**. [b] Efficacy determined at 3 μM. ND = not determined (plateau was not reached at 10 μM dose). NA = not active at 10 μM dose.

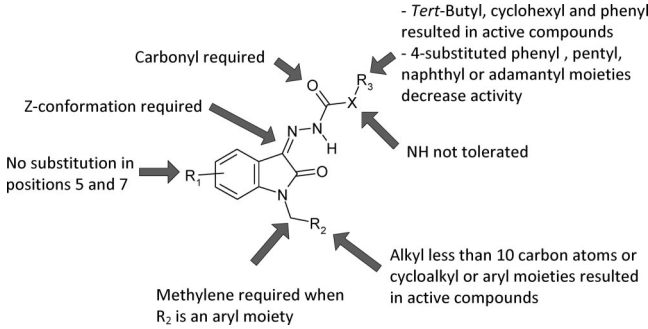


Figure 4. Structure−activity relationships.

Table 6. Radioligand Competitive Binding Assays (mean ± SEM)

compd	human CB_2 K_i (nM)
6	1.8 ± 1.1
28	58.8 ±1.7
33	44.3 ± 10.2
40	13.9 ±0.5
48	19.3 ± 9.6
53	18.5 ±8.4

scattering factors and values used to calculate the linear absorption coefficient were from the International Tables for X-ray Crystallography (1992).[57] All figures were generated using SHELXTL/PC.[56] Tables of positional and thermal parameters, bond lengths and angles, torsion angles, figures, and lists of observed and calculated structure factors are located in Tables 1 through 7.

X-Ray Data for Compound 33. Empirical formula: $C_{21}H_{23}N_3O_2$. Formula weight: 349.42. Temperature: 298(2) K. Wavelength: 0.71073 Å. Crystal system: Triclinic. Space group: $P\bar{1}$. Unit cell dimensions: a = 7.9246(4) Å, α = 87.074(1)°, b = 8.7859(8) Å, β = 85.771(1)°, c = 28.0323(12) Å, γ = 73.203(1)°. Volume: 1862.5(2) Å^3. Z: 4. Density (calculated): 1.246 Mg/m^3. Absorption coefficient: 0.082 mm^{-1}. F(000): 744. Crystal size: 0.27 mm × 0.25 mm × 0.20 mm. θ range for data collection: 2.69−27.49°. Index ranges: $-10 \le h \le 9$, $-11 \le k \le 7$, $-36 \le l \le 36$. Reflections collected: 10657. Independent reflections: 8267 [R(int) = 0.0240]. Completeness to θ = 27.49°: 96.7%. Absorption correction: none. Refinement method: full-matrix least-squares on F^2. Data/restraints/parameters: 8267/0/480. Goodness of fit on F^2: 1.028. Final R indices [$I > 2\sigma(I)$]: $R1$ = 0.0504, $wR2$ = 0.1212. R indices (all data): $R1$ = 0.0964, $wR2$ = 0.1440. Extinction coefficient: 4.2(4) × 10^{-5}. Largest diff. peak and hole: 0.278 and −0.138 e·Å^{-3}.

General Procedure for the Synthesis of the *N*-Alkyl Isatin: Method A. First, 60% sodium hydride (526 mg, 0.013 mol) was added portionwise to a mixture of isatin (1.75 g, 0.012 mol) dissolved in 35 mL of dimethylformamide. The reaction medium was stirred at room temperature for 2 h. A solution of 1-bromo-2-cyclohexylethane (2.5 g, 0.013 mol) dissolved in dimethylformamide (3 mL) was then added dropwise. The reaction medium was stirred at room temperature overnight. After extraction with ethyl acetate, the organic layer was washed with hydrochloric acid (0.4 N) and water. The organic fraction was dried over $MgSO_4$ and concentrated under vacuum.

General Procedure for the Synthesis of the *N*-Alkyl Isatin: Method B. A mixture of cesium carbonate (732 mg, 2.25 mmol), isatin (111 mg, 0.75 mmol), and 1-(bromomethyl)cyclohexane (200 mg, 1.125 mmol) in dimethylformamide (15 mL) in sealed vessels was irradiated at 140 °C for 10−15 min. After extraction with ethyl acetate, the organic layer was washed with hydrochloric acid (0.4 N) and water. The organic fraction was dried over $MgSO_4$ and concentrated under vacuum.

General Procedure for the Synthesis of the *N*-Alkyl Isatin: Method C. A mixture of cesium carbonate (6.52 g, 20 mmol), 5-iodoisatin (1.47 g, 5.4 mmol), and 1-bromopentane (1.81 g, 12 mmol) in dimethylformamide (90 mL) was stirred at room temperature overnight. After extraction with ethyl acetate, the organic layer was washed with hydrochloric acid (0.4 N) and water. The organic fraction was dried over $MgSO_4$ and concentrated under vacuum.

1-(2-Cyclohexylethyl)-isatin (10). The title compound was prepared as an orange solid, using isatin and 1-bromo-2-cyclohexylethane according to the synthetic method A. The resulting solid was washed with a mixture of heptane/AcOEt. Yield: 100%. mp 56−57 °C. ^{1}H NMR ($CDCl_3$): δ 0.92−1.05 (m, 2H), 1.12−1.41 (m, 4H), 1.57 (q, J = 6.9 Hz, 2H), 1.63−1.82 (m, 5H), 3.74 (t, J = 7.6 Hz, 2H), 6.88 (d, J = 7.8 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 7.56 − 7.61 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 26.09 (CH_2), 26.4 (CH_2), 33.08 (CH_2), 34.42 (CH_2), 35.42 (CH), 38.21 (CH_2), 110.12 (CH), 117.66 (C), 123.57 (CH), 125.41 (CH), 138.3 (CH), 151.01 (C), 158.03 (C═O). 178.71 (C═O).

1-Hexyl-isatin (11). The title compound was prepared as an orange solid, using isatin and 1-bromohexane according to the synthetic method A. The resulting solid was washed with a mixture of heptane/AcOEt. Yield: 93%. mp 40−41 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.9 Hz, 3H), 1.25−1.38 (m, 6H), 1.7 (m, 2H), 3.72 (t, J = 7.5 Hz, 2H), 6.90 (d, J = 7.8 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 7.56−7.61 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 13.97 (CH_3), 22.50 (CH_2), 26.55 (CH_2), 27.21 (CH_2), 31.38 (CH_2), 40.28 (CH_2), 110.16 (CH), 117.59 (C), 123.59 (CH), 125.44 (CH), 138.3 (CH), 151.09 (C), 158.13 (C═O). 183.69 (C═O).

1-Propyl-isatin (12). The title compound was prepared as a red solid, using isatin and 1-bromopropane according to the synthetic method B. Yield: 99%. mp 70−71 °C. ^{1}H NMR ($CDCl_3$): δ 1.00 (t, J = 7.2 Hz, 3H), 1.32−1.40 (m, 2H), 1.72 (m, 2H), 3.70 (t, J = 7.5 Hz, 2H), 6.90 (d, J = 7.8 Hz, 1H), 7.11 (td, J = 0.9 Hz, J = 7.8 Hz, 1H), 7.56−7.62 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 11.35 (CH_3), 20.64 (CH_2), 41.81 (CH_2), 110.18 (CH), 117.58 (C), 123.61 (CH), 125.45 (CH), 138.31 (CH), 151.13 (C), 158.21 (C═O). 183.66 (C═O).

1-Butyl-isatin (13). The title compound was prepared using isatin and 1-bromobutane according to the synthetic method B. The product was purified by flash chromatography (eluent: AcOEt/heptane: 2/8) to afford a red oil. Yield: 93%. ^{1}H NMR ($CDCl_3$): δ 0.98 (t, J = 7.2 Hz, 3H), 1.36−1.48 (m, 2H), 1.64−1.74 (m, 2H), 3.73 (t, J = 7.2 Hz, 2H), 6.90 (d, J = 8.1 Hz, 1H), 7.16 (td, J = 0.6 Hz, J = 7.5 Hz, 1H), 7.56−7.62 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 13.67 (CH_3), 20.14 (CH_2), 29.29 (CH_2), 40.02 (CH_2), 110.16

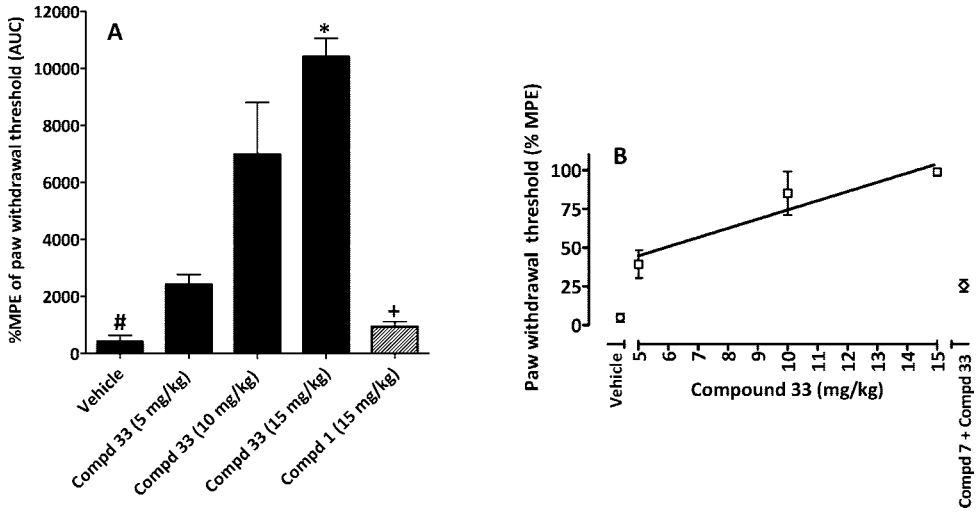


Figure 5. Effects of compound **33** (administered intraperitoneally) and compound **1** on tactile allodynia in a spinal nerve ligation neuropathic pain model in rats (six rats per group). Compound **33** increased the withdrawal threshold of the nerve-injured paw in a dose-dependent manner. (A) Area under the curve (AUC) of the effects of 5, 10, and 15 mg/kg of compound (compd) **33** and 15 mg/kg of compound **1**. (B) Dose−response curve of the antiallodynic effects of compound **33** calculated as described by Tallarida and Murray.[48] The calculated ED_{50} was 5.9 mg/kg (95% CI, 4.5−7.9 mg/kg) and the calculated ED_{90} was 12 mg/kg (95% CI, 9.6−15.5 mg/kg) when compound **33** was delivered intraperitoneally. Intraperitoneal administration of 5 mg/kg of a selective CB2 antagonist, compound **7**, before administration of compound **33**, antagonized the effects of compound **33**. Data are expressed as mean ± SEM. $^{*}P < 0.01$ versus all other groups (one-way ANOVA followed by Tukey−Kramer post hoc analysis for multiple group comparison). $^{+}P < 0.001$ versus 10 and 15 mg/kg of compound **33**. $^{\#}P < 0.001$ versus 5, 10, and 15 mg/kg of compound **33**. The AUC was calculated using the trapezoidal rule. %MPE = % of maximium peak effect.

