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Design, Synthesis and Biological Evaluation of Aminoalkylindole Derivatives as Cannabinoid Receptor Ligands with Potential for Treatment of Alcohol Abuse

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Abstract

Attenuation of increased endocannabinoid signaling with a CB1R neutral antagonist might offer a new therapeutic direction for treatment of alcohol abuse. We have recently reported that a mono-hydroxylated metabolite of the synthetic aminoalkylindole cannabinoid JHW-073 (**3**) exhibits neutral antagonist activity at CB1Rs and thus may serve as a promising lead for the development of novel alcohol abuse therapies. In the current study, we show that systematic modification of an aminoalkylindole scaffold identified two new compounds with dual CB1R antagonist/CB2R agonist activity. Similar to the CB1R antagonist/inverse agonist rimonabant, analogues **27** and **30** decrease oral alcohol self-administration, without affecting total fluid intake and block the development of alcohol-conditioned place preference. Collectively, these initial findings suggest that design and systematic modification of aminoalkylindoles such as **3** may lead to development of novel cannabinoid ligands with dual CB1R antagonist/CB2R agonist activity with potential for use as treatments of alcohol abuse.

Introduction

The abuse of drugs and alcohol is often associated with substantial psychiatric comorbidity and exacerbates the spread of HIV/AIDS and drug resistant tuberculosis.[1] Estimates of the total overall cost of substance abuse in the United States, including productivity, health- and crime-related costs, exceeds $600 billion annually.[2] This includes approximately $193 billion for use of illicit drugs, $193 billion for tobacco, and $235 billion for alcohol.[3–5]

The World Health Organization estimates that approximately 2.5 million people die from alcohol use every year.[6] While several therapeutic options are available for the treatment of alcohol abuse, all existing therapies have only modest efficacy.[7] A growing body of evidence suggests that the endocannabinoid system is involved in some of the abuse related effects of drug and alcohol dependence.[8] For example, studies have shown that chronic

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Supporting Information

Supporting data including HPLC characterization of compounds **17** – **37**. This material is available free of charge via the Internet at http://pubs.acs.org.

ethanol (EtOH) exposure down-regulates cannabinoid 1 receptors (CB1Rs) and increases the brain concentration of endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG).[9, 10] It has also been demonstrated that CB1R knock-out mice exhibit reduced voluntary alcohol consumption as compared to wild type mice.[11] Furthermore, the CB1R antagonist/inverse agonist rimonabant (SR141716A, Figure 1) reduces EtOH intake in C57B1/6J mice to levels comparable with that of $CB1^{-/-}$ mice.[12] Cannabinoid 2 receptors (CB2Rs) have also been implicated in substance abuse. Systematic administration of the CB2R agonist **1** (JWH-133) dose-dependently inhibits intravenous cocaine self-administration in wild-type and CB1R-deficient mice, but not in CB2R-deficient mice and this effect was blocked by the CB2R antagonist **2** (AM630).[13, 14] To determine if this effect was mediated by antagonism of central or peripheral CB2Rs, the authors microinjected **2** into the nucleus accumbens and again observed antagonism of the reduction in cocaine self-administration induced by systemic administration of the CB2R agonist **1**.[13] Collectively, these studies suggest that targeting the central cannabinoid signaling system has potential in treating drug and alcohol dependence.

One approach to developing cannabinoid-based drugs for treatment of alcohol abuse is to target a molecule that possesses both CB1R neutral antagonist and CB2R agonist activity. This combined pharmacological profile offers two advantages over presently available CB1R antagonists/inverse agonists. Firstly, it provides an opportunity to avoid the side effect profile seen with rimonabant. During the first year of a RIO-Europe clinical trial several moderate side effects of rimonabant treatment were observed, including nausea, dizziness, and diarrhea.[15] However, upon completion of a meta-analysis study in 2007, it was concluded that a therapeutic dose of rimonabant produced very serious psychiatric side effects such as depression, anxiety and suicide.[16, 17] However, subsequent studies in animals indicate that a neutral antagonist might be expected to retain the therapeutic properties of rimonabant, but be associated with fewer adverse effects resulting from inverse agonism at CB1Rs.[18–20] Secondly, incorporation of CB2 agonism into the same molecule provides an opportunity to harness a potential new direction in developing anti-addiction therapies. Our approach to identifying such a molecule has been to investigate synthetic cannabinoids.

Synthetic cannabinoids derived from the aminoalkylindole structural class have received increased attention due to recent widespread abuse of the incense blends known as K2/Spice.[21] Despite the recreational popularity of these commercial preparations, very little is known regarding K2/Spice metabolism, pharmacology, and toxicity. Among the most common components of K2/Spice are naphthalen-1-yl(1-pentyl-1*H*-indol-3-yl)methanone (JWH-018), (1-butyl-1*H*-indol-3-yl)(naphthalen-1-yl)methanone (JWH-073), (1-(2-morpholinoethyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone (JWH-200), and 2-((1*S*,3*R*)-3-hydroxycyclohexyl)-5-(2-methyloctan-2-yl)phenol (CP-47,497, Figure 2).[22] These agents exhibit differential selectivity towards CB1Rs and CB2Rs and have been shown to be both more potent than the classical cannabinoid Δ^9-THC.[23, 24] Recently, our group reported that a mono-hydroxylated metabolite of aminoalkylidole **3** (JWH-073), compound **4** (JWH-073-M4), retains relatively high affinity for CB1Rs, but lacks intrinsic activity when examined in the [^{35}S]GTPγS functional assay.[25] These results indicate that this metabolite may act as a neutral antagonist at CB1Rs.

Given our results that **4** acts as a neutral antagonist at CB1Rs and the known promiscuity of the aminolakylindoles for both CB1 and CB2 receptors, we hypothesize that further structure-activity relationships (SAR) would identify analogues with dual CB1R neutral antagonist/CB2R agonist activity.[23, 24, 26] Results from our initial results are described below.

Chemistry

Synthesis of analogues based on scaffold **4** began with the use of commercially available 7-methoxyindole (**5**), which was subjected to mild alkylating conditions to afford compound (**6**), an important intermediate in 74–93% yield for the different substituents, respectively (Scheme 1). In addition, commercially available 7-ethylindole was also subjected to the aforementioned conditions to yield compound (**6**) with an ethyl substitution at the 7-position of the indole ring in 93% yield. Intermediate **6** was then subjected to Friedel-Crafts like acylation conditions, using dimethyaluminum chloride and the appropriate acid chloride at 0 °C to afford many of the analogues prepared, structurally represented as intermediate (**7**), in yields ranging from 9–68%. Compound **7** was shown to be a versatile intermediate that can undergo several reactions to yield various scaffolds. Specifically, it undergoes O-demethylation in the presence of BBr^3 to afford compound (**8**) in yields ranging from 38%–90% as well as LAH reduction conditions to afford compound (**9**) in 20% yield. 1-butyl-5-methoxy-1*H*-indole, **11** synthesized from N-alkylation of commercially available 5-methoxyindole **10**, was subjected to Friedel-Crafts like acylation conditions to produce compound (**12**) in 58% yield, which was then O-demethylated using BBr^3 to afford compound (**13**) in 90% yield (Scheme 2). Compound **5** was additionally used in the synthesis of intermediate (**14**) utilizing a one-pot *N*-alkylation and 3-indole iodination in 69% yield. Intermediate **14** was then subjected to Suzuki coupling conditions utilizing different boronic acids to afford analogues (**15**) and (**16**) in 37% and 50% yield, respectively. Twenty-one analogues were synthesized and the structures of the analogues are schematically represented in Table 1.

Results and Discussion

In our quest for novel dual activity compounds with CB1R neutral antagonist/CB2R agonist properties, twenty-one analogues were prepared using step-wise molecular investigation of the elements present in the scaffold of compound **4**. The analogues prepared were designed to investigate the SAR at several positions on the scaffold of interest. Firstly, we wanted to examine the necessity of the carbonyl moiety as well as the optimal length of the linker from the indole core to the naphthalene ring. We also sought out to explore the possibility for addition of rotational bonds at this position of the molecule. Secondly, we set to explore the requirement of the naphthalene ring through selective introduction of electron withdrawing and electron donating groups. Our initial design strategy was to limit the use of charged species and keep steric demands to a minimum. Lastly, we wanted to investigate the positioning of the hydroxyl moiety around the indole core and its necessity for activity at CBRs. The prepared analogues were subjected to *in vitro* pharmacological characterization, which included receptor binding and functional assays for both cannabinoid receptors. Initial receptor binding screens for both CB1Rs and CB2Rs were conducted for all twenty-one analogues prepared. These initial binding screens were conducted by examining the ability of a single analogue concentration of 1 μM to compete for receptor binding with the high affinity CB1/CB2R agonist [^{3}H]CP-55,940.[27, 28] This approach allowed us to quickly attain an approximate affinity of all compounds tested at the cannabinoid receptors. Compounds were selected for further assays to determine intrinsic activity at CB1 and CB2Rs if a 1 μM concentration produced over 50% displacement of a 0.2 nM concentration of [^{3}H]CP-55,940, indicating a relatively high sub-micromolar affinity. For example, employing the conditions used for this screen, it would be predicted by the Cheng-Prusoff equation that the concentration of a compound producing 50% displacement of [^{3}H]CP-55,940 from a receptor will estimate the compounds affinity for that receptor.[29] Data presented in Table 1 shows that a 1μM concentration of most analogues examined produced greater than 50% displacement of [^{3}H]CP-55,940 from both CB1R and CB2Rs. As a result, out of the twenty-one analogues that were screened, sixteen bind to CB1R and

eighteen bind to CB2R with sub-micromolar affinity. Several analogues exhibited very high affinity for either of the two receptors tested, which can be predicted from their near 100% displacement of [^{3}H]CP-55,940 from the receptors. Based on the above criteria, thirteen compounds were chosen for further evaluation.

A functional assay to screen for the inhibition of adenylyl cyclase (AC) activity by CBRs was selected for determination of intrinsic activity of the analogues that demonstrated sub-micromolar affinity for CB1R and CB2Rs.[28] Since all analogues examined are predicted to bind to CBRs with sub-micromolar affinity, a 10 μM concentration of all compounds was used to achieve full-receptor occupancy. It is assumed that full-receptor occupancy will produce maximal efficacy. The non-selective CB1R/CB2R full agonist CP-55,940 inhibited AC-activity via CB1Rs endogenously expressed in Neuro2A cells by 45% and 37% in CHO cells transfected with CB2 receptors (Table 1). Most compounds examined exhibited levels of AC-inhibition similar to that produced by the full agonist CP-55,940. Unlike our prior report demonstrating that metabolite **4** exhibited neutral antagonist properties in assays examining G-protein activation, in this study **4** interestingly acts as a full CB1R agonist to inhibit AC-inhibition in Neuro2A cells.[25] However, compounds **17**, **27**, and **30**, produced significantly less AC-inhibition via CB1Rs when compared to the full agonist CP-55,940 of −4, 18, and 16% inhibition, respectively. Despite little or no AC-inhibition when examined at CB1Rs, these compounds exhibited significantly different results when tested for activity at CB2Rs. Specifically, the three compounds **17**, **27**, and **30** produced levels of AC-inhibition that were similar to inhibition resulting from CP-55,940 administration of 22.1, 33.2, and 20.8%, respectively. Collectively, these data indicate that compounds **17**, **27**, and **30** display only neutral antagonist to weak partial agonist activity at CB1Rs, while exhibiting partial to full agonist efficacy at CB2Rs.

Based on efficacy predicted from functional experiments examining AC-inhibition, together with the initial screen for sub-micromolar receptor affinity, several compounds were selected to determine actual K_i values by conducting full concentration effect curves for inhibition of [^{3}H]CP-55,940 binding to CBRs. Even though compound **17** produced minimal AC-inhibition via CB1Rs, it was shown to bind to both CB1 and CB2Rs with a relatively low affinity of 387 and 281 nM, respectively (Table 2). However, analogue **19** demonstrated high affinity for CB1 and CB2Rs in the low nanomolar range of 1.7 nM and 0.81 nM, respectively. Compounds **27** and **30** displayed similar affinity for both receptors, however binding with slightly higher affinity to the CB2R. Specifically, **27** bound to CB1Rs with a K_i value of 15.4 nM, while binding to CB2Rs with a K_i value of 10.9 nM. Compound **30** demonstrated an affinity of 37.2 nM and 26.5 nM for CB1R and CB2Rs, respectively.