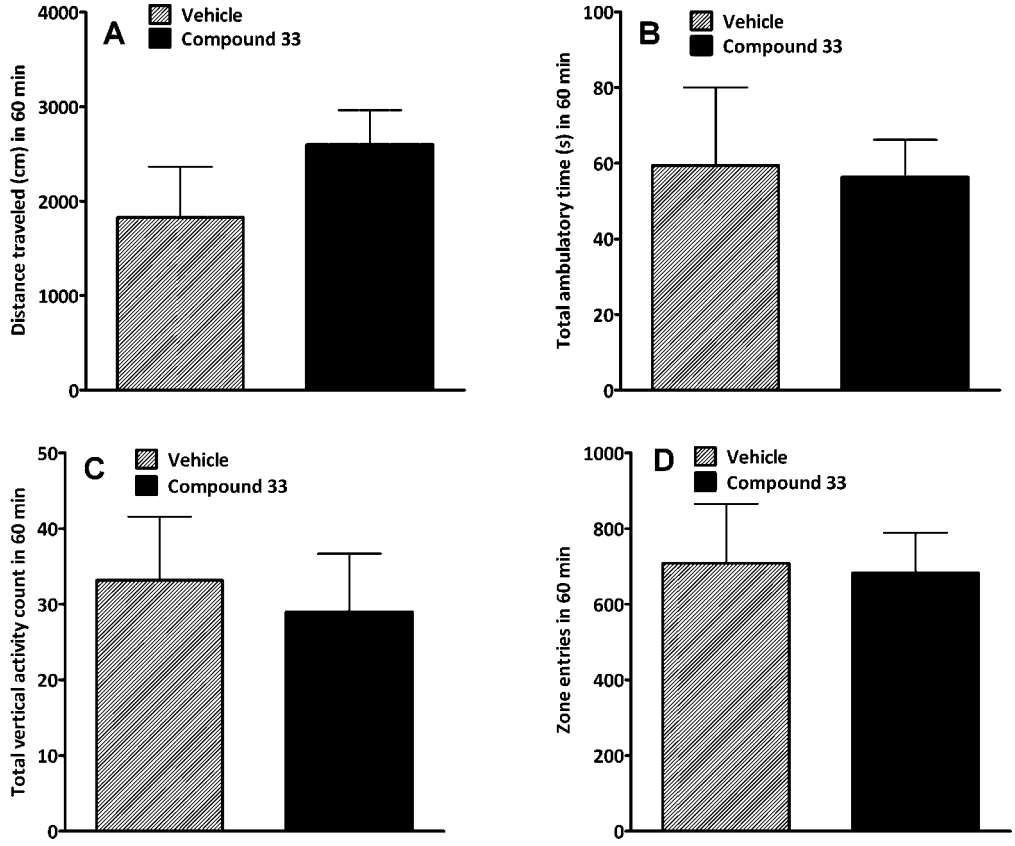


Figure 6. Absence of psychoactive cannabinoid effect of compound **33**. Exploratory behavior was tested in an open-field chamber following intraperitoneal administration of 15 mg/kg of compound **33** or vehicle (six rats per group). The following parameters were scored for 60 min: distance traveled (A), ambulatory time (B), vertical activity (C), and number of zone entries (D). No significant differences were observed between compound **33** and its vehicle (*t* test).

(CH), 117.62 (C), 123.58 (CH), 125.43 (CH), 138.28 (CH), 151.10 (C), 158.14 (C═O). 183.66 (C═O).

1-Pentyl-isatin (14). The title compound was prepared as a red solid, using isatin and 1-bromopentane according to the synthetic method B. The resulting solid was washed with a mixture of heptane and AcOEt. Yield: 95%. mp 45−46 °C. ^{1}H NMR ($CDCl_3$): δ 0.91 (t, J = 6.9 Hz, 3H), 1.34−1.39 (m, 4H), 1.69−1.73 (m, 2H), 3.72 (t, J = 7.2 Hz, 2H), 6.89 (d, J = 7.8 Hz, 1H), 7.11 (t, J = 7.8 Hz, 1H), 7.56−7.62 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 13.90 (CH_3), 22.29 (CH_2), 26.94 (CH_2), 28.98 (CH_2), 40.24 (CH_2), 110.17 (CH), 117.60

(C), 123.57 (CH), 124.40 (CH), 138.31 (CH), 151.09 (C), 158.13 (C═O), 183.67 (C═O).

1-(Cyclohexylmethyl)-isatin (15). The title compound was prepared as a red solid, using isatin and 1-bromomethylcyclohexane according to the synthetic method B. The resulting solid was washed with a mixture of heptane and AcOEt. Yield: 95%. mp 150−151 °C. ^{1}H NMR (DMSO-d_6): δ 0.86−1.3 (m, 6H), 1.62−1.74 (m, 5H), 3.51 (d, J = 6.9 Hz, 2H), 7.13 (t, J = 7.5 Hz, 1H), 7.20 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 7.5 Hz, 1H), 7.66 (t, J = 7.8 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ 25.70 (CH_2), 26.26 (CH_2), 30.65 (CH_2), 36.20 (CH), 46.15 (CH_2), 111.43 (CH), 117.84 (C), 123.55 (CH), 124.85 (CH), 138.64 (CH), 151.70 (C), 158.78 (C═O), 183.91 (C═O).

1-Benzyl-isatin (16). The title compound was prepared using isatin and benzyl bromide according to the synthetic method B. Column chromatography (silica gel, heptane/EtOAc: 6/4) yielded the title compound as an orange solid, M = 433 mg. Yield: 81%. mp 131−132 °C. ^{1}H NMR ($CDCl_3$): δ 4.94 (s, 2H), 6.78 (d, J = 7.8 Hz, 1H), 7.08 (td, J = 1 Hz, J = 7.8 Hz, 1H), 7.32−7.36 (m, 5H), 7.48 (td, J = 1.5 Hz, J = 7.8 Hz, 1H), 7.62 (dd, J = 1.2 Hz, J = 6.9 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 44.06 (CH2), 111.02 (CH), 117.70 (C), 123.88 (CH), 125.42 (CH), 127.44 (CH), 128.18 (CH), 129.07 (CH), 134.52 (C), 138.33 (CH), 150.74 (C), 158.29 (C═O), 183.25 (C═O).

1-[(3*Z*)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-ethyl]-isatin (17). The title compound was prepared using isatin and *N*-(2-Bromoethyl)phthalimide according to the synthetic method B. The product was purified by flash chromatography (eluent: AcOEt/heptane: 3/7) to afford an orange solid. mp 154−155 °C. Yield: 71%. ^{1}H NMR (DMSO-d_6): δ 3.86 (t, J = 6 Hz, 2H), 3.97 (t, J = 6 Hz, 2H), 7.12 (d, J = 7.5 Hz, 1H), 7.24 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 7.2 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.82−7.86 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 35.10 (CH_2), 38.59 (CH_2), 110.71 (CH), 117.68 (C), 123.48 (CH), 123.87 (CH), 125.63 (CH), 131.70 (C), 134.25 (CH), 138.33 (CH), 150.42 (C), 158.41 (C═O), 168.07 (C═O), 182.76 (C═O).

1-Hexyl-7-methyl-isatin (18). The title compound was prepared as a red solid, using 7-methyl-isatin and 1-bromohexane according to the synthetic method B. Yield 89%. mp 48−51 °C. ^{1}H NMR ($CDCl_3$): δ 0.87−0.91 (m, 3H), 1.24−1.41 (m, 6H), 1.60−1.68 (m, 2H), 3.67 (t, J = 7.2 Hz, 2H), 3.93 (s, 3H), 6.36 (d, J = 2.1 Hz, 1H), 6.54 (dd, J = 2.1 Hz, J = 8.7 Hz, 1H), 7.59 (d, J = 8.7 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 13.97 (CH_3), 18.81 (CH_3), 22.53 (CH_2), 26.34 (CH_2), 29.44 (CH_2), 31.43 (CH_2), 42.10 (CH_2), 118.90 (C), 121.45 (C), 123.57 (CH), 123.72 (CH), 142.43 (CH), 148.63 (C), 159.31 (C═O), 184.18 (C═O).

7-Chloro-1-hexyl-isatin (19). The title compound was prepared as an orange solid, using 7-chloro-isatin and 1-bromohexane according to the synthetic method B. mp 56−58 °C. Yield 85%. ^{1}H NMR (DMSO-d_6): δ 0.89 (t, J = 6.9 Hz, 3H), 1.26−1.41 (m, 6H), 1.69−1.79 (m, 2H), 4.09 (t, J = 7.8 Hz, 2H), 7.05 (dd, J = 7.5 Hz, J = 8.1 Hz, 1H), 7.5−7.55 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 13.98 (CH_3), 22.54 (CH_2), 26.27 (CH_2), 29.42 (CH_2), 31.39 (CH_2), 41.99 (CH_2), 117.17 (C), 120.48 (C), 124.10 (CH), 124.61 (CH), 140.63 (CH), 146.48 (C), 158.47 (C═O), 182.85 (C═O).

1-Hexyl-7-iodo-isatin (20). The title compound was prepared using 7-iodo-isatin and 1-bromohexane according to the synthetic method B. The product was purified by flash chromatography (eluent: AcOEt/heptane: 3/7) to afford a red oil. Yield 44%.[1] H NMR (DMSO-d_6): δ 0.87 (t, J = 6.9 Hz, 3H), 1.28−1.38 (m, 6H), 1.60−1.70 (m, 2H), 3.99 (t, J = 7.8 Hz, 2H), 6.88 (t, J = 7.5 Hz, 1H), 7.55 (dd, J = 0.9 Hz, J = 7.5 Hz, 1H), 7.59 (dd, J = 0.9 Hz, J = 7.5 Hz, 2H).^{13}C NMR ($CDCl_3$): δ 14.35 (CH_3), 22.50 (CH_2), 25.87 (CH_2), 29.36 (CH_2), 31.40 (CH_2), 40.13 (CH_2), 75.10 (C), 121.27 (C), 124.86 (CH), 125.53 (CH), 150.46 (CH), 150.80 (C), 159.39 (C═O), 182.94 (C═O).

1-Benzyl-7-iodo-isatin (21). The title compound was prepared using 7-iodo-isatin and benzyl bromide according to the synthetic method B. The product was purified by flash chromatography (eluent: AcOEt/heptane: 5/5) to afford an orange solid. Yield 61%. mp 114−117 °C. ^{1}H NMR ($CDCl_3$): δ 5.94 (s, 2H), 6.86 (dd, J = 7.2 Hz, J = 8.1 Hz, 1H), 7.21−7.37 (m, 5H), 7.65 (dd, J = 1.2 Hz, J = 7.2 Hz, 1H), 7.97 (dd, J = 1.2 Hz, J = 8.1 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 43.58 (CH_2), 73.66 (C), 120.77 (C), 125.46 (CH), 125.67 (CH), 126.37 (CH), 127.56 (CH), 128.81 (CH), 135.84 (C), 150.95 (C), 151.12 (CH), 159.11 (C═O), 182.34 (C═O).

1-Hexyl-5-methyl-isatin (22). The title compound was prepared as a red solid, using 5-methyl-isatin and 1-bromohexane according to the synthetic method B. Yield 99%. mp 81−82 °C. ^{1}H NMR (DMSO-d_6): δ 0.88 (t, J = 6.9 Hz, 3H), 1.32−1.40 (m, 6H), 1.66−1.70 (m, 2H), 2.33 (s, 3H), 3.69 (t, J = 7.2 Hz, 2H), 6.78 (d, J = 7.8 Hz, 1H), 7.37−7.41 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 13.99 (CH_3), 20.66 (CH_3), 22.51 (CH_2), 26.56 (CH_2), 27.24 (CH_2), 31.40 (CH_2), 40.26 (CH_2), 109.99 (CH), 117.63 (C), 125.79 (CH), 133.39 (C), 138.66 (CH), 148.91 (C), 158.24 (C═O), 183.98 (C═O).

1-Hexyl-5-methoxy-isatin (23). The title compound was prepared as an orange solid, using 5-methoxy-isatin and 1-bromohexane according to the synthetic method B. Yield 26%. mp 40−41 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.5 Hz, 3H), 1.26−1.38 (m, 6H), 1.66−1.71 (m, 2H), 3.68 (t, 7.5 Hz, 2H), 3.83 (s, 3H), 6.82 (d, J = 8.0 Hz, 1H), 7.14−7.15 (m, 2H).^{13}C NMR ($CDCl_3$): δ 13.98 (CH_3), 22.51 (CH_2), 26.56 (CH_2), 27.24 (CH_2), 31.39 (CH_2), 40.27 (CH_2), 55.99 (CH_3), 109.61 (CH), 111.16 (CH), 118.03 (C), 124.68 (CH), 145.00 (C), 156.39 (C), 158.20 (C═O), 182.07 (C═O).

5-Fluoro-1-hexyl-isatin (24). The title compound was prepared, using 5-fluoro-isatin and 1-bromohexane according to the synthetic method B. Column chromatography (silica gel, heptane/EtOAc: 6/4) afforded the title compound as an orange solid. Yield 100%. mp 46−49 °C. ^{1}H NMR ($CDCl_3$): δ 0.89 (t, J = 6.9 Hz, 3H), 1.25−1.42 (m, 6H), 1.63−1.74 (m, 2H), 3.71 (t, 7.5 Hz, 2H), 6.84−6.88 (m, 1H), 7.27−7.33 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 13.97 (CH_3), 22.50 (CH_2), 26.54 (CH_2), 27.12 (CH_2), 31.36 (CH_2), 40.40 (CH_2), 111.28 (d, J = 25 Hz, CH), 112.50 (d, J = 95 Hz, CH), 118.20 (d, J = 25 Hz, C), 124.59 (d, J = 95 Hz, CH), 147.11 (s, C), 158.07 (d, J = 180 Hz, C═O), 160.21 (s, C), 183.09 (d, J = 10 Hz, C═O).

5-Chloro-1-hexyl-isatin (25). The title compound was prepared as an orange solid, using 5-chloro-isatin and 1-bromohexane according to the synthetic method B. Yield 100%. mp 86 °C. ^{1}H NMR ($CDCl_3$): δ 0.89 (t, J = 7.2 Hz, 3H), 1.22−1.42 (m, 6H), 1.63−1.73 (m, 2H), 3.71 (t, 7.2 Hz, 2H), 6.85 (d, J = 8.1 Hz, 1H), 7.53−7.57 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 13.94 (CH_3), 22.47 (CH_2), 26.51 (CH_2), 27.13 (CH_2), 31.33 (CH_2), 40.43 (CH_2), 111.46 (CH), 118.42 (C), 125.31 (CH), 129.41 (C), 137.65 (CH), 149.34 (C), 157.58 (C═O), 182.64 (C═O).

5-Iodo-1-pentyl-isatin (26). The title compound was prepared as an orange solid, using 5-iodo-isatin and 1-bromopentane according to the synthetic method C. The resulting solid was washed with a mixture of heptane/AcOEt. Yield 15%. mp 100−101 °C. ^{1}H NMR ($CDCl_3$): δ 0.90 (t, J = 6.6 Hz, 3H), 1.30−1.42 (m, 6H), 1.62−1.73 (m, 2H), 3.70 (t, 7.2 Hz, 2H), 6.85 (d, J = 8.7 Hz, 1H), 7.86−7.90 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 13.89 (CH_3), 22.27 (CH_2), 26.86 (CH_2), 28.94 (CH_2), 40.37 (CH_2), 85.76 (C), 112.32 (CH), 119.15 (C), 133.87 (CH), 146.33 (CH), 150.39 (C), 157.13 (C═O), 182.29 (C═O).

General Procedure for the Synthesis of Hydrazone Derivatives from the Corresponding Isatins: Method D. A solution of 1-(2-cyclohexyl-ethyl)-isatin (180 mg, 0.70 mmol) and 4-phenylsemicarbazide (106 mg, 0.70 mmol) in a solution of acetic acid 20%, ethanol 40%, and tetrahydrofuran (THF) 40% (18 mL) was stirred at room temperature for 24 h. The mixture was then concentrated under vacuum to afford the desired product.

General Procedure for the Synthesis of Hydrazone Derivatives from the Corresponding Isatins: Method E. A solution of 1-pentyl-isatin (109 mg, 0.5 mmol) and benzhydrazide (68 mg, 0.5 mmol) in a solution of acetic acid 10%, ethanol 45%, and THF 45% (6.5 mL) was stirred at room temperature for 24 h. The mixture was then concentrated under vacuum to afford the desired product.

(3*Z*)-1-(2-Cyclohexylethyl)-1*H*-indole-2,3-dione 3-(*N*-phenylsemicarbazone) (27). The title compound was prepared as a yellow solid, using **10** and 4-phenylsemicarbazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 73%. mp 168 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 to 1.30 (m, 6H), 1.46 (q, J = 6.6 Hz, 2H), 1.64−1.80 (m, 5 H), 3.76 (t, J = 7.2 Hz,

2H), 7.07 to 7.18 (m, 3H), 7.36 (t, J = 7.5 Hz, 2H), 7.47 (t, J = 7.8 Hz, 1H), 7.58 (d, J = 7.5 Hz, 2H), 8.13 (d, J = 7.5 Hz, 1H), 9.51 (s, 1H), 10.45 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 26.14 (CH_2), 26.49 (CH_2), 33.00 (CH_2), 34.77 (CH_2), 35.12 (CH), 37.61 (CH_2), 110.56 (CH), 119.71 (C), 121.28 (CH), 123.70 (CH), 127.89 (CH), 129.66 (CH), 132.26 (CH), 132.43 (C), 133.4 (CH), 143.28 (C═N), 161.45 (C═O). HRMS (ES+) calcd for $C_{23}H_{27}N_4O_2$ (M + H^+) *m/e*, 391.2138; found, 391.2129.

***N′*-[(3*Z*)-1-(2-Cyclohexylethyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (28).** The title compound was prepared as a yellow solid, using **10** and benzhydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 100%. mp 145−146 °C. ^{1}H NMR (DMSO-d_6): δ 0.90−1.00 (m, 2H), 1.11−1.36 (m, 4 H), 1.50−1.80 (m, 7H), 3.79 (t, J = 7.5 Hz, 2H), 7.16 to 7.21 (m, 2H), 7.49 (td, J = 1.2 Hz, J = 7.8 Hz, 1H), 7.602 to 7.73 (m, 4H), 7.91 (dd, J = 1.5 Hz, J = 6.9 Hz, 2H), 13.9 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 26.10 (CH2), 26.47 (CH_2), 32.97 (CH_2), 34.64 (CH_2), 35.21 (CH), 37.73 (CH_2), 110.56 (CH), 119.71 (C), 121.28 (CH), 123.70 (CH), 127.89 (CH), 129.66 (CH), 132.26 (CH), 132.43 (C), 133.4 (CH), 143.28 (C═N), 161.45 (C═O). HRMS (ES+) calcd for $C_{23}H_{26}N_3O_2$ (M + H^+) *m/e*, 376.2025; found, 376.2020.