The four lead compounds exhibiting relatively high affinity as indicated by K_i values were examined together with metabolite 4 to determine their potency to inhibit AC-activity via CB1 and CB2Rs. Similar to data reported in the initial screen for AC-inhibition, **4** and **19** produced 50–60% inhibition of AC-activity via CB1Rs with IC_{50} values of 225 and 45 nM, respectively. Conversely, **27** and **30** produced very little inhibition of AC-activity, which was only observed at very high concentrations of both analogues (Figure 3A). In support of the observed functional activity indicating neutral antagonism, the two lead compounds and the CB1R antagonist O-2050 (1 μM concentration) significantly antagonized AC-inhibition produced by the CB1R full agonist **3** (Figure 3B). In contrast to neutral antagonism at CB1Rs, all of the compounds examined produced 40–50% inhibition of AC-activity at CB2Rs in CHO-*h*CB2 cells with potency similar to their rank order of K_i values reported in Table 2 (Figure 4A). To examine if the observed agonist activity in CHO-*h*CB2 cells is due to activation of CB2Rs, the AC-inhibition assay was conducted in CHO-WT cells not expressing CB2Rs. As observed in Figure 4B, neither **27** nor **30** significantly modified AC-

activity in CHO-WT cells indicating that the agonist activity observed for both compounds in CHO-*h*CB2 cells is indeed due to their activation of CB2Rs.

The dual CB1R neutral antagonist/CB2 agonist activity displayed by compounds **27** and **30** in the *in vitro* assays prompted us to select these analogues for further evaluation *in vivo* using murine subjects in two complementary models relevant to alcohol abuse. Our hypothesis is that an analogue with dual activity as CB1R neutral antagonist/CB2R agonist will reduce both the reinforcing effects of EtOH and the conditioned rewarding effects of EtOH in mice. The compounds selected were initially tested for potential cannabinoid receptor antagonist activity using a thermoregulation assay. The hypothermic effects of cannabinoid agonists are well established, and are blunted by prior treatment with CB1R antagonists, allowing us to rapidly determine whether or not our selected compounds display *in vivo* effects consistent with CB1R antagonism.[30, 31] The assay was conducted using glass-encapsulated radiotelemetry probes surgically implanted into the peritoneal cavity of each mouse, which monitored core body temperature in response to drug administration.[25] Intraperitoneal administration of 10 mg/kg of the full CB1R agonist **3**, elicited a profound hypothermic effect as seen in Figure 5 (black circles). Importantly, thirty minute pretreatment with either **27** (Figure 5, gray triangle) or **30** (Figure 5, white circles), significantly decreased the hypothermic effects elicited by subsequent administration of compound **3**, confirming the *in vivo* cannabinoid activity of **27** and **30**, and illustrating apparent antagonist effects against CB1R agonist-induced hypothermia.

Having established apparent *in vivo* antagonism, **27** and **30** were further tested in two complementary models of alcohol abuse: oral self-administration (SA) and alcohol conditioned place preference (CPP). These assays are important models for the study of alcoholism because they capture several aspects of this condition including voluntary EtOH drinking (SA) and conditioned EtOH reward (CPP). The effects of compounds **27** and **30** on voluntary 10% EtOH drinking were studied using a two-bottle choice procedure (ethanol vs. water) similar to those described by Keane and coworkers, as well as by Cunningham and coworkers, using 10 mg/kg rimonabant as a positive control.[32, 33] Across five observations conducted under baseline “no injection” conditions it can be observed in Figures 6A, and 6D (black circles) that EtOH preference and total fluid consumption (Figure 6B, Figure 6E; black circles) were steadily maintained during the entire treatment period. Consistent with previous reports, daily treatment with 10 mg/kg rimonabant decreased EtOH preference (Figure 6A, Figure 6D, triangles) without altering total fluid intake (Figure 6B, Figure 6E, triangles).[34] However, consisted with previously published reports, rimonabant decreased body weight across the treatment period (Figure 6C, Figure 6F, triangles).[35, 36] After ten day treatment with rimonabant, mice were returned to baseline “no injection” conditions where their weights increased and voluntary EtOH drinking resumed. After five such observations, representing a two week drug “washout” period, daily treatments with 10 mg/kg of **27** or **30** were initiated. As previously observed with rimonabant, both compounds **27** and **30** reduced EtOH preference (Figure 6A, inverted triangles; Figure 6D, gray triangles, respectively) without affecting total fluid consumption (Figure 6B, inverted triangles; Figure 6E, gray triangles). However, unlike rimonabant, compounds **27** and **30** did not decrease body weight of mice tested (Figure 6C, inverted triangles; Figure 6E, gray triangles). The last treatment was a daily injection of the rimonabant/**27**/**30** vehicle, which was 8% Tween/92% sterile water. With the exception of the first observation period for compound **27** (perhaps representing a persistent effect of **27**), EtOH preference (Figure 6A, gray circles-compound **27**; Figure 6D, gray circles-compound **30**), total fluid consumption (Figure 6B, gray circles-compound **27**; Figure 6E, gray circles-compound **30**), and mean body weight (Figure 6C, gray circles-compound **27**; Figure 6F, gray circles-compound **30**) were not affected by vehicle injections. This study demonstrates that a JWH-derived compound devoid of inverse

agonist activity can replicate the effects of rimonabant on alcohol self-administration in mice.

To further explore the *in vivo* effects of these compounds, an alcohol conditioned place preference assay was performed with compounds **27**, **30**, and rimonabant. This assay tested the effects of these compounds on the conditioned rewarding effects of EtOH. Training of mice involved daily pairings of 2 mg/kg EtOH with novel contextual cues within a spatial conditioning chamber, and daily pairings of saline with distinct contextual cues within the same chamber. Following four such pairings, an increase in time spent in EtOH-paired chamber was observed, indicating the development of conditioned place preference (Figure 7, "No inj" bar). Separate groups of mice were conditioned as previously described, with the exception that 10 mg/kg **27,** 10 mg/kg **30** or 10 mg/kg rimonabant were administered one hour before each EtOH pairing. In contrast to the ~400 sec preference elicited by EtOH in the absence of cannabinoid administration, mice treated with 10 mg/kg rimonabant prior to each EtOH pairing did not exhibit a significant preference for the EtOH-paired chamber, which is in agreement with previously reported data (Figure 7, "10 RIM" bar).[37, 38] Similar to the effects of rimonabant produced in the present study, the conditioned rewarding effects of EtOH were also blocked by the administration of either 10 mg/kg **27** or 10 mg/kg **30** prior to each EtOH pairing. In total, these studies demonstrate that a novel indole-derived compound with cannabinoid activity lacking inverse agonist activity can replicate the effects of rimonabant on alcohol induced CPP in mice.

Conclusions

CBR ligands that attenuate endocannabinoid signaling have been shown to produce effects in several disorders, among which are drug abuse and alcohol dependence.[16, 39] Our goal is to design and synthesize a dual activity CB1R neutral antagonist/CB2R agonist that would reduce increased endocannabinoid signaling without affecting intrinsic CB1R activity, and also be able to modulate the rewarding effects of alcohol abuse. As an initial step towards achieving this goal, we conducted experiments to synthesize compounds that exhibit dual CB1R neutral antagonist/CB2R agonist activity based on the scaffold of compound **4**. Initial studies explored the scaffold of **4** by utilizing a molecular dissection approach to better understand the elements involved in producing cannabinoid activity. The necessity of the naphthalene ring and the electronic potential at this position, the necessity of the carbonyl moiety, the length of the linker between the indole core and the naphthalene substituent, as well as the necessity of the hydroxyl moiety on the 7-position of the indole ring were all explored. Similar to the parent compound **3,** the majority of the analogues examined exhibited relatively high affinity for both CB1R and CB2Rs. Of all of the compounds tested, two analogues, **27** and **30**, appeared to have the most promise. For example, compounds **27** and **30** displayed similar high affinity for both receptors, with K_i values of **27** of 15.4 nM at CB1R and 10.9 nM for CB2R and K_i values of **30** of 37.2 and 26.5 nM for CB1R and CB2R, respectively. In AC-inhibition assays, both compounds produced very little inhibition of AC-activity via CB1Rs, consistent with neutral antagonism. However, **27** and **30** both produced 40–50% inhibition of AC-activity via CB2Rs, similar to that of the full agonist CP-55,940 and thus consistent with partial to full agonism. Based on promising results from these *in vitro* studies, compounds **27** and **30** were selected for testing in two *in vivo* models of alcohol abuse: oral self-administration and alcohol conditioned place preference. Similar to the actions produced by the CB1R antagonist/inverse agonist rimonabant, both **27** and **30** were shown to decrease alcohol self-administration, without affecting total fluid intake. Unlike rimonabant, **27** and **30** did not alter body weight during the treatment period. Interestingly, both **27** and **30** decreased alcohol conditioned place preference in a similar manner to rimonabant, despite not possessing any apparent inverse agonist activity *in vitro*. As has been previously reported for rimonabant, results from the present study collectively

demonstrate that both the reinforcing and conditioning rewarding effects of alcohol can also be significantly blunted by treatment with novel JWH-derived cannabinoids, devoid of inverse agonist activity at CB1Rs.[40] Such compounds with dual CB1R neutral antagonist/CB2R agonist activity may indeed represent potential leads in an ongoing search for new and improved alcohol abuse therapies. Additional data will be reported in due course.

Experimental section

General Methods

Unless otherwise indicated, all reagents were purchased from commercial sources and were used without further purification. Melting points were determined on a Thomas-Hoover capillary melting apparatus. NMR spectra were recorded on a Bruker DRX-400 with qnp probe or a Bruker AV-500 with cryoprobe using δ values in ppm (TMS as internal standard) and *J* (Hz) assignments of ^{1}H resonance coupling. High resolution mass spectrometry data were collected on either a LCT Premier (Waters Corp., Milford, MA) time of flight mass spectrometer or an Agilent 6890 N gas chromatograph in conjunction with a quarto Micro GC mass spectrometer (Micromass Ltd, Manchester UK). Thin-layer chromatography (TLC) was performed on 0.25 mm plates Analtech GHLF silica gel plates using ethyl acetate/*n*-hexanes, in 20%:80% ratio as the solvent unless otherwise noted. Spots on TLC were visualized by UV (254 or 365 nm), if applicable, and phosphomolybdic acid in ethanol. Column chromatography was performed with Silica Gel (40–63 μ particle size) from Sorbent Technologies (Atlanta, GA). Analytical HPLC was carried out on an Agilent 1100 Series Capillary HPLC system with diode array detection at 254 nm on an Agilent Eclipse XDB-C18 column (250 × 10 mm, 5 μm) with isocratic elution in 80% CH_3CN/20% H_2O (0.1% Formic acid) unless otherwise specified.

General Procedure A: Indole *N*-alkylation

To a suspension of KOH (5 equiv) in DMF (13 mL) was added 5-methoxyindole, 7-methoxyindole or 7-ethylindole (1 equiv). After stirring at 21 °C for an hour, 1-bromobutane (1.375 equiv) was added and the reaction mixture was heated to 50 °C and stirred overnight. Upon completion, the resulting mixture was poured into H_2O (15 mL) and extracted with DCM (3 × 15 mL). Combined organic extracts were washed with water, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and the resulting residue was purified by flash column chromatography on silica gel using EtOAc/*n*-hexanes.

1-Butyl-7-methoxy-1H-indole (6ac)—Compound **6ac** was synthesized from commercially available 7-methoxyindole using general procedure A and 1-bromobutane to afford 0.51 g (74% yield) as a clear oil. TLC system: 10% EtOAc/90% *n*-hexanes. Spectral data matched previously reported data.[41]

1-Butyl-7-ethyl-1H-indole (6bc)—Compound **6bc** was synthesized from commercially available 7-ethylindole using general procedure A and 1-bromobutane to afford 0.64 g (93% yield) as a clear oil. TLC system: 10% EtOAc/90% *n*-hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.05 – 6.95 (m, 3H), 6.47 (d, *J* = 3.1 Hz, 1H), 4.31 – 4.21 (m, 2H), 3.03 (q, *J* = 7.5 Hz, 2H), 1.83 – 1.69 (m, 2H), 1.35 (td, *J* = 7.6, 2.7 Hz, 5H), 0.94 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 133.57, 130.09, 129.60, 127.30, 122.42, 119.49, 119.00, 101.40, 48.75, 34.47, 25.67, 20.04, 16.08, 13.77. [M+H] calcd for $C_{14}H_{19}N$, 202.1590; found 202.1596.

4-(2-(7-Methoxy-1H-indol-1-yl)ethyl)morpholine (6ad)—Compound **6ad** was synthesized from commercially available 7-methoxyindole using general procedure A and 4-(2-bromoethyl)morpholine that was made utilizing conditions reported by Cai J., *et al*.[42] to

afford 0.51 g (74% yield) as an oil with a slightly orange tint. TLC system: 40% EtOAc/60% *n*-hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (dd, *J* = 7.9, 0.9 Hz, 1H), 7.01 – 6.99 (m, 1H), 6.97 (d, *J* = 7.8 Hz, 1H), 6.61 (dd, *J* = 7.8, 0.9 Hz, 1H), 6.42 (d, *J* = 3.1 Hz, 1H), 4.53 – 4.46 (m, 2H), 3.93 (s, 3H), 3.74 – 3.68 (m, 4H), 2.78 – 2.71 (m, 2H), 2.53 – 2.47 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.33, 130.92, 129.19, 125.48, 119.75, 113.77, 102.14, 101.33, 67.02, 60.15, 55.15, 53.93, 46.65. [M+H] calcd for $C_{15}H_{21}N_2O_2$, 261.1598; found 261.1603.

1-Butyl-5-methoxy-1H-indole (11)—Compound **11** was synthesized from commercially available 5-methoxyindole using general procedure A and 1-bromobutane to afford 1.28 g (92% yield) as a clear oil. TLC system: 10% EtOAc/90% *n*-hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (d, *J* = 8.9, 1H), 7.02 (dd, *J* = 2.7, 13.3, 2H), 6.81 (dd, *J* = 2.4, 8.9, 1H), 6.34 (dd, *J* = 0.7, 3.0, 1H), 4.02 (t, *J* = 7.1, 2H), 3.79 (s, 3H), 1.75 (ddd, *J* = 7.3, 11.2, 14.8, 2H), 1.32 – 1.23 (m, 2H), 0.87 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.09, 131.56, 129.06, 128.55, 111.93, 110.35, 102.71, 100.53, 56.12, 46.54, 32.63, 20.43, 13.95. [M+H] calcd for $C_{13}H_{18}NO$, 204.1383; found 204.1386.

Preparation of 1-butyl-3-iodo-7-methoxy-1H-indole (14)—A round bottom flask containing 7-methoxyindole (0.22 mL, 1.70 mmol, 1 equiv) in DMF at 21 °C was stirred with KOH (0.10 g, 1.78 mmol, 1.05 equiv) for 15 min and then treated with I_2 (0.44g, 1.73 mmol, 1.02 equiv). After 30 min, NaH (60% dispersion in mineral oil; 0.082 g, 2.04 mmol, 1.2 equiv) was added portion-wise. After an additional 15 min, 1-bromobutane (0.2 mL, 1.87 mmol, 1.1 equiv) was added and the reaction mixture was stirred until completion. Upon completion (TLC monitoring), water was added and allowed to stir for 15 min, upon which the mixture was extracted with DCM and the layers were separated. Aqueous layer was washed with DCM (3 × 15 mL) and the combined organic layers were washed with H_2O (2 × 15 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography using EtOAc/*n*-hexanes to afford 0.45g (80% yield) as an clear oil with a yellow tint that was used immediately. TLC system: 10% EtOAc/90% *n*-hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (t, *J* = 3.8, 2H), 7.04 (dd, *J* = 1.1, 8.0, 1H), 6.68 (d, *J* = 7.5, 1H), 4.38 (t, *J* = 7.2, 2H), 3.95 (s, 3H), 1.78 (dt, *J* = 7.5, 12.7, 2H), 1.33 (td, *J* = 7.5, 15.0, 2H), 0.94 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 146.67, 132.31, 131.99, 125.18, 119.88, 113.27, 102.43, 54.80, 48.90, 33.67, 19.20, 13.13.

General Procedure B: *O*-Demethylation procedure

A solution of BBr_3 (1 M in DCM, 6 equiv) in DCM was added dropwise to a solution of methyl ether (1 equiv) in DCM at −78 °C. The mixture was then allowed to warm to 21 °C overnight, and upon completion, $NaHCO_3$ (6 equiv) was added. The resulting mixture was then cooled in an ice-bath and MeOH (20 mL) was added dropwise and then stirred for 30 min. The reaction mixture was then warmed up to 21 °C and stirred for an additional hour. Upon that, the reaction was quenched with H_2O and the separated aqueous phase was extracted with DCM (3 × 15 mL). Combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel using mixtures of EtOAc/*n*-hexanes.

General Procedure C: Acid chloride formation

The appropriate acid (1 equiv) was placed in a round bottom flask and flushed twice with Argon (Ar). Anhydrous DCM (7 mL) was then added followed by the dropwise addition of oxalyl chloride (2 M in DCM, 3.1 equiv). After 5 min, 3 drops of anhydrous DMF were added to the reaction mixture and once the fizzing stopped, the reaction was allowed to stir

overnight at 21 °C. Solvent was then evaporated using reduced pressure and the crude residue was used immediately without any further purification.

General Procedure D: 3′-indole acylation

To a solution of the appropriate indole (1 equiv) in DCM at 0 °C under an Ar atmosphere was added Me_2AlCl (1.5 equiv) dropwise and the solution was allowed to stir at that temperature for 30 min, after which a solution of the appropriate acid chloride (1.2 equiv) in DCM was added dropwise. Reaction was monitored by TLC and upon completion, was carefully poured into an ice-cold 1N HCl solution and then extracted with DCM (3 × 15 mL). Combined organic layers were then washed with $NaHCO_3$ (2 × 15 mL), brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the resulting residue was purified by flash column chromatography on silica gel using mixtures of EtOAc/*n*-hexanes to afford the desired product.

1-(1-Butyl-7-methoxy-1H-indol-3-yl)-2-(4-fluorophenyl)ethanone (17)— Compound **17** was synthesized from compound **6** using general procedure D and 4-fluorophenylacetyl chloride to afford 0.08g (9% yield) isolated as an brown oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 0.8, 8.1, 1H), 7.64 (s, 1H), 7.29 – 7.25 (m, 2H), 7.16 (t, *J* = 8.0, 1H), 7.02 – 6.96 (m, 2H), 6.71 (d, *J* = 7.5, 1H), 4.39 (t, *J* = 7.2, 2H), 4.09 (s, 2H), 3.93 (s, 3H), 1.85 – 1.77 (m, 2H), 1.36 – 1.27 (m, 2H), 0.94 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.32, 162.72, 160.77, 147.31, 135.78, 131.58, 131.55, 130.88, 130.82, 129.07, 126.33, 123.30, 115.76, 115.38, 115.21, 115.12, 104.31, 55.36, 50.28, 45.87, 33.81, 19.78, 13.68. HRMS (*m/z*): [M+K] calcd for $C_{21}H_{22}FKNO_2$, 378.1272; found 378.1315. HPLC t_R = 15.031 min; purity = 95.4% using 70% CH_3CN/30% H_2O (0.1% Formic acid).

1-(1-Butyl-7-methoxy-1H-indol-3-yl)-2-(4-methoxyphenyl)ethanone (18)— Compound **18** was synthesized from compound **6** using general procedure D and 4-methoxyphenylacetyl chloride to afford 0.63 g (59.2% yield) isolated as a reddish solid, mp = 72–74 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 0.8, 8.1, 1H), 7.63 (s, 1H), 7.25 – 7.21 (m, 2H), 7.15 (t, *J* = 8.0, 1H), 6.87 – 6.81 (m, 2H), 6.70 (d, *J* = 7.8, 1H), 4.37 (t, *J* = 7.2, 2H), 4.06 (s, 2H), 3.92 (s, 3H), 3.77 (s, 3H), 1.84 – 1.76 (m, 2H), 1.35 – 1.26 (m, 2H), 0.93 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 193.44, 158.73, 147.69, 136.29, 130.73, 129.59, 128.50, 126.69, 123.58, 116.22, 115.62, 114.40, 104.62, 55.76, 55.69, 50.63, 46.48, 34.22, 20.19, 14.11. HRMS (*m/z*): [M+Na] calcd for $C_{22}H_{25}NNaO_3$, 374.1732; found 374.1783. HPLC t_R = 7.490 min; purity = 99.9%.

1-(1-Butyl-7-methoxy-1H-indol-3-yl)-2-(2,3-difluorophenyl)ethanone (19)— Compound **19** was synthesized from compound **6** using general procedure D and 1-naphthoyl chloride to afford 2.00 g (53% yield) isolated as pale brown solid, mp = 108–110 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.4, 1H), 8.11 (d, *J* = 7.4, 1H), 7.96 (d, *J* = 8.2, 1H), 7.91 (d, *J* = 7.8, 1H), 7.65 (dd, *J* = 1.1, 7.0, 1H), 7.56 – 7.45 (m, 3H), 7.28 – 7.24 (m, 2H), 7.22 (s, 1H), 6.79 (d, *J* = 7.7, 1H), 4.30 (t, *J* = 7.3, 2H), 3.96 (s, 3H), 1.80 – 1.71 (m, 2H), 1.32 – 1.23 (m, 2H), 0.89 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.08, 147.45, 139.32, 139.07, 133.81, 130.89, 129.96, 129.46, 128.22, 126.79, 126.61, 126.34, 126.14, 125.89, 124.65, 123.60, 117.42, 115.35, 104.66, 55.50, 50.34, 33.86, 19.83, 13.73. HRMS (*m/z*): [M+Na+CH_3CN] calcd for $C_{26}H_{26}N_2NaO_2$ 421.1892; found 421.1843. HPLC t_R = 14.576; purity = 95.8%.

1-(1-Butyl-7-hydroxy-1H-indol-3-yl)-2-(4-fluorophenyl)ethanone (20)— Compound **20** was synthesized from **7** using general procedure B to afford 0.10g (38% yield) isolated as an off-brown solid, mp = decomposition at 204–206 °C. ^{1}H NMR (500 MHz, DMSO) δ 9.91 (s, 1H), 8.44 (s, 1H), 7.62 (dd, *J* = 0.9, 8.0, 1H), 7.39 – 7.33 (m, 2H),

7.12 (ddd, *J* = 2.6, 5.9, 8.9, 2H), 6.94 (t, *J* = 7.8, 1H), 6.61 (dd, *J* = 0.9, 7.7, 1H), 4.44 (t, *J* = 7.0, 2H), 4.09 (s, 2H), 1.85 – 1.75 (m, 2H), 1.31 – 1.21 (m, 2H), 0.90 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, DMSO) δ 191.61, 161.72, 159.80, 144.49, 137.94, 132.50, 132.48, 131.05, 130.99, 128.73, 125.46, 122.80, 114.76, 114.59, 114.50, 112.34, 108.26, 48.70, 44.52, 33.29, 18.97, 13.39. HRMS (*m/z*): [M+Na+CH_3CN] calcd for $C_{22}H_{23}FN2NaO_2$, 389.1641; found 389.1711. HPLC t_R = 6.491 min; purity = 99.9% using 70% CH_3CN/30% H_2O (0.1% Formic acid).

1-(1-Butyl-7-methoxy-1H-indol-3-yl)-2-phenylethanone (21)—Compound **21** was synthesized from compound **6** using general procedure D and phenyacetyl chloride to afford 0.13 g (17% yield) isolated as an off-white solid with a pinkish tint, mp = 65–68 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 0.8, 8.1, 1H), 7.66 (s, 1H), 7.36 – 7.30 (m, 4H), 7.26 – 7.22 (m, 1H), 7.18 (t, *J* = 8.0, 1H), 6.72 (d, *J* = 7.8, 1H), 4.39 (t, *J* = 7.2, 2H), 4.14 (s, 2H), 3.94 (s, 3H), 1.87 – 1.77 (m, 2H), 1.32 (dq, *J* = 7.4, 14.8, 2H), 0.95 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.72, 147.37, 136.13, 136.06, 129.43, 129.25, 128.62, 126.64, 126.38, 123.30, 115.96, 115.30, 104.34, 55.44, 50.32, 47.09, 33.88, 19.86, 13.78. HRMS (*m/z*): [M+Na] calcd for $C_{21}H_{23}NNaO_2$, 344.1626; found 344.1656. HPLC t_R = 8.114 min; purity = 99.9%.

1-(1-Butyl-7-hydroxy-1H-indol-3-yl)-2-phenylethanone (22)—Compound **22** was synthesized from compound **7** using general procedure B to afford 0.17 g (68% yield) isolated as a fluffy brown solid, mp = decomposition at 197–200 °C. ^{1}H NMR (500 MHz, DMSO) δ 9.91 (s, 1H), 8.44 (s, 1H), 7.62 (dd, *J* = 0.9, 8.0, 1H), 7.36 – 7.31 (m, 2H), 7.28 (dd, *J* = 4.9, 10.3, 2H), 7.20 (dd, *J* = 4.3, 11.6, 1H), 6.93 (t, *J* = 7.8, 1H), 6.61 (dd, *J* = 0.9, 7.7, 1H), 4.44 (t, *J* = 7.0, 2H), 4.08 (s, 2H), 1.84 – 1.75 (m, 2H), 1.30 – 1.20 (m, 2H), 0.90 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, DMSO) δ 191.75, 144.49, 137.99, 136.39, 129.20, 128.76, 128.00, 126.01, 125.46, 122.77, 114.60, 112.35, 108.24, 48.67, 45.62, 33.28, 18.95, 13.38. HRMS (*m/z*): [M+Na] calcd for $C_{20}H_{21}NNaO_2$, 330.1470; found 330.1514. HPLC t_R = 6.458 min; purity = 99.8% using 70% CH_3CN/30% H_2O (0.1% Formic acid).