***N′*-[1-(2-Cyclohexylethyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]-2-phenylacetohydrazide (29).** The title compound was prepared as an orange solid, using **10** and phenylacetic hydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 80%. mp 82−83 °C. ^{1}H NMR (DMSO-d_6): δ 0.85−1.31 (m, 6 H), 1.48 (q, J = 6.6 Hz, 2H), 1.62−1.76 (m, 5H), 3.71 (t, J = 7.2 Hz, 2H), 3.82 (br s, 1H, minor isomer (28%)), 4.11 (br s, 1H, major isomer (72%)), 7.11−7.17 (m, 2H), 7.27−7.34 (m, 5H), 7.44 (t, J = 7.8 Hz, 1H), 7.62 (br s, 1H), 12.45 (br s, 1H, major isomer), 12.90 (br, s, 1H, minor isomer). At 75 °C, peaks with a displacement of 3.82 ppm and 4.11 ppm corresponding to the benzylic CH_2 are converted to a singlet at 4.02 ppm. Identically, peaks with a displacement of 12.45 ppm and 12.90 ppm are converted to a singlet with a displacement of 12.50 ppm. ^{13}C NMR (DMSO-d_6): δ 26.09 (CH_2), 26.46 (CH_2), 32.95 (CH_2), 34.64 (CH_2), 35.08 (CH), 37.55 (CH_2), 110.41 (CH), 119.71 (C), 120.93 (CH), 123.51 (CH), 127.21 (CH), 128.86 (CH), 129.47 (C), 130.00 (CH), 131.95 (CH), 135.05 (C), 143.20 (C═N), 160.85 (C═O). HRMS (ES+) calcd for $C_{24}H_{28}N_3O_2$ (M + H^+) *m/e*, 390.2181; found, 390.2176.

(3*Z*)-1-(2-Cyclohexylethyl)-1*H*-indole-2,3-dione 3-(*N*-phenylthiosemicarbazone) (30). The title compound was prepared as an orange solid, using **10** and 4-phenylthiosemicarbazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 73%. mp 169−170 °C. ^{1}H NMR (DMSO-d_6): δ 0.88−1.00 (m, 2H), 1.1−1.33 (m, 4H) 1.50−1.80 (m, 7 H), 3.78 (t, J = 7.5 Hz, 2H), 7.15 to 7.20 (m, 2H), 7.30 (t, J = 7.5 Hz, 1H), 7.40 to 7.48 (m, 3H), 7.61 (d, J = 8.4 Hz, 2H), 7.83 (d, J = 7.5 Hz, 1H), 10.85 (s, 1H), 12.75 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 26.12 (CH_2), 26.50 (CH_2), 32.99 (CH_2), 34.71 (CH_2), 35.10 (CH), 37.62 (CH_2), 110.42 (CH), 119.82 (C), 121.70 (CH), 123.28 (CH), 126.08 (CH), 126.59 (CH), 128.87 (CH), 131.85 (CH), 138.91 (C), 143.31 (C), 161.13 (C═O), 176.81 (C═S). HRMS (ES+) calcd for $C_{23}H_{27}N_4OS$ (M + H^+) *m/e*, 407.1808; found, 407.1900.

***N′*-[(3*Z*)-1-(2-Cyclohexylethyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzenesulfonohydrazide (31).** The title compound was prepared as a yellow solid, using **10** and benzenesulfonyl hydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 83%. mp 160 °C. ^{1}H NMR (DMSO-d_6): δ 0.85−0.96 (m, 2H), 1.09−1.24 (m, 4H), 1.46 (q, J = 6.9 Hz, 2H), 1.62−1.76 (m, 5 H), 3.70 (t, J = 7.2 Hz, 2H), 7.07 to 7.12 (m, 2H), 7.40 to 7.49 (m, 2H), 7.61 to 7.72 (m, 3H), 7.99 (d, J = 7.2 Hz, 1H), 12.55 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 26.08 (CH_2), 26.46 (CH_2), 32.94 (CH_2), 34.60 (CH_2), 35.07 (CH), 37.61 (CH_2), 110.42 (CH), 119.24 (C), 121.70 (CH), 123.28 (CH), 126.08 (CH), 126.59 (CH), 128.87 (CH), 131.85 (CH), 138.91 (C), 143.31 (C), 161.13 (C═O), 176.81 (C═S). HRMS (ES+) calcd for $C_{22}H_{26}N_3O_3S$ (M + H^+) *m/e*, 412.1699; found, 412.1689.

1-Hexyl-1*H*-indole-2,3-dione-3-(*N*-phenylsemicarbazone) (32). The title compound was prepared as a yellow solid, using **11** and 4-phenylsemicarbazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 100%. mp 142−143 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, J = 6.6 Hz, 3H), 1.28 (m, 6H), 1.57 (t, J = 6.6 Hz, 2H), 3.73 (t, J = 6.9 Hz, 2H), 7.07 (t, J = 7.2 Hz, 1H), 7.13−7.17 (m, 2H), 7.35 (t, J = 7.8 Hz, 2H), 7.47 (t, J = 7.8 Hz, 1H), 7.59 (d, J = 7.8 Hz, 2H), 8.13 (d, J = 7.5 Hz, 1H), 9.52 (s, 1H), 10.46 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.31 (CH_3), 22.47 (CH_2), 26.37 (CH_2), 27.42 (CH_2), 31.33 (CH_2), 39.68 (CH_2), 109.81 (CH), 115.46 (C), 119.72 (CH), 119.98 (CH), 122.49 (CH), 123.51 (CH), 125.55 (CH), 129.21 (CH), 129.32 (CH), 132.34 (CH), 134.19 (C), 138.98 (C), 143.94 (C═N), 152.66 (C═O), 163.96 (C═O). HRMS (ES+) calcd for $C_{21}H_{25}N_4O_2$ (M + H^+) *m/e*, 365.1980; found, 365.1972.

***N′*-[(3*Z*)-1-(1-Hexyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (33).** The title compound was prepared as a yellow solid, using **11** and benzhydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 78%. mp 103 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, J = 6.6 Hz, 3H), 1.23−1.29 (m, 6H), 1.65 (t, J = 6.6 Hz, 2H), 3.77 (t, J = 7.2 Hz, 2H), 7.15−7.24 (m, 2H), 7.48 (td, J = 1.2, J = 6.6 Hz, 1H), 7.60−7.73 (m, 4H), 7.90−7.93 (m, 2H), 13.90 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.32 (CH_3), 22.43 (CH_2), 26.38 (CH_2), 27.39 (CH_2), 31.32 (CH_2), 39.78 (CH_2), 110.65 (CH), 119.64 (C), 121.25 (CH), 123.68 (CH), 127.88 (CH), 129.65 (CH), 132.22 (CH), 132.42 (C), 133.38 (CH), 143.43 (C═N), 161.58 (C═O). HRMS (ES+) calcd for $C_{21}H_{24}N_3O_2$ (M + H^+) *m/e*, 350.1861; found, 350.1863. HRMS (ES+) calcd for $C_{21}H_{24}N_3O_2$ (M + H^+) *m/e*, 350.1866; found, 350.1863.

***N′*-[1-(1-Hexyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]-2-phenylacetohydrazide (34).** The title compound was prepared as a yellow solid, using **11** and phenylacetic hydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 80%. mp 94−95 °C. ^{1}H NMR (DMSO-d_6): δ 0.84 (t, J = 6.6 Hz, 3H), 1.27 (br s, 6H), 1.61 (t, J = 6.3 Hz, 2H), 3.72 (t, J = 6.9 Hz, 2H), 3.83 (br s, minor isomer 30%, 2H), 4.13 (br s, major isomer 70%, 2H), 7.13 to 7.35 (m, 7H), 7.45 (t, J = 7.5 Hz, 1H), 7.64 (br s, 1H), 12.48 (br s, major isomer 70%, 1H), 12.92 (br s, minor isomer 30%, 1H). ^{13}C NMR (DMSO-d_6): δ 14.31 (CH_3), 22.42 (CH_2), 26.32 (CH_2), 27.35 (CH_2), 31.28 (CH_2), 39.61 (CH_2), 110.51 (CH), 119.65 (C), 120.86 (CH), 123.48 (CH), 127.19 (CH), 128.84 (CH), 129.99 (CH), 131.92 (CH), 135.06 (C), 143.36 (C═N), 160.97 (C═O). HRMS (ES+) calcd for $C_{22}H_{26}N_3O_2$ (M + H^+) *m/e*, 364.2023; found, 364.2020.

(3*Z*)-1-(1-Hexyl)-1*H*-indole-2,3-dione-3-(*N*-phenylthiosemicarbazone) (35). The title compound was prepared as an orange solid following, using **11** and 4-phenylthiosemicarbazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 87%. mp 135−136 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, J = 6.3 Hz, 3H), 1.29 (br s, 6H), 1.65 (br t, 2H), 3.76 (t, J = 6.9 Hz, 2H), 7.15 to 7.22 (m, 2H), 7.28 (t, J = 7.5 Hz, 1H), 7.41−7.48 (m, 3H), 7.61 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 7.2 Hz, 1H), 10.85 (s, 1H), 12.75 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.34 (CH_3), 22.44 (CH_2), 26.35 (CH_2), 27.41 (CH_2), 31.31 (CH_2), 39.50 (CH_2), 110.49 (CH), 119.76 (C), 121.68 (CH), 123.26 (CH), 126.08 (CH), 126.58 (CH), 128.87 (CH), 131.82 (CH), 138.91 (C), 143.45 (C═N), 161.25 (C═O), 176.80 (C═S). HRMS (ES+) calcd for $C_{21}H_{25}N_4OS$(M + H^+) *m/e*, 381.1752; found, 381.1744.

***N′*-[(3*Z*)-1-(1-Hexyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzenesulfonohydrazide (36).** The title compound was prepared as a yellow solid, using **11** and benzenesulfonyl hydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 20%. mp 117−118 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, J = 6.6 Hz, 3H), 1.25 (br s, 6H), 1.58 (br t, 2H), 3.68 (t, J = 6.9 Hz, 2H), 7.10 (t, J =7.8 Hz, 1H), 7.15 (d, J = 7.8 Hz, 1H), 7.43 (t, J = 7.8 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.61−7.72 (m, 3H), 7.98−8.00 (m, 2H), 12.54 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.28 (CH_3), 22.38 (CH_2), 26.31 (CH_2), 27.30 (CH_2), 31.25 (CH_2), 39.74 (CH_2), 110.50 (CH), 119.17 (C), 120.93 (CH), 123.42 (CH), 125.96 (CH), 128.09 (CH), 128.38 (CH), 129.68 (CH), 129.93 (CH), 132.28 (CH), 134.31 (CH), 137.14 (C), 138.43 (C), 143.51

(C═N), 160.25 (C═O). HRMS (ES+) calcd for $C_{20}H_{24}N_3O_3S$ (M + H^+) *m/e*, 386.1541; found, 386.1533.

***N′*-[(3*Z*)-1-Hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]cyclohexanecarbohydrazide (37).** The title compound was prepared as a yellow solid, using **11** and cyclohexanecarbohydrazide according to the synthetic method E. The resulting solid was washed with ethanol. Yield: 45%. mp 58−60 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, *J* = 6.8 Hz, 3H), 1.24−1.83 (m, 19H), 3.74 (t, *J* = 6.9 Hz, 2H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 7.5 Hz, 1H), 7.59 (d, 3H), 12.34 (br s, isomer 1: 50%, 0.5 H), 13.00 (br s, isomer 2: 50%, 0.5 H), both isomer are in the same proportion. ^{13}C NMR (DMSO-d_6): δ 14.33(CH_3), 22.42 (CH_2), 25.48 (CH_2), 26.34 (CH_2), 25.83 (CH_2), 27.37 (CH_2), 31.29 (CH_2), 39.64 (CH_2), 110.52 (CH), 119.78 (C), 123.48 (CH), 131.79 (CH), 143.27 (C═N), (C═O). HRMS (ES+) calcd for $C_{21}H_{30}N_3O_2$ (M + H^+) *m/e*, 356.2328; found, 356.2333.

***N′*-[(3*Z*)-1-Hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]hexanohydrazide (38).** The title compound was prepared as a yellow solid, using **11** and hexanecarbohydrazide according to the synthetic method E. The resulting solid was washed with ethanol. Yield: 32%. mp 61−62 °C. ^{1}H NMR (DMSO-d_6): δ 0.82−0.91 (m, 6H), 1.27−1.33 (m, 10H), 1.62 (m, 4H), 2.71 (br s, 1H), 3.74 (t, *J* = 7.2 Hz, 2H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.58 (d, *J* = 7.5 Hz, 1H), 12.44 (br s, 1 H). ^{13}C NMR (DMSO-d_6): δ 14.25 (CH_3), 14.29 (CH_3), 22.30 (CH_2), 22.42 (CH_2), 26.33 (CH_2), 27.36 (CH_2), 31.26 (CH_2), 31.28 (CH_2), 39.59 (CH_2), 110.46 (CH), 119.72 (C), 120.62 (CH), 123.41 (CH), 131.73 (CH), 143.22 (C═N), 161.01 (C═O). HRMS (ES+) calcd for $C_{20}H_{30}N_3O_2$ (M + H^+) *m/e*, 344.2335; found, 344.2333.

4-Chloro-*N′*-[(3*Z*)-1-hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (39). The title compound was prepared as a yellow solid, using **11** and 4-chlorobenzhydrazide according to the synthetic method E. The resulting solid was washed with ethanol. Yield: 80%. mp 139 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, *J* = 6.9 Hz, 3H), 1.25−1.38 (m, 6H), 1.64 (m, 2H), 3.76 (t, *J* = 7.2 Hz, 2H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.23 (d, *J* = 7.8 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.64−7.72 (m, 3H), 7.91−7.94 (m, 2H), 13.85 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.32 (CH_3), 22.41 (CH_2), 26.37 (CH_2), 27.38 (CH_2), 31.31 (CH_2), 39.80 (CH_2), 110.69 (CH), 119.56 (C), 121.33 (CH), 123.72 (CH), 129.75 (CH), 131.20 (C), 132.35 (CH), 138.23 (C), 143.52 (C═N), 161.55 (C═O). HRMS (ES+) calcd for $C_{21}H_{23}N_3O_2Cl$ (M + H^+) *m/e*, 384.1484; found, 384.1473.

***tert*-Butyl(2*Z*)-2-(1-hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) hydrazinecarboxylate (40).** The title compound was prepared using **11** and *tert*-butoxycarbonyl hydrazide according to the synthetic method E. The product was purified by flash chromatography (eluent: AcOEt/heptane: 4/6) to afford a yellow oil which crystallized (Rf: 0.5).Yield: 8.5%. mp 77−78 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 7.2 Hz, 3H), 1.25 to 1.33 (m, 6H), 1.56 (s, 9H), 1.65−1.72 (m, 2H), 3.74 (t, *J* = 7.2 Hz, 2H), 6.87 (d, *J* = 7.8 Hz, 1H), 7.10 (t, *J* = 7.5 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.74 (d, *J* = 7.5 Hz, 1H), 12.33 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.01 (CH_3), 22.49 (CH_2), 26.63 (CH_2), 27.54 (CH_2), 28.15 (CH_3), 31.41 (CH_2), 39.77 (CH_2), 82.46 (C), 108.98 (CH), 120.15 (C), 121.31 (CH), 123.10 (CH), 130.54 (CH), 134.02 (C), 142.39 (C═N), 152.32 (C═O), 161.46 (C═O). HRMS (ES+) calcd for $C_{19}H_{28}N_3O_3$ (M + H^+) *m/e*, 346.2138; found, 346.2125.

***tert*-Butyl(2*E*)-2-(1-hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) hydrazinecarboxylate (41).** The title compound was obtained in the same experiment as compound **40** using **11** and *tert*-butoxycarbonyl hydrazide according to the synthetic method E and isolated by flash chromatography (eluent: AcOEt/heptane: 4/6) to afford a yellow solid (Rf: 0.1). Yield: 76.5%. mp 119−120 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, *J* = 6.9 Hz, 3H), 1.27−1.37 (m, 6H), 1.59 (s, 9H), 1.61−1.69 (m, 2H), 3.75 (t, *J* = 7.2 Hz, 2H), 6.91 (d, *J* = 8.1 Hz, 1H), 7.10 (t, *J* = 7.5 Hz, 1H), 7.41 (t, *J* = 8.1 Hz, 1H), 7.58 (d, *J* = 7.5 Hz, 1H), 8.75 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.00 (CH_3), 22.50 (CH_2), 26.54 (CH_2), 27.41 (CH_2), 28.11 (CH_3), 31.47 (CH_2), 40.04 (CH_2), 83.49 (C), 109.47 (CH), 115.51 (C), 122.33 (CH), 124.10 (CH), 123.22 (CH), 135.22 (C), 144.46 (C═N), 151.90 (C═O), 163.55 (C═O). HRMS (ES+) calcd for $C_{19}H_{28}N_3O_3$ (M + H^+), *m/e*, 346.2138; found, 346.2125.

***N′*-[(3*Z*)-1-Hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]-2-naphthohydrazide (42).** The title compound was prepared as a yellow solid, using **11** and 2-naphthhydrazide according to the synthetic method E. The resulting solid was washed with ethanol. Yield: 86%. mp 127−128 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, *J* = 6.9 Hz, 3H), 1.22−1.38 (m, 6H), 1.66 (m, 2H), 3.79 (t, *J* = 7.2 Hz, 2H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.24 (d, *J* = 7.8 Hz, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.63−7.73 (m, 3H), 7.94 (dd, *J* = 1.8 Hz, *J* = 8.4 Hz, 1H), 8.05 (d, *J* = 7.5 Hz, 1H), 8.13−8.17 (m, 2H), 8.55, (s, 1H), 14.01 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.32 (CH_3), 22.44 (CH_2), 26.39 (CH_2), 27.43 (CH_2), 31.33 (CH_2), 39.79 (CH_2), 110.66 (CH), 119.69 (C), 121.27 (CH), 123.68 (CH), 127.74 (CH), 128.24 (CH), 129.03 (CH), 129.38 (CH), 129.70 (CH), 129.74 (C), 132.21 (CH), 132.63 (C), 135.22 (C), 143.45 (C═N), 161.58 (C═O). HRMS (ES+) calcd for $C_{25}H_{26}N_3O_2$ (M + H^+) *m/e*, 400.2031; found, 400.2020.

***N′*-[(3*Z*)-1-Hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]-4-methoxybenzohydrazide (43).** The title compound was prepared as a yellow solid, using **11** and 4-methoxybenzhydrazide according to the synthetic method E. The resulting solid was washed with ethanol. Yield: 79%. mp 124−125 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, *J* = 6.9 Hz, 3H), 1.26−1.36 (m, 6H), 1.65 (m, 2H), 3.79 (t, *J* = 7.2 Hz, 2H), 3.87 (s, 3H), 7.14−7.24 (m, 4H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.65 (d, *J* = 7.5 Hz, 1H), 7.89 (d, *J* = 9 Hz, 2H), 13.86 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.33 (CH_3), 22.43 (CH_2), 26.39 (CH_2), 27.41 (CH_2), 31.32 (CH_2), 39.77 (CH_2), 56.07 (O-CH_3), 110.63 (CH), 114.97 (CH), 119.76 (C), 121.13 (CH), 123.64 (CH), 124.46 (C), 129.98 (CH), 132.02 (CH), 143.30 (C═N), 161.63 (C═O), 163.68 (C═O). HRMS (ES+) calcd for $C_{22}H_{26}N_3O_3$ (M + H^+) *m/e*, 380.1974; found, 380.1969.