1-(1-Butyl-7-methoxy-1H-indol-3-yl)-2-(naphthalen-1-yl)ethanone (23)—Compound **23** was synthesized from compound **6** using general procedure D and 1-naphthoylacetyl chloride to afford 0.09 g (12% yield) isolated as an amorphous yellow solid, mp = 110–113 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 – 8.00 (m, 2H), 7.89 – 7.85 (m, 1H), 7.79 (dd, *J* = 2.4, 7.0, 1H), 7.72 (s, 1H), 7.51 – 7.46 (m, 2H), 7.46 – 7.43 (m, 2H), 7.18 (t, *J* = 8.0, 1H), 6.73 (d, *J* = 7.6, 1H), 4.61 (s, 2H), 4.38 (t, *J* = 7.1, 2H), 3.95 (s, 3H), 1.85 – 1.77 (m, 2H), 1.34 – 1.24 (m, 2H), 0.94 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.76, 147.41, 135.82, 133.97, 132.76, 132.55, 129.27, 128.76, 127.93, 127.63, 126.32, 126.30, 125.73, 125.57, 124.28, 123.31, 115.99, 115.33, 104.33, 55.45, 50.32, 44.80, 33.84, 19.84, 13.77. HRMS (*m/z*): [M+K] calcd for $C_{25}H_{25}KNO_2$, 410.1522; found 410.1527. HPLC t_R = 11.439 min; purity = 99.8%

1-(1-Butyl-7-hydroxy-1H-indol-3-yl)-2-(naphthalen-1-yl)ethanone (24)—Compound **24** was synthesized from compound **7** using general procedure B to afford 0.07 g (55% yield) isolated as an off brown solid, mp = decomposition at 213–216 °C. TLC system: 30% EtOAc/70% *n*-hexanes. ^{1}H NMR (500 MHz, DMSO) δ 9.32 (s, 1H), 8.85 (s, 1H), 8.65 – 8.58 (m, 1H), 8.39 – 8.33 (m, 1H), 8.28 (dt, *J* = 4.3, 8.6, 2H), 8.00 – 7.88 (m, 4H), 7.42 (t, *J* = 7.8, 1H), 7.15 (d, *J* = 7.6, 1H), 5.13 (s, 2H), 5.02 (t, *J* = 7.1, 2H), 2.41 – 2.33 (m, 2H), 1.81 (dq, *J* = 7.4, 14.9, 2H), 1.40 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, DMSO) δ 191.47, 144.32, 136.79, 133.93, 133.52, 132.76, 129.74, 128.34, 128.04, 127.00, 125.69, 125.40, 125.36, 124.74, 122.71, 115.59, 113.81, 108.35, 49.30, 43.73, 33.85, 19.44, 13.07. HRMS (*m/z*): [M+Na+CH_3CN] calcd for $C_{26}H_{26}N_2NaO_2$, 421.1892; found

421.1924. HPLC t_R = 6.458 min; purity = 99.8% using 70% CH_3CN/30% H_2O (0.1% Formic acid).

(1-Butyl-5-methoxy-1H-indol-3-yl)(naphthalen-1-yl)methanone (25)—Compound **25** was synthesized from compound **11** using general procedure D and 1-naphthoylacetyl chloride to afford 0.36 g (16% yield) isolated as an amorphous yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.5, 1H), 8.01 (d, *J* = 2.5, 1H), 7.96 (d, *J* = 8.2, 1H), 7.90 (d, *J* = 7.6, 1H), 7.64 (dd, *J* = 1.2, 7.0, 1H), 7.54 – 7.44 (m, 3H), 7.28 (d, *J* = 2.2, 1H), 7.25 (s, 1H), 6.98 (dd, *J* = 2.6, 8.9, 1H), 4.02 (t, *J* = 7.2, 2H), 3.92 (s, 3H), 1.81 – 1.71 (m, 2H), 1.32 – 1.22 (m, 2H), 0.88 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.12, 156.75, 139.25, 138.00, 133.81, 131.99, 130.88, 129.96, 128.24, 127.90, 126.80, 126.37, 126.11, 125.83, 124.65, 117.25, 114.28, 110.95, 103.99, 55.93, 47.23, 31.95, 20.11, 13.65. HRMS (*m/z*): [M +Na] calcd for $C_{26}H_{26}N_2NaO_2$, 421.1892; found 421.1930. HPLC t_R = 16.771 min; purity = 99.9% using 70% CH_3CN/30% H_2O (0.1% Formic acid).

(1-Butyl-5-hydroxy-1H-indol-3-yl)(naphthalen-1-yl)methanone (26)—Compound **26** was synthesized from compound **12** using general procedure B to afford 0.59 g (90% yield) isolated as a lightly yellow solid, mp = 209–212 °C. TLC system: 30% EtOAc/70% *n*-hexanes. ^{1}H NMR (500 MHz, DMSO) δ 9.20 (s, 1H), 8.07 (d, *J* = 7.8, 1H), 8.02 (d, *J* = 7.9, 1H), 7.98 (d, *J* = 8.5, 1H), 7.74 (d, *J* = 2.4, 1H), 7.65 – 7.59 (m, 3H), 7.58 – 7.54 (m, 1H), 7.50 (ddd, *J* = 1.4, 6.8, 8.2, 1H), 7.41 (d, *J* = 8.8, 1H), 6.80 (dd, *J* = 2.4, 8.8, 1H), 4.11 (t, *J* = 7.2, 2H), 1.69 – 1.61 (m, 2H), 1.23 – 1.14 (m, 2H), 0.81 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, DMSO) δ 190.38, 153.63, 138.93, 138.61, 133.14, 130.83, 129.92, 129.30, 128.09, 127.39, 126.46, 126.06, 125.31, 125.20, 124.82, 115.24, 112.81, 111.40, 106.01, 45.86, 31.33, 19.16, 13.27. HRMS (*m/z*): [M+Na+CH_3CN] calcd for $C_{25}H_{24}N_2NaO_2$, 407.1735; found 407.1777. HPLC t_R = 13.460 min; purity = 100.0% using 60% CH_3CN/40% H_2O (0.1% Formic acid).

1-Butyl-7-methoxy-3-(naphthalen-1-ylmethyl)-1H-indole (27)—$LiAlH_4$ (4.9 mL, 4.85 mmol, 4 equiv) was dissolved in THF (1M) and a solution of $AlCl_3$ (1.94 g, 14.5 mmol, 12 equiv) in THF (8 mL) was added dropwise at 0 °C. After 30 min, indole **3** (0.433 g, 1.21 mmol, 1 equiv) in THF (9 mL) was added to the reaction mixture and allowed to stir at 21 °C for 48 hrs. Upon completion, reaction mixture was cooled in an ice-bath and carefully quenched with H_2O and acidified with 1 N HCl to pH = 3. The organic phase was then separated and washed with $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the resulting residue was purified by flash column chromatography on silica gel using mixtures of EtOAc/*n*-hexanes to afford 0.07 g (18% yield) isolated as an pinkish oil. TLC system: 10% EtOAc/90% *n*-hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.0, 1H), 7.91 – 7.85 (m, 1H), 7.76 (d, *J* = 8.0, 1H), 7.46 (tt, *J* = 3.5, 8.3, 2H), 7.42 – 7.34 (m, 2H), 7.21 (dd, *J* = 0.8, 8.0, 1H), 7.00 (t, *J* = 7.8, 1H), 6.65 (d, *J* = 7.5, 1H), 6.50 (s, 1H), 4.51 (s, 2H), 4.24 (t, *J* = 7.2, 2H), 3.94 (s, 3H), 1.70 (dt, *J* = 7.4, 14.8, 2H), 1.24 (dq, *J* = 7.4, 14.7, 2H), 0.87 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.93, 137.47, 134.22, 132.61, 130.65, 128.93, 128.32, 127.11, 126.92, 126.31, 126.09, 126.05, 125.83, 124.87, 119.55, 113.82, 112.31, 102.72, 55.68, 49.26, 34.70, 29.37, 20.24, 14.16. HRMS (*m/z*): [M^+] calcd for $C_{24}H_{25}NO$, 343.1936; found 343.1887. HPLC t_R = 12.038 min; purity = 99.9% using 90% CH_3CN/10% H_2O (0.1% Formic acid).

(7-Methoxy-1-(2-morpholinoethyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone (28)—Compound **28** was synthesized from compound **6** using general procedure D and 1-naphthoylacetyl chloride to afford 0.16 g (16% yield) isolated as an yellow solid, mp = 149–152 °C. TLC system: 50% EtOAc/50% *n*-hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 – 8.10 (m, 2H), 7.95 (d, *J* = 8.2, 1H), 7.89 (d, *J* = 7.5, 1H), 7.63 (dd, *J* = 1.2, 7.0, 1H), 7.54 –

7.41 (m, 3H), 7.27 – 7.23 (m, 3H), 6.77 (d, *J* = 7.4, 1H), 4.40 (t, *J* = 6.5, 2H), 3.94 (s, 3H), 3.60 – 3.49 (m, 4H), 2.67 (t, *J* = 6.6, 2H), 2.42 – 2.33 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.18, 147.29, 140.00, 139.28, 133.80, 130.86, 130.00, 129.34, 128.26, 126.86, 126.45, 126.38, 126.06, 125.82, 124.55, 123.67, 117.60, 115.47, 104.70, 66.99, 59.29, 55.48, 53.77, 47.37. HRMS (*m/z*): [M+H] calcd for $C_{26}H_{27}N_2O_3^+$, 415.2016; found 415.1985. HPLC t_R = 7.049 min; purity = 95.0% using 30% CH_3CN/70% H_2O (0.1% Formic acid).

1-(1-Butyl-7-hydroxy-1H-indol-3-yl)-2-(4-hydroxyphenyl)ethanone (29)— Compound **29** was synthesized from compound **7** using general procedure B to afford 0.05 g (17% yield) isolated as an off-white solid, mp = decomposition at 197–200 °C. TLC system: 30% EtOAc/70% *n*-hexanes. ^{1}H NMR (500 MHz, Acetone) δ 8.86 (s, 1H), 8.23 (s, 1H), 8.16 (s, 1H), 7.86 (dd, *J* = 0.9, 8.0, 1H), 7.21 (d, *J* = 8.6, 2H), 6.98 (t, *J* = 7.8, 1H), 6.80 – 6.73 (m, 2H), 6.68 (dd, *J* = 0.9, 7.6, 1H), 4.54 (t, *J* = 7.1, 2H), 4.01 (s, 2H), 1.94 – 1.85 (m, 2H), 1.39 – 1.29 (m, 2H), 0.94 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, Acetone) δ 193.14, 156.81, 145.21, 137.93, 131.20, 130.75, 128.46, 126.87, 123.57, 116.43, 115.91, 115.82, 114.76, 109.25, 50.17, 46.31, 34.79, 20.37, 13.99. HRMS (*m/z*): [M+Na] calcd for $C_{22}H_{24}N_2NaO_3$, 387.1685; found 387.1658. HPLC t_R = 4.998 min; purity = 99.4% using 60% CH_3CN/40% H_2O (0.1% Formic acid).