***N′*-[(3*Z*)-1-Hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]-1-benzothiophene-2-carbohydrazide (44).** The title compound was prepared as a yellow solid, using **11** and Benzo[*b*]thiophene-2-carboxylic hydrazide according to the synthetic method E. The resulting solid was washed with ethanol. Yield: 82%. mp 164 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (t, *J* = 6.9 Hz, 3H), 1.26−1.36 (m, 6H), 1.64−1.66 (m, 2H), 3.79 (t, *J* = 7.2 Hz, 2H), 7.19−7.27 (m, 2H), 7.48−7.59 (m, 3H), 7.75 (d, *J* = 7.5 Hz, 1H), 8.13 (d, *J* = 7.5 Hz, 2H), 8.31 (br s, 1H), 13.65 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.33 (CH_3), 22.44 (CH_2), 26.40 (CH_2), 27.41 (CH_2), 31.34 (CH_2), 39.81 (CH_2), 110.71 (CH), 115.52 (C), 119.55 (C), 121.52 (CH), 123.35 (CH), 123.69 (CH), 125.75 (CH), 126.51 (CH), 127.83 (CH), 132.38 (CH), 143.64 (C═N), 161.60 (C═O). HRMS (ES+) calcd for $C_{23}H_{24}N_3O_2S$ (M + H^+) *m/e*, 406.1596; found, 406.1584.

***N′*-[(3*Z*)-1-Hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]-1-naphthohydrazide (45).** The title compound was prepared using **11** and 1-naphthhydrazide according to the synthetic method E. The product was purified by flash chromatography (eluent: AcOEt/heptane: 3/7) to afford a yellow oil which crystallized. Yield: 58%. mp 114−115 °C. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, *J* = 6.9 Hz, 3H), 1.26 (m, 6H), 1.59−1.62 (m, 2H), 3.73 (t, *J* = 6.9 Hz, 2H), 7.17−7.23 (m, 2H), 7.48 (t, *J* = 7.2 Hz, 1H), 7.63−7.70 (m, 3H), 7.90 (d, *J* = 6.6 Hz, 1H), 8.07 (t, *J* = 6.6 Hz, 1H), 8.19 (d, *J* = 8.7 Hz, 1H), 8.35 (br s, 1H), 13.51 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.29 (CH_3), 22.39 (CH_2), 26.32 (CH_2), 27.34 (CH_2), 31.27 (CH_2), 39.50 (CH_2), 110.61 (CH), 119.61 (C), 121.22 (C), 123.65 (CH), 125.36 (CH), 125.51 (CH), 126.83 (CH), 127.20 (CH), 128.03 (CH), 129.04 (CH), 130.34 (C), 131.26 (C), 132.26 (CH), 133.82 (C), 143.49 (C═N), 161.34 (C═O). HRMS (ES+) calcd for $C_{25}H_{26}N_3O_2$ (M + H^+) *m/e*, 400.2033; found, 400.2020.

***N′*-[(3*Z*)-1-Hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]adamantane-1-carbohydrazide (46).** The title compound was prepared as a yellow solid, using **11** and adamantanecarbohydrazide according to the synthetic method E. The resulting solid was washed with ethanol. Yield: 59%. mp 72 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, *J* = 6.9 Hz, 3H), 1.27 (m, 6H), 1.60−1.65 (m, 2H), 1.72 (s, 6H), 1.91 (s, 6H), 2.05 (s, 3H), 3.75 (t, *J* = 7.2 Hz, 2H), 7.16 (t, *J* = 7.5 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 7.46 (t, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 13.28 (br s, 1H). ^{13}C NMR (DMSO-d_6):

δ 14.32 (CH_3), 22.44 (CH_2), 26.38 (CH_2), 27.41 (CH_2), 27.85 (CH), 31.31 (CH_2), 36.32 (CH), 38.80 (CH_2), 39.52 (CH_2), 110.59 (CH), 119.75 (C), 121.03 (CH), 123.61 (CH), 131.96 (CH), 143.27 (C═N), 161.39 (C═O). HRMS (ES+) calcd for $C_{25}H_{34}N_3O_2$ (M + H^+) *m/e*, 408.2660; found, 408.2646.

***N′*-[(3*Z*)-1-(1-Propyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (47).** The title compound was prepared as a yellow solid, using **12** and benzhydrazide according to the synthetic method E. Yield: 86%. mp 188 °C. ^{1}H NMR (DMSO-d_6): δ 0.92 (t, *J* = 7.5 Hz, 3H), 1.68 (dt, *J* = 7.5 Hz, 2H), 3.75 (t, *J* = 7.0 Hz, 2H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.62−7.67 (m, 3H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 2H), 13.93 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 11.67 (CH_3), 20.89 (CH_2), 41.32 (CH_2), 110.74 (CH), 119.64 (C), 121.27 (CH), 123.70 (CH), 127.89 (CH), 129.69 (CH), 132.24 (CH), 132.44 (C), 133.41 (CH), 143.54 (C═N), 161.71 (C═O). HRMS (ES+) calcd for $C_{18}H_{18}N_3O_2$ (M + H^+) *m/e*, 308.1397; found, 308.1394.

***N′*-[(3*Z*)-1-(1-Butyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (48).** The title compound was prepared as a yellow solid, using **13** and benzhydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 65.3%. mp 158−159 °C. ^{1}H NMR (DMSO-d_6): δ 0.95 (t, *J* = 7.5 Hz, 3H), 1.36−1.41 (m, 2H), 1.64−1.69 (m, 2H), 3.78 (t, *J* = 7.2 Hz, 2H), 7.19 (t, Hz, *J* = 7.5 Hz, 1H), 7.24 (d, *J* = 7.8 Hz, 1H), 7.49 (td, *J* = 1.2 Hz, *J* = 7.8 Hz, 1H), 7.60−7.73 (m, 4H), 7.90−7.92 (m, 2H), 13.90 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.06 (CH_3), 20.04 (CH_2), 29.56 (CH_2), 39.49 (CH_2), 110.69 (CH), 119.66 (C), 121.26 (CH), 123.69 (CH), 127.88 (CH), 129.67 (CH), 132.22 (CH), 132.43 (C), 133.40 (CH), 137.40 (C), 143.43 (C═N), 161.58 (C═O), 163.34 (C═O). HRMS (ES+) calcd for $C_{19}H_{20}N_3O_2$ (M + H^+) *m/e*, 322.1548; found, 322.1550.

***N′*-[(3*Z*)-1-(1-Pentyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (49).** The title compound was prepared as a pale-orange solid, using **14** and benzhydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 78.1%. mp 134−135 °C. ^{1}H NMR (DMSO-d_6): δ 0.90 (t, *J* = 6.5 Hz, 3H), 1.34−1.36 (m, 4H), 1.63−1.68 (m, 2H), 3.80 (t, *J* = 7 Hz, 2H), 7.22 (t, Hz, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.5 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.65−7.70 (m, 3H), 7.74 (t, Hz, *J* = 7 Hz, 1H), 7.96 (d, (d, *J* = 7, 2H), 13.95 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.30 (CH_3), 22.27 (CH_2), 27.15 (CH_2), 28.89 (CH_2), 39.75 (CH_2), 110.66 (CH), 119.65 (C), 121.26 (CH), 123.69 (CH), 127.88 (CH), 129.67 (CH), 132.23 (CH), 132.42 (C), 133.40 (CH), 137.80 (C), 143.43 (C═N), 161.58 (C═O), 163.36 (C═O). HRMS (ES+) calcd for $C_{20}H_{22}N_3O_2$ (M + H^+) *m/e*, 336.1713; found, 336.1707.

***N′*-[(3*Z*)-1-(1-Dodecyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (50).** The title compound was prepared as a pale-orange solid, using 1-dodecyl-isatin and benzhydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 100%. mp 59−60 °C. ^{1}H NMR (DMSO-d_6): δ 0.82 (t, *J* = 6.6 Hz, 3H), 1.20−1.28 (m, 18H), 1.62−1.66 (m, 2H), 3.77 (t, *J* = 6.9 Hz, 2H), 7.16−7.24 (m, 2H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.60−7.73 (m, 4H), 7.92 (d, *J* = 7.8 Hz, 2H), 13.91 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.38 (CH_3), 22.53 (CH_2), 26.67 (CH_2), 27.38 (CH_2), 29.07 (CH_2), 29.14 (CH_2), 29.32 (CH_2), 39.36 (CH_2), 29.43 (CH_2), 29.45 (CH_2), 31.73 (CH_2), 39.75 (CH_2), 110.68 (CH), 119.65 (C), 121.27 (CH), 123.70 (CH), 127.88 (CH), 129.65 (CH), 132.22 (CH), 132.41 (C), 133.40 (CH), 143.44 (C═N), 161.61 (C═O). HRMS (ES+) calcd for $C_{27}H_{36}N_3O_2$ (M + H^+) *m/e*, 434.2812; found, 434.2802.

***N′*-[(3*Z*)-2-oxo-1-Phenyl-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (51).** The title compound was prepared as a pale-orange solid, using 1-phenylisatin and benzhydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 66.8%. mp 227−228 °C. ^{1}H NMR (DMSO-d_6): δ 6.87 (d, *J* = 8.1 Hz, 1H), 7.25 (td, *J* = 0.6 Hz, *J* = 7.5 Hz, 1H), 7.44 (td, *J* = 1.2 Hz, *J* = 7.8 Hz, 1H), 7.50−7.56 (m, 3H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.64 (d, *J* = 7.5 Hz, 2H), 7.70 (t, *J* = 7.5 Hz, 1H), 7.76 (d, *J* = 7.5 Hz, 1H), 7.92 d, *J* = 7.5 Hz, 2H), 13.88 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 110.73 (CH), 116.77 (C), 119.85 (C), 121.51 (CH), 124.30 (CH), 127.18 (CH), 127.90 (CH), 129.20 (CH), 129.72 (CH), 130.22 (CH), 132.24 (C), 132.36 (C), 133.24 (CH), 133.49 (CH), 137.71 (C), 144.11 (C═N), 161.17 (C═O), 163.49 (C═O). HRMS (ES+) calcd for $C_{21}H_{16}N_3O_2$ (M + H^+) *m/e*, 342.1250; found, 342.1237.