1-Butyl-7-methoxy-3-(naphthalen-2-yl)-1H-indole (30)—$Pd(OAc)_2$ (2 mg, 0.01 mmol, 0.01 equiv), SPhos (7.4 mg, 0.02 mmol, 0.02 equiv), K_3PO_4 (379 mg, 1.79 mmol, 2 equiv), and 2-naphthaleneboronic acid (230 mg, 1.34 mmol, 1.5 equiv) were placed into a round bottom pressure flask and flushed with Ar. Intermediate **14** (294 mg, 0.89 mmol, 1 equiv) in toluene (1 mL) was then added and the reaction mixture was allowed to stir at 100 °C overnight. Reaction was monitored via TLC and upon completion, it was allowed to cool to 21 °C and diluted with Et_2O (10mL). Mixture was then filtered through a pad of Celite and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using mixtures of DCM/pentane, to afford 0.15 g (50% yield) isolated as an clear amorphous oil, TLC system: 15% DCM/85% *n*-hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.93 – 7.84 (m, 4H), 7.80 (dd, *J* = 1.8, 8.4, 1H), 7.65 (dt, *J* = 3.2, 6.3, 1H), 7.47 (dddd, *J* = 1.3, 6.9, 8.0, 16.2, 2H), 7.29 (s, 1H), 7.12 (t, *J* = 7.9, 1H), 6.72 (d, *J* = 7.7, 1H), 4.46 (t, *J* = 7.2, 2H), 3.99 (s, 3H), 1.92 – 1.83 (m, 2H), 1.45 – 1.36 (m, 2H), 0.98 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.84, 134.10, 133.46, 131.93, 128.72, 128.20, 127.80, 127.75, 127.51, 126.71, 126.56, 126.12, 125.11, 125.07, 120.43, 116.57, 112.74, 102.83, 55.45, 49.49, 34.43, 20.06, 13.91. HRMS (*m/z*): [M^+] calcd for $C_{23}H_{23}NO$, 329.1780; found 329.1747. HPLC t_R = 12.570 min; purity = 96.7% using 90% CH_3CN/10% H_2O (0.1% Formic acid).

(1-Butyl-7-methoxy-1H-indol-3-yl)(4-fluorophenyl)methanone (31)—Compound **31** was synthesized from compound **6** using general procedure D and 4-fluorobenzoyl chloride to afford 0.28 g (49% yield) isolated as an pinkish solid, mp = 91–94 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (dd, *J* = 0.8, 8.0, 1H), 7.89 – 7.84 (m, 2H), 7.45 (s, 1H), 7.26 (t, *J* = 8.0, 1H), 7.22 – 7.17 (m, 2H), 6.80 (d, *J* = 7.4, 1H), 4.44 (t, *J* = 7.2, 2H), 4.00 (s, 3H), 1.91 – 1.81 (m, 2H), 1.43 – 1.32 (m, 2H), 0.98 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 189.45, 165.64, 163.64, 147.43, 137.73, 137.34, 137.32, 131.17, 131.10, 129.80, 126.50, 123.39, 115.45, 115.31, 115.28, 115.13, 104.57, 55.49, 50.37, 33.97, 19.90, 13.79. HRMS (*m/z*): [M+Na+CH_3CN] calcd for $C_{22}H_{23}FN_2NaO_2$, 389.1641; found 389.1608. HPLC t_R = 8.765 min; purity = 99.8%.

1-(1-Butyl-7-methoxy-1H-indol-3-yl)-2-(2-fluorophenyl)ethanone (32)— Compound **32** was synthesized from compound **6** using general procedure D and an acid

chloride made in situ from 2-(2-fluorophenyl)acetic acid[43] to afford 0.30 g (35% yield) isolated as a darker yellow solid, mp = 86–88 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 0.8, 8.1, 1H), 7.78 (s, 1H), 7.41 (td, *J* = 1.7, 7.6, 1H), 7.29 – 7.23 (m, 1H), 7.21 (t, *J* = 8.0, 1H), 7.16 – 7.07 (m, 2H), 6.75 (d, *J* = 7.4, 1H), 4.44 (t, *J* = 7.2, 2H), 4.20 (s, 2H), 3.97 (s, 3H), 1.91 – 1.82 (m, 2H), 1.42 – 1.32 (m, 2H), 0.99 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.37, 160.78, 158.83, 146.34, 135.04, 135.03, 130.71, 130.67, 128.14, 127.54, 127.48, 125.39, 123.22, 123.19, 122.30, 122.13, 122.01, 114.61, 114.37, 114.19, 114.17, 103.31, 54.40, 49.30, 38.49, 38.48, 32.82, 18.80, 12.73. [M+Na] calcd for $C_{21}H_{22}FNNaO_2$, 362.1532; found 362.1513. HPLC t_R = 8.732 min; purity = 99.6%.

1-(1-Butyl-7-methoxy-1H-indol-3-yl)-2-(3-fluorophenyl)ethanone (33)— Compound **33** was synthesized from compound **6** using general procedure D and an acid chloride made *in situ* from 2-(3-fluorophenyl)acetic acid[43] to afford 0.21 g (27% yield) isolated as an yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 0.8, 8.1, 1H), 7.63 (s, 1H), 7.28 – 7.21 (m, 2H), 7.16 (t, *J* = 8.0, 1H), 7.08 (d, *J* = 8.1, 1H), 7.03 (d, *J* = 9.8, 1H), 6.91 (td, *J* = 1.8, 8.3, 1H), 6.71 (d, *J* = 7.5, 1H), 4.38 (t, *J* = 7.2, 2H), 4.10 (s, 2H), 3.92 (s, 3H), 1.85 – 1.75 (m, 2H), 1.36 – 1.25 (m, 2H), 0.93 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.85, 163.92, 161.97, 147.41, 138.42, 138.36, 135.97, 130.00, 129.94, 129.16, 126.44, 125.18, 125.16, 123.45, 116.54, 116.37, 115.86, 115.21, 113.69, 113.52, 104.46, 55.46, 50.40, 46.55, 46.54, 33.88, 19.87, 13.77. HRMS (*m/z*): [M+Na] calcd for $C_{21}H_{22}FNNaO_2$, 362.1532; found 362.1503. HPLC t_R = 13.931 min; purity = 99.9% using 50% CH_3CN/50% H_2O (0.1% Formic acid).

1-(1-Butyl-7-ethyl-1H-indol-3-yl)-2-(4-fluorophenyl)ethanone (34)—Compound **34** was synthesized from compound **6** using general procedure D and 4-fluorophenylacetyl chloride to afford 0.34 g (39% yield) isolated as an white powder, mp = 67–70 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (dd, *J* = 1.2, 8.0, 1H), 7.72 (s, 1H), 7.32 – 7.27 (m, 2H), 7.25 – 7.20 (m, 1H), 7.10 (d, *J* = 6.5, 1H), 7.04 – 6.98 (m, 2H), 4.35 – 4.28 (m, 2H), 4.12 (s, 2H), 3.02 (q, *J* = 7.5, 2H), 1.87 – 1.78 (m, 2H), 1.42 – 1.32 (m, 5H), 0.98 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.35, 162.81, 160.87, 136.80, 134.58, 131.73, 131.70, 130.95, 130.88, 128.22, 127.79, 124.78, 123.06, 120.82, 115.82, 115.50, 115.33, 49.80, 46.00, 34.07, 25.52, 20.01, 16.28, 13.75. HRMS (*m/z*): [M+Na+CH_3CN] calcd for $C_{24}H_{27}FN_2NaO$, 401.2005; found 401.1976. HPLC t_R = 9.645 min; purity = 99.9%.

1-Butyl-7-methoxy-3-(naphthalen-1-yl)-1H-indole (35)—Intermediate **14** (512 mg, 1.56 mmol, 1 equiv) and $Pd(PPh_3)_4$ (54 mg, 0.05 mmol, 0.03 equiv) were placed into a round bottom flask and flushed with Ar. Solvent (DME, 6 mL) was then added and allowed to stir for 10 min upon which the solution was degassed by bubbling with Ar for 15 min. Sodium carbonate (2M [1.06 g in 5 mL H_2O, 1.6 ml], 3.11mmol, 2 equiv) and 1-naphthaleboronic acid (401 mg, 2.33 mmol, 1.5 equiv) in EtOH (1 mL) were added and the reaction mixture was refluxed. Reaction was monitored via TLC and upon completion, it was allowed to cool to 21 °C and EtOAc was added. Mixture was then filtered through a pad of Celite and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using mixtures of EtOAc/*n*-hexanes, to afford 0.17 g (37% yield) isolated as an yellow solid, mp = 74–76 °C. TLC system: 10% DCM/ 90% n-hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.2, 1H), 7.92 (d, *J* = 8.2, 1H), 7.85 (d, *J* = 8.0, 1H), 7.53 (dddd, *J* = 1.3, 6.9, 8.1, 23.6, 3H), 7.41 (ddd, *J* = 1.3, 6.8, 8.2, 1H), 7.19 (s, 1H), 7.09 (dd, *J* = 0.9, 8.0, 1H), 7.00 (t, *J* = 7.8, 1H), 6.70 (d, *J* = 7.3, 1H), 4.50 (t, *J* = 7.1, 2H), 4.01 (s, 3H), 1.95 – 1.86 (m, 2H), 1.47 – 1.37 (m, 2H), 0.99 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.75, 134.08, 133.36, 132.64, 130.54, 128.76, 128.28, 127.75, 126.92, 126.80, 125.90, 125.70, 125.67, 125.65, 119.90, 114.86, 113.25, 102.56, 55.47, 49.36, 34.47, 20.08, 13.93. HRMS (*m/z*): [M^+] calcd for $C_{23}H_{23}NO$,

329.1780; found 329.1747. HPLC t_R = 11.727 min; purity = 95.4% using 90% CH_3CN/10% H_2O (0.1% Formic acid).

1-(1-Butyl-7-methoxy-1H-indol-3-yl)-2-(3,4-difluorophenyl)ethanone (36)— Compound **36** was synthesized from compound **6** using general procedure D and an acid chloride made *in situ* from 2-(3,4-difluorophenyl)acetic acid[43] to afford 0.07 g (8% yield) isolated as an off-white solid, mp = 88–90 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (dd, *J* = 0.8, 8.1, 1H), 7.68 (s, 1H), 7.21 (t, *J* = 8.0, 1H), 7.19 – 7.14 (m, 1H), 7.14 – 7.09 (m, 1H), 7.06 (d, *J* = 2.2, 1H), 6.76 (d, *J* = 7.8, 1H), 4.44 (t, *J* = 7.2, 2H), 4.11 (s, 2H), 3.97 (s, 3H), 1.92 – 1.79 (m, 2H), 1.37 (dq, *J* = 7.4, 14.8, 2H), 0.99 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.44, 150.14 (dd, *J* = 247.8, 12.8 Hz), 149.30 (dd, *J* = 246.7, 12.6 Hz), 147.32, 135.70, 132.64 (dd, *J* = 6.0, 4.0 Hz), 128.98, 126.38, 125.38 (dd, *J* = 6.1, 3.6 Hz), 123.43, 118.36 (d, *J* = 17.3 Hz), 117.08 (d, *J* = 17.1 Hz), 115.66, 115.02, 104.41, 55.37, 50.34, 46.07 – 44.76 (m), 33.81, 19.80, 13.68. HRMS (*m/z*): [M+Na+CH_3CN] calcd for $C_{23}H_{24}F_2N_2NaO_2$, 421.1704; found 421.1678. HPLC t_R = 8.678 min; purity = 100.0%.

1-(1-Butyl-7-methoxy-1H-indol-3-yl)-2-(2,3-difluorophenyl)ethanone (37)— Compound **37** was synthesized from compound **6** using general procedure D and an acid chloride made *in situ* from 2-(2,3-difluorophenyl)acetic acid[43] to afford 0.23 g (24% yield) isolated as an off-brown solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (dd, *J* = 0.8, 8.1, 1H), 7.78 (s, 1H), 7.21 (t, *J* = 8.0, 1H), 7.16 (t, *J* = 6.7, 1H), 7.13 – 7.03 (m, 2H), 6.76 (d, *J* = 7.4, 1H), 4.45 (t, *J* = 7.2, 2H), 4.23 (d, *J* = 1.3, 2H), 3.98 (s, 3H), 1.92 – 1.83 (m, 2H), 1.43 – 1.33 (m, 2H), 0.99 (t, *J* = 7.4, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.40, 150.68 (dd, *J* = 222.0, 13.0 Hz), 148.72 (dd, *J* = 220.5, 13.1 Hz), 147.32, 135.89 (d, *J* = 1.6 Hz), 129.00, 126.37, 126.29 (t, *J* = 3.2 Hz), 125.38 (d, *J* = 12.6 Hz), 123.91 (dd, *J* = 6.9, 4.6 Hz), 123.37, 115.65 (d, *J* = 17.1 Hz), 115.44, 115.03, 104.36, 55.36, 50.32, 39.10 (t, *J* = 1.8 Hz), 33.78, 19.76, 13.67. HRMS (*m/z*): [M+Na+CH_3CN] calcd for $C_{23}H_{24}F_2N_2NaO_2$, 421.1704; found 421.1687. HPLC t_R = 8.827 min; purity = 100.0%.

Membrane preparation.[27]

Mouse brain homogenates for *in vitro* assays were prepared as previously described.[27] Briefly, whole brains were harvested from B6SJL mice, snap-frozen in liquid nitrogen and stored at −80 °C. On the day membrane homogenates were to be prepared, brains were thawed on ice, then pooled in a 40 mL Dounce glass homogenizer and suspended in 5 volumes of ice-cold homogenization buffer (50mM HEPES, pH 7.4, 3 mM $MgCl_2$, and 1 mM EGTA). Brains were then subjected to 10 complete strokes with an A pestle, followed by centrifugation at 40,000 × g for 10 min at +4 °C. Resulting supernatants were discarded, and the pellet was resuspended, homogenized and centrifuged similarly twice more, with supernatants being discarded. For the final resuspension and homogenization with a B pestle, ice-cold 50 mM HEPES was used in place of homogenization buffer and homogenates were aliquoted and stored at −80 °C. Protein concentrations of homogenates were determined using the BCA™ Protein Assay (Thermo Scientific, Rockford, IL).

Competition receptor binding assay.[28]

Competition receptor binding was performed as previously described.[28] Briefly, 50 μg of mouse brain homogenates were incubated for 90 min to attain equilibrium binding at room temperature with 0.2 nM [3H]CP-55,940, 5 mM $MgCl_2$, and either increasing cannabinoid concentrations (0.1 nM to 10 μM), 10 μM WIN-55,212-2 (for non-specific binding) or vehicle (for total binding), in triplicate, in a volume of 1 mL of buffer containing 50 mM Tris, 0.05% bovine serum albumin (BSA) and 0.1% ethanol vehicle. Reactions were terminated by rapid vacuum filtration through Whatman GF/B glass fiber filters, followed by five washes with ice-cold buffer (50 mM Tris, 0.05% BSA). Filters were immediately

placed into 7 mL scintillation vials to which 4 mL of ScintiVerse™ BD Cocktail scintillation fluid (Fisher Scientific, Fair Lawn, NJ) was added. Bound radioactivity was determined after overnight incubation at room temperature and shaking, by liquid scintillation spectrophotometry with an efficiency of 44% (TriCarb 2100 TR Liquid Scintilation Analyzer, Packard Instrument Company, Meriden, CT). Specific binding is expressed as total binding minus non-specific binding, and is graphed for each data point as a percentage of specific binding occurring in the absence of any competitor.

Adenylyl cyclase assay.[28]

Four million Neuro2AWT or CHO-*h*CB2 cells were plated into a 24-well plate and allowed to attach overnight. At 80–90% confluency (the following morning), 0.5 mL of warm incubation media composed of DMEM with 0.9g/L NaCl, 2.5 μCi/mL [^{3}H]Adenine and 0.5 mM IBMX was added to the cells. After a 4-hour incubation period at 37°C in a 5% CO_2 incubator, the media was removed and the plate was briefly floated on an ice-water bath while 0.5 mL of an assay mix was quickly added to the cells in triplicate. The assay mix consisted of a Krebs Ringer HEPES buffered saline solution containing 0.5 mM IBMX, 10 μM forskolin and either vehicle (0.2% ethanol) or at least 6 concentrations (0.1 nM to 10 μM) of each test compound. The plate was then transferred to a 37°C water bath for a 15 minute incubation and the reaction was terminated by addition of 50 μL of 2.2 N HCl. Intracellular [^{3}H]cAMP was separated by column chromatography employing acidic alumina. Four mL of the final eluent was added to 10 mL of ScintiVerse™ BD Cocktail Scintillation Fluid (Fisher Scientific, Fair Lawn, NJ) and radioactivity was immediately measured employing liquid scintillation spectrophotometry (Tri Carb 2100 TR Liquid Scintillation Analyzer, Packard Instrument Company, Meriden, CT). Data are expressed as the percent of intracellular [^{3}H]cAMP relative to that observed in vehicle samples. In experiments where agonists and antagonists were co-incubated, antagonists were added 5 minutes prior to addition of agonists. Statistical comparison of mean IC_{50} and I_{MAX} values (± SEM) for test compounds was accomplished using one-way ANOVA followed by a Tukey's post-test.

Animal care and use

Prior to surgery (see below), male NIH Swiss mice (Harlan Sprague Dawley Inc., Indianapolis, IN), weighing approximately 25–30 g, were housed 3 animals per Plexiglass cage (15.24 × 25.40 × 12.70 cm) in a temperature-controlled room at the University of Arkansas for Medical Sciences, Little Rock, AR. Room conditions were maintained at an ambient temperature of 22 ± 2 °C at 45–50% humidity. Lights were set to a 12 h light/dark cycle. Animals were fed Lab Diet rodent chow (laboratory rodent Diet #5001, PMI Feeds, Inc., St. Louis, MO) and water *ad libitum* until immediately before testing. Animals were acclimated to the laboratory environment 2 days prior to experiments and were tested in groups of 4–8 mice per condition. All studies were carried out in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of Health. Experimental protocols were approved by the Animal care and Use Committee at the University of Arkansas for Medicinal Sciences.

Core temperature measurements

Following appropriate anesthetization with inhaled isoflurane, the abdominal area of each mouse was shaved and sanitized with iodine swabs. A rostral-caudal cut approximately 1.5 cm in length was made with skin scissors, providing access to the intraperitoneal cavity. A cylindrical glass encapsulated radiotelemetry probe (model ER-4000 E-Mitter, Mini Mitter, Bend, OR) was then inserted, and then incision was closed using absorbable 5-0 chromic gut

suture material. At least 7 days were imposed between surgery and experimental observation of drug effects to allow incision to heal and mice to recover normal body weights. Following surgery, implanted mice were individually housed in Plexiglass mouse cages (15.24 × 25.40 × 12.70 cm) for the duration of all temperature experiments. Implanted transmitters produced temperature-modulated signals that were transmitted to a receiver (model ER-4000 receiver, Mini Mitter Co., Inc) underneath each mouse cage. Receivers were housed in light- and sound attenuating cubicles (med Associates model ENV-022MD, St. Albans, VT) equipped with exhaust fans, which further masked ambient laboratory noise. On experimental days, mice were weighted, marked, and returned to their individual cages for at least 1 hr of baseline data collection. Cannabinoid doses were then calculated and drugs were prepared for injection. Animals were subsequently removed from their cage and injected with various doses of drug or an equivalent volume of vehicle. Temperature data were collected at regular 5-min intervals and processed simultaneously by the Vital View data acquisition system (Mini Mitter Co., Inc.) for at least 8 hrs.

Oral EtOH self-administration (two-bottle EtOH choice).[44]

The standard two-bottle EtOH choice protocol is a widely used oral self-administration model of ongoing EtOH drinking that captures aspects of voluntary alcohol consumption in humans. In our procedure, each home cage contained two 25 mL Pyrex glass bottles, capped with rubber stoppers fitted with stainless steel tips. One bottle contained water while the other bottle contained a 10% ethanol solution (diluted with tap water). Volumetric consumption data were recorded from both drinking bottles every Monday and Thursday at approximately the same time. After recording consumption data, bottles were emptied, cleaned, refilled to 25 ml, and switched to eliminate a position preference. All animals were given unrestricted food access (Purina rodent lab diet) during testing, and all mice had 24 h access to 10% (v/v) EtOH and water for the duration of the study. Drinking preference was assessed as the amount of EtOH consumed divided by total fluid consumed × 100. Baseline drinking was established and considered stable when EtOH drinking varied by <10% for three consecutive days. After baseline criterion drinking was achieved, mice were injected (i.p.) with 10 mg/kg of the CB1 antagonist rimonabant for 10 consecutive days. After a two week drug “washout” period, mice were injected with 10 mg/kg **27** or 10 mg/kg **30**. After 10 days of treatment with either **27** or **30** was conducted, mice were injected with the drug vehicle solution for 10 consecutive days. Solutions were prepared fresh weekly with 8%tween and 92% distilled water.

Conditioned place preference.[45]

Conditioned place preference (CPP) is one of the most popular models to study the motivational effects of drugs and non-drug treatments in experimental animals, and has been widely used to evaluate the appetitive effects of abused drugs in mice.[45] Our procedure for establishing CPP involved 3 distinct phases, using Panlab 3-compartment spatial discrimination chambers. Briefly, these chambers consist of a box with two equally-sized conditioning compartments connected by a rectangular corridor. The compartments are differentiated by visual pattern on the walls (dots vs. stripes), color (distinct shades of grey), floor texture (smooth grid vs. rough), and shape (square vs. multi-angled), providing the subjects with multiple contextual dimensions across sensory modalities with which to differentiate the distinct compartments. During the pre-conditioning preference test, mice were allowed to explore the chamber for 30 min while their behavior was recorded and scored. These tests occurred on Friday afternoons, and on the next two days mice were housed in the colony room and not used experimentally. During this time, mice were assigned to receive EtOH pairings (see below) on their non-preferred side, and saline in their preferred compartment. On Monday, the conditioning phase began with all mice receiving a saline injection, then being placed in their preferred compartment for 30 min. During saline

conditioning, the hallway was blocked so that mice could not leave the conditioning compartment. After the saline conditioning trial, mice were removed from the chamber and placed back in their home cage. Approximately 4 hours later, mice were injected with 2 g/kg EtOH, and then placed into their non-preferred compartment for 30 min. In this manner, mice were conditioned with both saline and drug each day, and these conditioning trials occurred Monday through Thursday. On Friday morning, the post-conditioning preference test was conducted in a manner identical to the preconditioning preference test. For studies involving CB antagonists, mice were injected with 10 mg/kg rimonabant, 10 mg/kg **27**, or 10mg/kg **30** one hour before each EtOH pairing. To calculate a preference (or aversion) score, the time spent in the drug-paired side during the pre-conditioning test is subtracted from the time spent in that compartment during the post-conditioning test.

Statistical analysis

Curve fitting and statistical analyses for *in vitro* experiments were performed using GraphPad Prism version 5.0b (GraphPad Software, Inc., San Diego, CA). The Cheng-Prusoff equation was used to convert the experimental IC_{50} values obtained from competition receptor binding experiments to *Ki* values, a quantitative measure of receptor binding.[29] Non-linear regression for one-site competition was used to determine the IC_{50} for competition binding. Data are expressed as mean ± SEM. A one-way ANOVA, followed by Tukey's Multiple Comparison post-hoc test, was used to determine statistical significance ($P<0.05$) between three or more groups. For core body temperature experiments, the area under the curve (AUC) was calculated using a trapezoidal rule from 0–10 hr. For temperature data, statistical significance ($P<0.05$) was determined using a one-way ANOVA, followed by Tukey's HSD post-hoc Test. All in vivo statistical calculations were performed using SigmaStat 3 (Systat Software, Inc., San Jose, CA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

AEA	anandamide
2-AG	2-arachidonoylglycerol
CB1R	cannabinoid 1 receptor
CB2R	cannabinoid 2 receptor
SR141716A	rimonabant
EtOH	ethanol
Δ^9-THC	Δ^9-tetrahydrocannabinol
AC	adenylyl cyclase
CHO-*h*CB2	Chinese hamster ovary cells expressing cannabinoid 2 receptors
SA	self-administration
CPP	conditioned place preference

EtOAc	ethyl acetate
TLC	thin-layer chromatography
MeOH	methanol
Ar	argon
DMEM	Dulbecco's modified Eagle's medium
IBMX	3-isobutyl-1-methylxanthine
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

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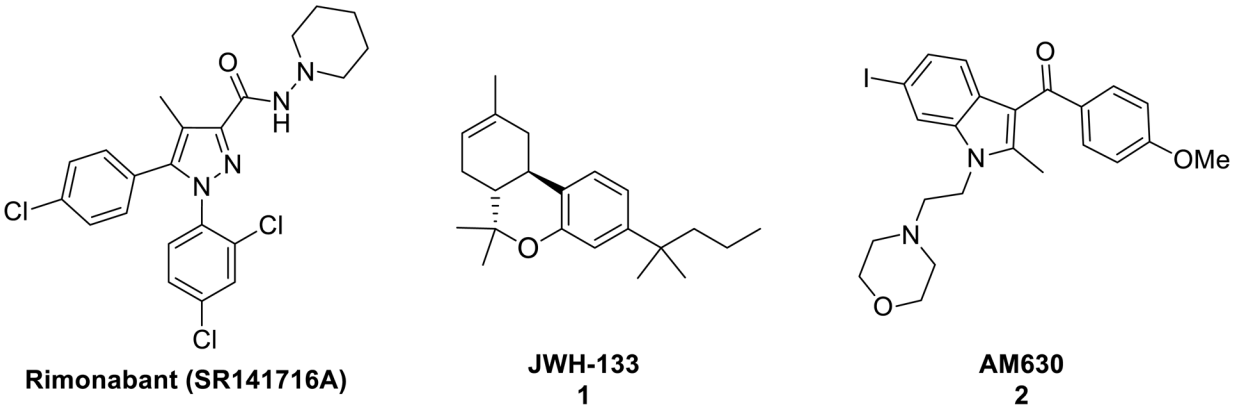