***N′*-[(3*Z*)-1-(Cyclohexylmethyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (52).** The title compound was prepared as a yellow solid, using **15** and benzhydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 71.4%. mp 140−141 °C. ^{1}H NMR (DMSO-d_6): δ 1.05−1.09 (m, 2H), 1.18−1.20 (m, 3H), 1.61−1.79 (m, 6H), 3.65 (d, *J* = 7.5 Hz, 2H), 7.22 (t, *J* = 8 Hz, 1H), 7.28 (d, *J* = 8 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.66−7.70 (m, 3H), 7.75 (t, *J* = 7 Hz, 1H), 7.96 (d, *J* = 7 Hz, 2H), 13.97 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 25.68 (CH_2), 26.26 (CH_2), 30.71 (CH_2), 36.40 (CH), 45.90 (CH_2), 110.95 (CH), 119.57 (C), 121.19 (CH), 123.67 (CH), 127.87 (CH), 129.68 (CH), 132.19 (CH), 132.44 (C), 133.40 (CH), 137.70 (C), 143.94 (C═N), 161.90 (C═O), 163.42 (C═O). HRMS (ES+) calcd for $C_{22}H_{24}N_3O_2$ (M + H^+) *m/e*, 362.1872; found, 362.1863.

***N′*-[(3*Z*)-1-Benzyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (53).** The title compound was prepared as a yellow solid, using **16** and benzhydrazide according to the synthetic method E. Yield: 96%. mp 192−193 °C. ^{1}H NMR (DMSO-d_6): δ 05.03 (s, 2H), 7.08 (d, *J* = 8 Hz, 1H), 7.17 (t, *J* = 7.5 Hz, 1H), 7.29 (t, *J* = 7.5 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 2H), 7.39−7.43 (m, 3H), 7.63 (t, *J* = 7.5 Hz, 2H), 7.66−7.72 (m, 2H), 7.94 (d, *J* = 7.5 Hz, 2H), 13.88 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 43.09 (CH_2), 111.02 (CH), 119.80 (C), 121.32 (CH), 123.92 (CH), 127.92 (CH), 128.14 (CH), 129.20 (CH), 129.68 (CH), 132.12 (CH), 132.43 (C), 133.43 (CH), 136.08 (C), 143.17 (C═N), 161.71 (C═O). HRMS (ES+) calcd for $C_{22}H_{18}N_3O_2$ (M + H^+) *m/e*, 356.1397; found, 356.1394.

***N′*-[(3*Z*)-1-(4-Chlorobenzyl)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (54).** The title compound was prepared as a yellow solid, using 1-(4-chlorobenzyl)-isatin and benzhydrazide according to the synthetic method D. The resulting solid was purified by crystallization in ethyl alcohol. Yield: 86%. mp 233−234 °C. ^{1}H NMR (DMSO-d_6): δ 5.03 (s, 2H), 7.08 (d, *J* = 7.8 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 7.39−7.47 (m, 5H), 7.60−7.74 (m, 4H), 7.94 (m, 2H), 13.84 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 42.43 (CH_2), 110.97 (CH), 119.87 (C), 121.35 (CH), 123.99 (CH), 127.93 (CH), 129.15 (CH), 129.70 (CH), 129.88 (CH), 132.14 (CH), 132.45 (C), 132.78 (C), 133.44 (CH), 135.15 (C), 143.04 (C═N), 161.74 (C═O). HRMS (ES+) calcd for $C_{22}H_{17}N_3O_2Cl$ (M + H^+) *m/e*, 390.1018; found, 390.1004.

***N′*-{[3*Z*]-1-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene}benzohydrazide (55).** The title compound was prepared as a yellow solid, using **17** and benzhydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 82.6%. mp 298 °C decomp. ^{1}H NMR (DMSO-d_6): δ 3.91 (t, *J* = 5.5 Hz, 2H), 4.09 (t, *J* = 5.5 Hz, 2H), 7.18 (t, *J* = 8 Hz, 1H), 7.27 (d, *J* = 8 Hz, 1H), 7.46 (t, *J* = 8 Hz, 1H), 7.55 (t, *J* = 7.5 Hz, 2H), 7.65 (d, *J* = 7.5 Hz, 2H), 7.69 (t, *J* = 7.5 Hz, 2H), 7.76−7.78 (m, 2H), 7.83−7.86 (m, 2H), 13.59 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 35.69 (CH_2), 38.35 (CH_2), 110.27 (CH), 119.64 (C), 121.41 (CH), 123.52 (CH), 123.96 (CH), 127.71 (CH), 129.65 (CH), 131.98 (C), 132.23 (CH), 132.27 (C), 133.44 (CH), 134.93 (CH), 143.01 (C═N), 162.12 (C═O), 168.31 (C═O). HRMS (ES+) calcd for $C_{25}H_{19}N_4O_4$ (M + H^+) *m/e*, 439.1404; found, 439.1401.

***N′*-[(3*Z*)-1-Hexyl-7-methyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (56).** The title compound was prepared as an orange solid, using **18** and benzhydrazide according to the synthetic method D. The resulting solid was purified by crystallization in ethyl alcohol. Yield: 80%. mp 141−142 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, *J* = 6.5 Hz, 3H), 1.29 to 1.36 (m, 6H), 1.61−1.67 (m, 2H), 3.92 (t, *J* = 8 Hz, 2H), 7.09 (t, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.5 Hz, 1H), 7.52 (d, *J* = 7.0 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 2H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 2H), 13.92 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.33 (CH_3), 18.57 (CH_3), 22.46 (CH_2), 26.30 (CH_2), 29.55 (CH_2), 31.28 (CH_2), 41.46 (CH_2), 119.21 (CH), 120.60 (C), 121.34 (C), 123.78 (CH), 127.90 (CH), 129.68 (CH), 132.46 (C), 133.40 (CH), 136.12 (CH), 141.05

(C═N), 162.21 (C═O). HRMS (ES+) calcd for $C_{22}H_{26}N_3O_2$ (M + H^+) *m/e*, 364.2028; found, 364.2020.

***N′*-[(3*Z*)-7-Chloro-1-hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (57).** The title compound was prepared as a yellow solid, using **19** and benzhydrazide according to the synthetic method D. The resulting solid was purified by crystallization in ethyl alcohol. Yield: 73%. mp 161−162 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 6.5 Hz, 3H), 1.30 to 1.37 (m, 6H), 1.68−1.71 (m, 2H), 4.05 (t, J = 7.0 Hz, 2H), 7.20 (t, J = 7.5 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.63−7.66 (m, 3H), 7.73 (t, J = 7.0 Hz, 1H), 7.93 (d, J = 7.5 Hz, 2H), 13.81 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.33 (CH_3), 22.43 (CH_2), 26.17 (CH_2), 29.54 (CH_2), 31.30 (CH_2), 41.39 (CH_2), 111.92 (C), 115.65 (C), 117.16 (C), 120.17 (CH), 123.10 (C), 125.05 (CH), 127.90 (CH), 127.97 (CH), 129.74 (CH), 132.25 (C), 133.58 (CH), 133.98 (CH), 138.73 (C═N), 161.88 (C═O). HRMS (ES+) calcd for $C_{21}H_{23}N_3O_2Cl$ (M + H^+) *m/e*, 384.1473; found, 384.1473.

***N′*-[(3*Z*)-1-Hexyl-7-iodo-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (58).** The title compound was prepared as a pale-yellow solid, using **20** and benzhydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 68.6%. mp 132−140 °C. ^{1}H NMR (DMSO-d_6): δ 0.89 (t, J = 7 Hz, 3H), 1.33 to 1.40 (m, 6H), 1.67−1.71 (m, 2H), 4.11 (t, J = 8 Hz, 2H), 6.92 (t, J = 8 Hz, 1H), 7.61 (t, J = 8 Hz, 2H), 7.69 (t, J = 7.5 Hz, 2H), 7.87 (d, J = 8 Hz, 1H), 7.93 (d, J = 7.5 Hz, 2H), 13.85 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.30 (CH_3), 22.50 (CH_2), 25.94 (CH_2), 29.60 (CH_2), 31.39 (CH_2), 39.57 (CH_2), 74.19 (C), 120.93 (CH), 122.90 (C), 125.36 (CH), 127.92 (CH), 129.50 (CH), 132.22 (C), 133.34 (CH), 143.07 (C═N), 144.23 (CH), 162.21 (C═O). HRMS (ES+) calcd for $C_{21}H_{22}IN_3O_2$ (M + H^+) *m/e*, 476.0848; found, 476.0830.

***N′*-[(3*Z*)-1-Hexyl-5-methyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (59).** The title compound was prepared as an orange solid, using **22** and benzhydrazide according to the synthetic method E. The resulting solid was washed with ethanol. Yield: 91%. mp 119−120 °C. ^{1}H NMR (DMSO-d_6): δ 0.84 (t, J = 7.0 Hz, 3H), 1.25 to 1.32 (m, 6H), 1.60−1.63 (m, 2H), 3.72 (t, J = 7.0 Hz, 2H), 7.09 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.46 (s, 1H), 7.61−7.63 (m, 2H), 7.92 (d, J = 7.5 Hz, 1H), 13.90 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.33 (CH_3), 20.94 (CH_3), 22.44 (CH_2), 26.38 (CH_2), 27.41 (CH_2), 31.33 (CH_2), 39.75 (CH_2), 110.45 (CH), 119.61 (C), 121.68 (CH), 127.86 (CH), 129.65 (CH), 132.45 (C), 132.56 (CH), 132.91 (C), 133.36 (CH), 141.24 (C═N), 161.57 (C═O). HRMS (ES+) calcd for $C_{22}H_{26}N_3O_2$ (M + H^+) *m/e*, 364.2024; found, 364.2020.