Figure 1.
Structures of rimonabant (CB1R antagonist/inverse agonist), **1** (CB2R agonist), and **2** (CB2R antagonist)

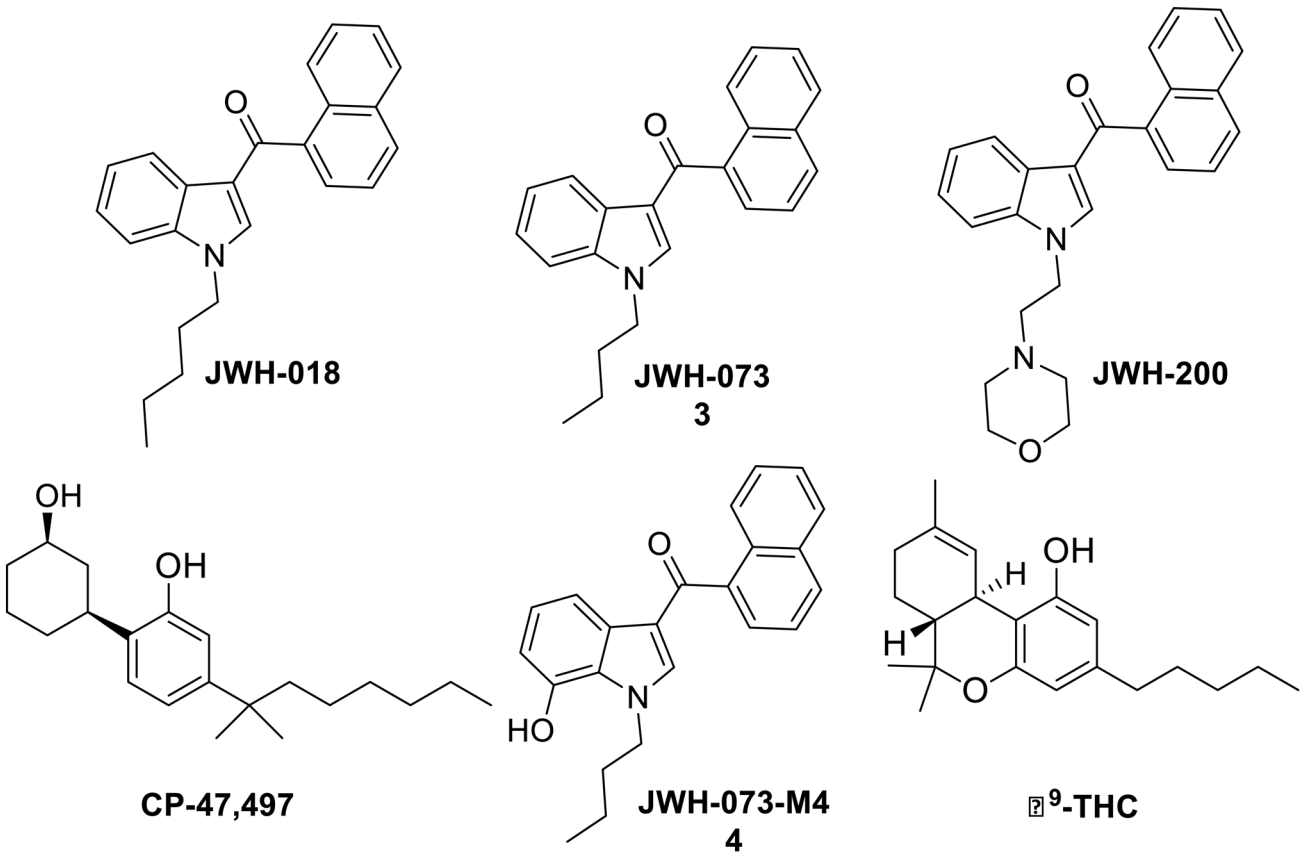


Figure 2.
Structures of common K2/Spice components and the metabolite of aminoalkylindole **3** (**4**) and the classical cannabinoid Δ^9-THC

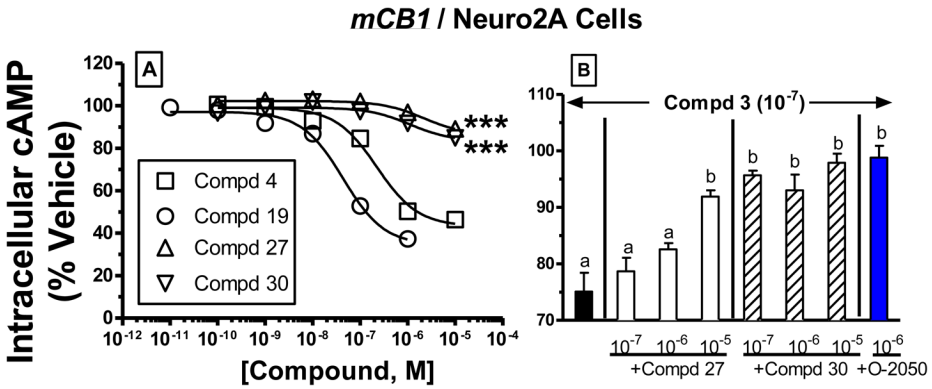


Figure 3.

Compounds **27** and **30** produce very little inhibition of AC-activity when tested alone at high concentrations and antagonize AC-inhibition produced by CB1R-agonist (black bar in panel B represents inhibition of AC-activation of 10^{-7} M concentration of **3**)

***Lead compounds selected for testing in animals

[a,b]Different letters signify statistical differences between groups

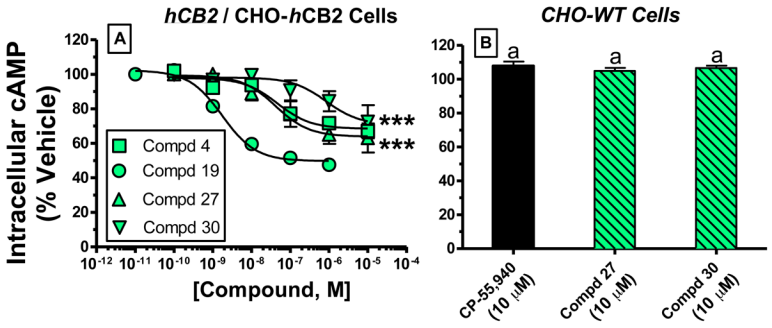


Figure 4.

Compounds **27** and **30** produce concentration-dependent inhibition of adenylyl cyclase activity by activation of CB2 receptors

***Lead compounds selected for testing in animals

[a]Letter signifies statistical differences between groups

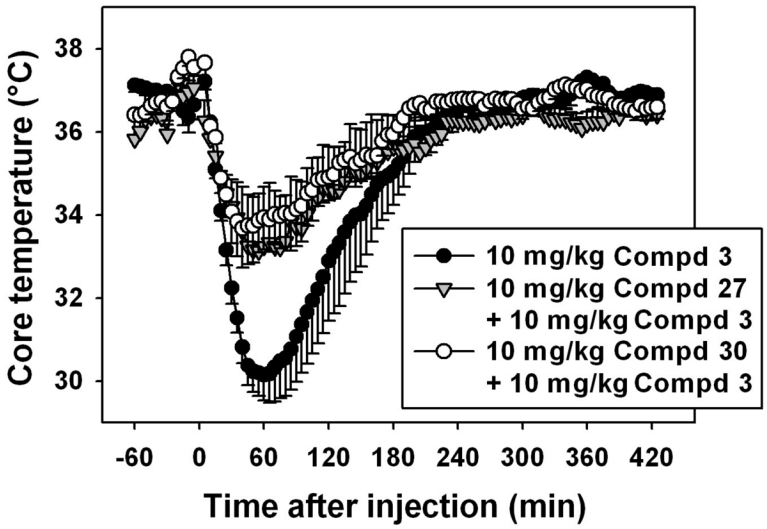


Figure 5.
Compounds **27** and **30** reduce agonist **3** induced hypothermic effects in rodents

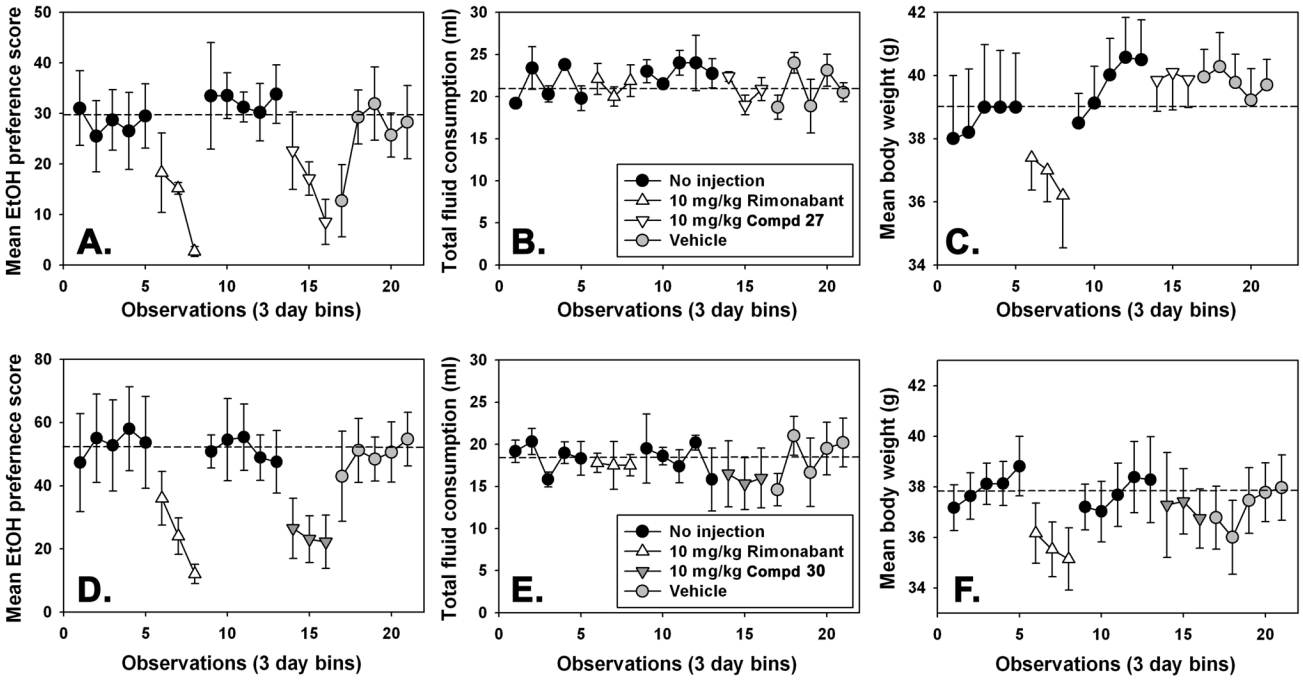


Figure 6.

Reduction of EtOH self-administration with daily administration of 10 mg/kg rimonabant or compounds **27** and **30**. Dotted lines represent group means from the 10 “no injection” control periods

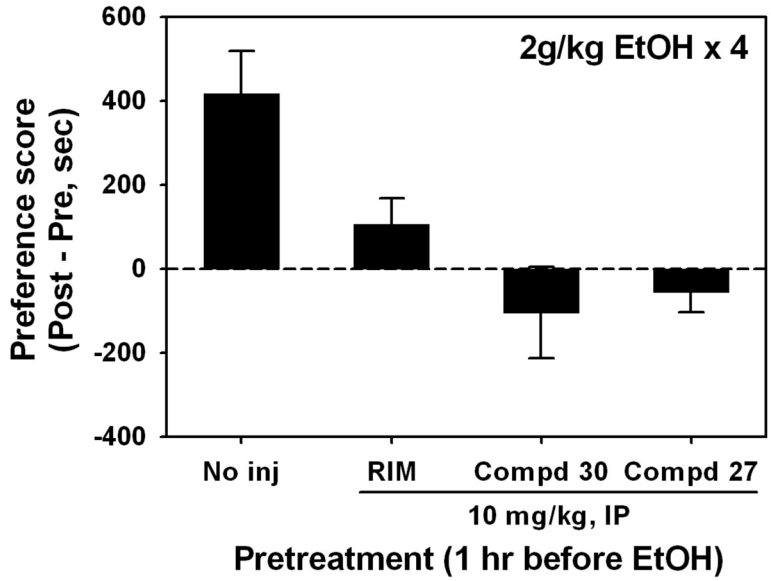
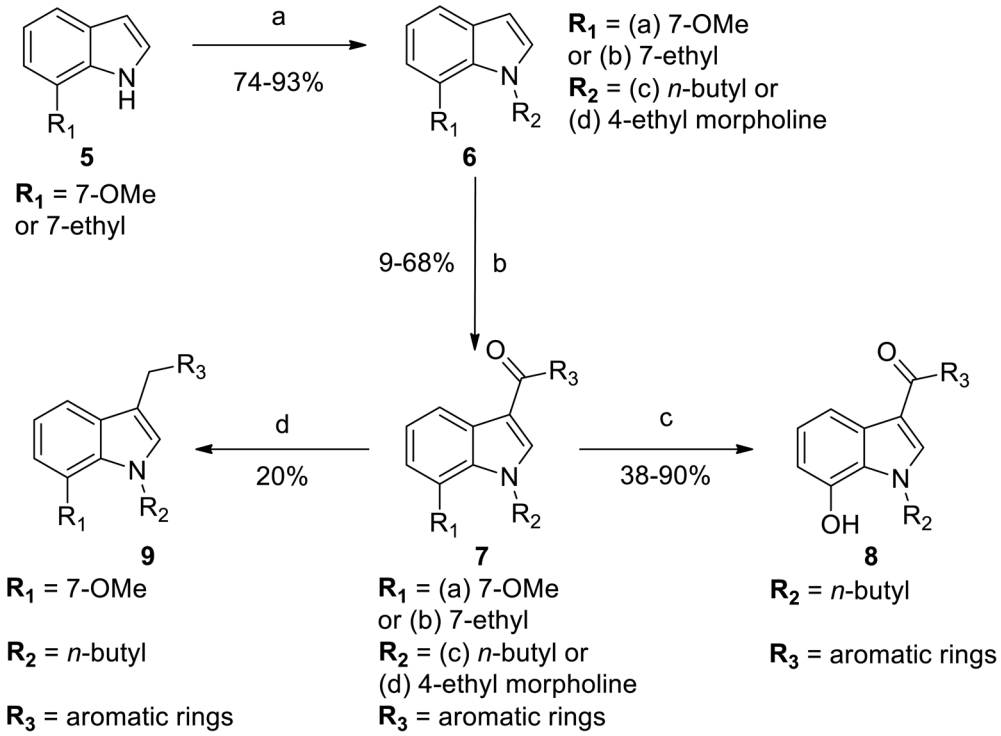


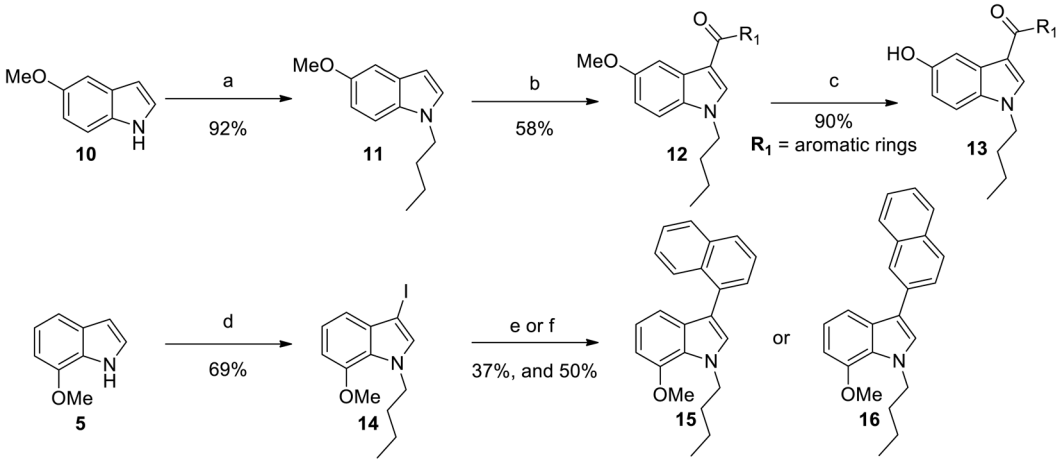
Figure 7.
Blockage of EtOH CPP with rimonabant and compounds **27** and **30**



Scheme 1.

Reagents and Conditions:

(a) 1-bromobutane or 4-(2-bromoethyl)morpholine, KOH, DMF, 50°C; (b) Me_2AlCl, RCOCl, DCM, 0°C; (c) BBr_3, DCM, −78°C; (d) $LiAlH_4$, $AlCl_3$, THF, 0°C



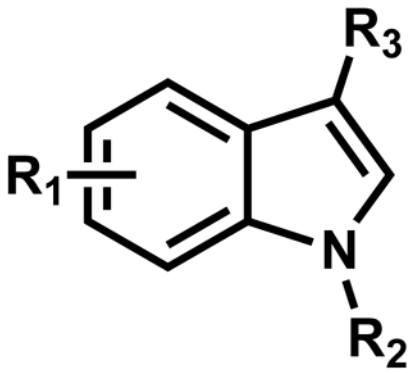
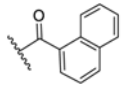
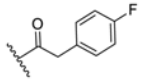
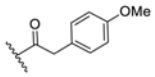
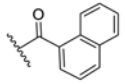
Scheme 2.

Reagents and Conditions:

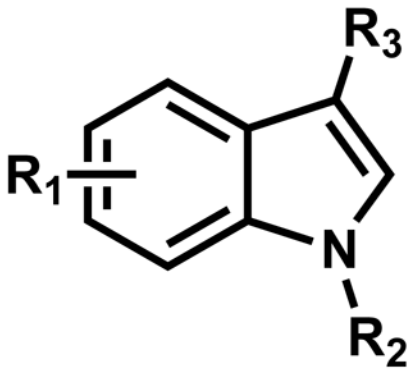
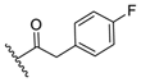
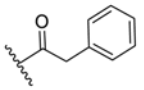
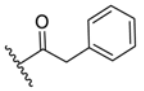
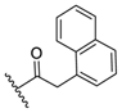
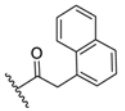
(a) 1-bromobutane, KOH, DMF, 50°C; (b) Me_2AlCl, RCOCl, DCM, 0°C; (c) BBr_3, DCM, −78°C; (d) 1-bromobutane, I_2, KOH, DMF, NaH, 50°C; (e) $Pd(PPh_3)_4$, 1-naphthaleneboronic acid, Na_2CO_3, DME, EtOH; (f) $Pd(OAc)_2$, SPhos, K_3PO_4, 2-naphthaleneboronic acid, toluene

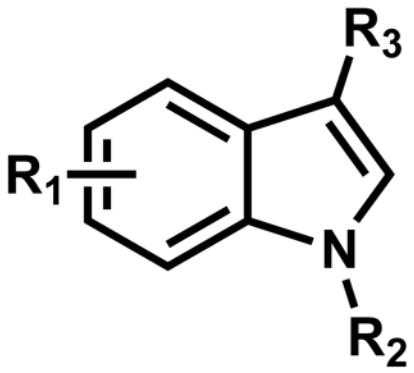
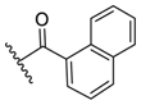
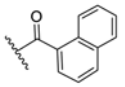
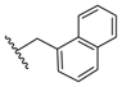
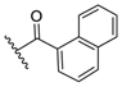
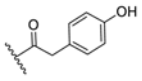
Table 1

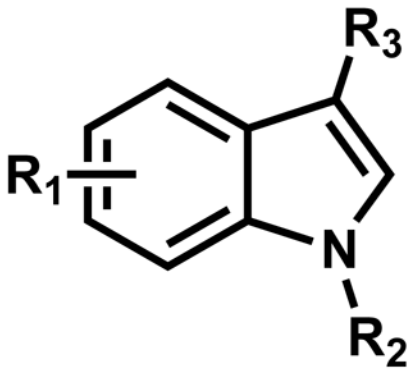
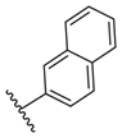
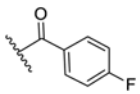
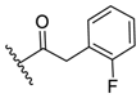
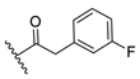
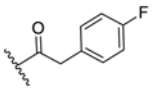
Structures of compounds synthesized and binding screen results for CBRs

General Structure				% displacement of [^{3}H]CP-55,940 at CB1Rs	% displacement of [^{3}H]CP-55,940 at CB2Rs	% vehicle inhibition of AC-activity at CB1Rs	% vehicle inhibition of AC-activity at CB2Rs
Compd	**R_1**	**R_2**	**R_3**	***1 μM concentration of compounds used***		***10 μM concentration of compounds used***	
CP55,940	**Not applicable**			ND[a]	ND[a]	45	37
4	7-OH	*n*-butyl		99	90	55	38
17	7-OMe	*n*-butyl		55	80	−4	22
18	7-OMe	*n*-butyl		38	64	ND[a]	ND[a]
19	7-OMe	*n*-butyl		93	100	51	52

J Med Chem. Author manuscript; available in PMC 2014 June 13.

General Structure				% displacement of [^{3}H]CP-55,940 at CB1Rs	% displacement of [^{3}H]CP-55,940 at CB2Rs	% vehicle inhibition of AC-activity at CB1Rs	% vehicle inhibition of AC-activity at CB2Rs
Compd	**R_1**	**R_2**	**R_3**	***1 μM concentration of compounds used***		***10 μM concentration of compounds used***	
20	7-OH	*n*-butyl		29	10	ND[a]	ND[a]
21	7-OMe	*n*-butyl		69	91	34	16
22	7-OH	*n*-butyl		21	20	ND[a]	ND[a]
23	7-OMe	*n*-butyl		81	91	34	−40
24	7-OH	*n*-butyl		37	21	ND[a]	ND[a]

General Structure				% displacement of [^{3}H]CP-55,940 at CB1Rs	% displacement of [^{3}H]CP-55,940 at CB2Rs	% vehicle inhibition of AC-activity at CB1Rs	% vehicle inhibition of AC-activity at CB2Rs
Compd	**R_1**	**R_2**	**R_3**	***1 μM concentration of compounds used***		***10 μM concentration of compounds used***	
25	5-OMe	*n*-butyl		90	90	47	31
26	5-OH	*n*-butyl		97	91	39	37
27	7-OMe	*n*-butyl		90	97	18	33
28	7-OMe	4-ethyl morpholine		100	100	39	37
29	7-OH	*n*-butyl		10	10	ND[a]	ND[a]

General Structure				% displacement of [^{3}H]CP-55,940 at CB1Rs	% displacement of [^{3}H]CP-55,940 at CB2Rs	% vehicle inhibition of AC-activity at CB1Rs	% vehicle inhibition of AC-activity at CB2Rs
Compd	**R_1**	**R_2**	**R_3**	***1 μM concentration of compounds used***		***10 μM concentration of compounds used***	
30	7-OMe	*n*-butyl		82	91	16	21
31	7-OMe	*n*-butyl		84	99	45	38
32	7-OMe	*n*-butyl		84	99	45	1
33	7-OMe	*n*-butyl		63	91	ND[a]	ND[a]
34	7-Ethyl	*n*-butyl		63	88	ND[a]	ND[a]

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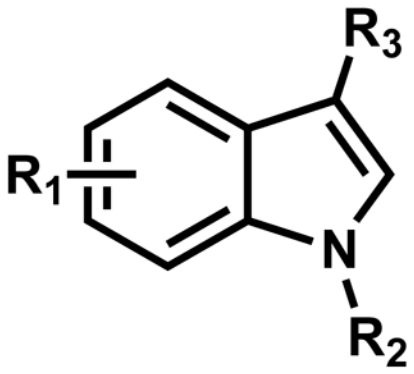
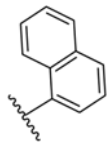
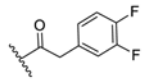
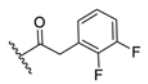
General Structure				% displacement of [^{3}H]CP-55,940 at CB1Rs	% displacement of [^{3}H]CP-55,940 at CB2Rs	% vehicle inhibition of AC-activity at CB1Rs	% vehicle inhibition of AC-activity at CB2Rs
Compd	**R_1**	**R_2**	**R_3**	***1 μM concentration of compounds used***		***10 μM concentration of compounds used***	
35	7-OMe	*n*-butyl		98	97	39	37
36	7-OMe	*n*-butyl		41	80	ND[a]	ND[a]
37	7-OMe	*n*-butyl		80	97	35	−6

[a]ND = not determined

Table 2

Affinity of selected compounds for mouse CB1 and human CB2 receptors

Compound	*m*CB1R K_i ± SEM, nM	*h*CB2R K_i ± SEM, nM
3 (JWH-073)	12.9 ± 3.4	9.8 ± 0.9
4 (JWH-073-M4)	24.2 ± 17.2	78.3 ± 36.2
17	387 ± 77.0	281 ± 51.0
19	1.7 ± 0.3	0.81 ± 0.4
27	15.4 ± 2.2	10.9 ± 3.1
30	37.3 ± 11.8	26.5 ± 1.5