***N′*-[(3*Z*)-1-Hexyl-5-methoxy-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (60).** The title compound was prepared as dark-orange solid, using **23** and benzhydrazide according to the synthetic method E. The resulting solid was washed with ethanol. mp 138−139 °C. Yield: 62%. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, J = 7.0 Hz, 3H), 1.26 to 1.31 (m, 6H), 1.61−1.64 (m, 2H), 3.73 (t, J = 7.0 Hz, 2H), 3.81 (s, 3H), 7.05 (dd, J = 2.5, J = 8.5 Hz, 1H), 7.15 (d, J = 8.5 Hz, 1H), 7.19 (s, 1H), 7.61−7.65 (m, 2H), 7.71 (t, J = 7.0 Hz, 1H), 7.92 (d, J = 7.5 Hz, 1H), 13.97 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.34 (CH_3), 22.43 (CH_2), 26.37 (CH_2), 27.40 (CH_2), 31.33 (CH_2), 39.50 (CH_2), 56.19 (CH_3), 106.49 (CH), 111.63 (CH), 118.09 (CH), 120.48 (C), 127.87 (CH), 129.68 (CH), 132.41 (C), 133.43 (CH), 137.13 (C), 156.34 (C═N), 161.55 (C═O). HRMS (ES+) calcd for $C_{22}H_{26}N_3O_3$ (M + H^+) *m/e*, 380.1977; found, 380.1969.

***N′*-[(3*Z*)-5-Fluoro-1-hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (61).** The title compound was prepared as a yellow solid, using **24** and benzhydrazide according to the synthetic method E. The resulting solid was washed with ethanol. Yield: 61.3%. mp 103 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, J = 6.6 Hz, 3H), 1.25 to 1.30 (m, 6H), 1.58−1.65 (m, 2H), 3.75 (t, J = 7.0 Hz, 2H), 7.24 (dd, J = 4 Hz, J = 9 Hz, 1H), 7.31 (td, J = 2.5 Hz, J = 9 Hz, 1H), 7.45 (dd, J = 2 Hz, J = 7.5 Hz, 1H), 7.61−7.64 (m, 2H), 7.71 (t, J = 7.5 Hz, 1H), 7.90 (d, J = 2 Hz, J = 7.5 Hz, 2H), 13.89 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.33 (CH_3), 22.44 (CH_2), 26.36 (CH_2), 27.34 (CH_2), 31.33 (CH_2), 39.89 (CH_2), 108.52 (d, J = 100 Hz, CH), 111.91 (d, J = 30 Hz, CH), 121.25 (d, J = 95 Hz, CH), 120.98 (d, J = 35 Hz, C), 127.93 (CH), 129.68 (CH), 132.24 (C), 133.50 (CH), 137.28 (C), 139.66 (C═N), 159.20 (d, J = 950 Hz, CF), 161.59 (C═O), 163.34 (C═O). HRMS (ES+) calcd for $C_{21}H_{23}FN_3O$ (M + H^+) *m/e*, 368.1778; found, 368.1769.

***N′*-[(3*Z*)-5-Chloro-1-hexyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (62).** The title compound was prepared as a yellow solid, using **25** and benzhydrazide according to the synthetic method E. The resulting solid was washed with ethanol. Yield: 13.5%. mp 161 °C. ^{1}H NMR (DMSO): δ 0.90 (t, J = 6.5 Hz, 3H), 1.32 (br s, 6H), 1.62−1.64 (m, 2H), 3.78 (t, J = 7 Hz, 2H), 7.25 (d, J = 8.5 Hz, 1H), 7.60 (dd, J = 1.5 Hz, J = 8 Hz, 1H), 7.63−7.68 (m, 2H), 7.72 (t, J = 7 Hz, 1H), 8.01 (d, J = 7.5 Hz, 1H), 8.164 (s, 1H), 11.92 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.34 (CH_3), 22.47 (CH_2), 26.32 (CH_2), 27.28 (CH_2), 31.32 (CH_2), 39.50 (CH_2), 111.34 (CH), 116.57 (C), 126.57 (C), 126.79 (CH), 128.99 (CH), 129.19 (CH), 132.42 (CH), 132.86 (CH), 133.32 (C), 139.01 (C), 143.48 (C═N), 163.54 (C═O), 167.36 (C═O). HRMS (ES+) calcd for $C_{21}H_{23}ClN_3O_2$ (M + H^+) *m/e*, 384.1480; found, 384.1473.

***N′*-[(3*Z*)-5-Iodo-2-oxo-1-pentyl-1,2-dihydro-3*H*-indol-3-ylidene]benzohydrazide (63).** The title compound was prepared as an orange solid, using **26** and benzhydrazide according to the synthetic method D. The resulting solid was washed with ethanol. Yield: 70.2%. mp 161−162 °C. ^{1}H NMR (DMSO-d_6): δ 0.91 (t, J = 6.5 Hz, 3H), 1.35 to 1.36 (m, 4H), 1.66−1.69 (m, 2H), 3.80 (t, J = 7 Hz, 2H), 7.15 (d, J = 8.5 Hz, 1H), 7.69 (t, J = 7.5 Hz, 2H), 7.77 (t, J = 7 Hz, 1H), 7.86 (dd, J = 1.5 Hz, J = 8.5 Hz, 1H), 7.91 (s, 1H), 7.97 (d, J = 7 Hz, 2H), 13.87 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.30 (CH_3), 22.27 (CH_2), 27.08 (CH_2), 28.83 (CH_2), 39.85 (CH_2), 86.79 (C), 113.11 (CH), 121.98 (C), 127.96 (CH), 129.01 (CH), 129.70 (CH), 132.25 (C), 133.53 (CH), 136.53 (C), 140.14 (CH), 142.99 (C═N), 161.05 (C═O), 163.61 (C═O). HRMS (ES+) calcd for $C_{20}H_{21}IN_3O_2$ (M + H^+) *m/e*, 462.0672; found, 462.0673.

In Vitro Receptor Radioligand Binding Studies. The compounds were screened in a competitive binding experiment using membranes of Chinese hamster ovarian cells selectively expressing the *h*CB1 or *h*CB2 receptor. [^{3}H]**6** at a concentration of 0.5 nM and [^{3}H]**8** at a concentration of 0.8 nM were used as radioligands for *h*CB1 and *h*CB2 assays, respectively. For CB1 binding studies, cell membrane homogenates (25 μg of protein) were incubated for 120 min at 37 °C with 0.5 nM [^{3}H]**6** in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 5 mM MgCl2, 2.5 mM EDTA, and 0.3% BSA. For CB2 binding studies, cell membrane homogenates (15 μg of protein) were incubated for 120 min at 37 °C with 0.8 nM [^{3}H]**8** in the absence or presence of the test compound in a buffer containing 50 mM Hepes/Tris (pH 7.4), 5 mM $MgCl_2$, 2.5 mM EGTA, and 0.1% BSA. Nonspecific binding was determined in the presence of 10 μM of compound **8**. Following incubation, the samples were filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with an ice-cold buffer containing 50 mM Tris-HCl (pH 7.4) and 0.5% BSA using a 96-sample cell harvester (Unifilter, Packard). The filters were dried and then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The standard reference compounds for CB1 and CB2 were **6**[58] and **8**,[9] respectively. Reference compounds were tested in each experiment at several concentrations to obtain a competition curve from which the IC_{50} was calculated. The specific ligand binding to the receptors was defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabeled ligand. The results are expressed as percentage inhibition of control specific binding (100 − ((measured specific binding/control specific binding) × 100)) obtained in the presence of the test compounds at a concentration of 1 or 10 uM.

[^{35}S]GTP-γ-S Functional Assays. Functional activity was evaluated using GTP-γ-[^{35}S] assay in Chinese hamster ovarian cell membrane extracts expressing recombinant *h*CB1 receptors or *h*CB2 receptors. The assay relies on the binding of GTP-γ-[^{35}S], a radiolabeled nonhydrolyzable GTP analogue, to the G protein upon binding

of an agonist of the G-protein-coupled receptor. In this system, agonists stimulate GTP-γ-[^{35}S] binding, whereas antagonists have no effect and inverse agonists decrease GTP-γ-[^{35}S] basal binding.

Compounds were solubilized in 100% DMSO at a concentration of 10 mM within 4 h of the first testing session (master solution). A predilution for the dose−response curve was performed in 100% DMSO and then diluted 100-fold in assay buffer at a concentration 4-fold higher than the concentration to be tested. Compounds were tested for agonist activity at eight concentrations in duplicate: 10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0.001 μM, with compound **6** (Tocris, 0949) as the reference agonist. For GTP-γ-[^{35}S], membranes (Euroscreen S.A., Gosselies, Belgium) were mixed with GDP diluted in assay buffer to give 30 μM solution (volume:volume) and incubated for at least 15 min on ice. In parallel, GTP-γ-[^{35}S] (Amersham, SJ1308) was mixed with the beads PVT-WGA (Amersham, RPNQ001) diluted in assay buffer at 50 mg/mL (0.5 mg/10 μL) (volume:volume) just before starting the reaction. The following reagents were successively added in the wells of an Optiplate (Perkin-Elmer): 50 μL of ligand, 20 μL of the membranes: GDP mix, 10 μL of assay buffer for agonist testing, and 20 μL of the GTP-γ-[^{35}S]:beads mix. The plates were covered with a topseal, shaken on an orbital shaker for 2 min, and then incubated for 1 h at room temperature. Then the plates were centrifuged for 10 min at 2000 rpm, incubated at room temperature for 1 h, and counted for 1 min/well with a PerkinElmer TopCount reader. Assay reproducibility was monitored by the use of a reference compound **6**. For replicate determinations, the maximum variability tolerated in the test was of ±20% around the average of the replicates. Efficacies (E_{max}) for CB1 and CB2 are expressed as a percentage relative to the efficacy of compound **6**.

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Supporting Information Available: Further characterization data (high-performance liquid chromatography [HPLC]) for all novel compounds, details of 2D NMR data for compounds **33**, **40**, and **41**, crystallographic data for compound **33**, and ^{1}H NMR spectrum in DMSO at room temperature and at 75 °C for compound **34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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