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The Synthesis and Pharmacological Evaluation of Adamantane-Derived Indoles: Cannabimimetic Drugs of Abuse

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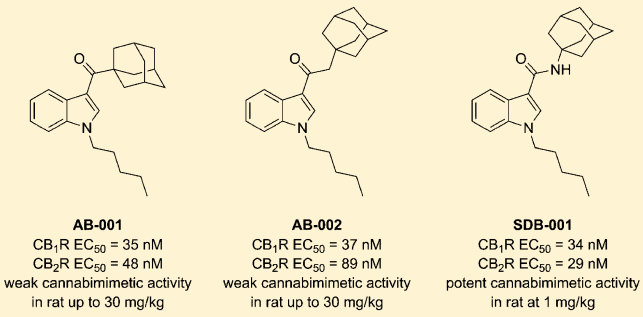
Supporting Information

ABSTRACT: Two novel adamantane derivatives, adamantan-1-yl(1-pentyl-1*H*-indol-3-yl)methanone (AB-001) and *N*-(adamtan-1-yl)-1-pentyl-1*H*-indole-3-carboxamide (SDB-001), were recently identified as cannabimimetic indoles of abuse. Conflicting anecdotal reports of the psychoactivity of AB-001 in humans, and a complete dearth of information about the bioactivity of SDB-001, prompted the preparation of AB-001, SDB-001, and several analogues intended to explore preliminary structure−activity relationships within this class. This study sought to elucidate which structural features of AB-001, SDB-001, and their analogues govern the cannabimimetic potency of these chemotypes in vitro and in vivo. All compounds showed similar full agonist profiles at CB_1 (EC_{50} = 16−43 nM) and CB_2 (EC_{50} = 29−216 nM) receptors in vitro using a FLIPR membrane potential assay, with the exception of SDB-002, which demonstrated partial agonist activity at CB_2 receptors. The activity of AB-001, AB-002, and SDB-001 in rats was compared to that of Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabimimetic indole JWH-018 using biotelemetry. SDB-001 dose-dependently induced hypothermia and reduced heart rate (maximal dose 10 mg/kg) with potency comparable to that of Δ^9-tetrahydrocannabinol (Δ^9-THC, maximal dose 10 mg/kg), and lower than that of JWH-018 (maximal dose 3 mg/kg). Additionally, the changes in body temperature and heart rate affected by SDB-001 are of longer duration than those of Δ^9-THC or JWH-018, suggesting a different pharmacokinetic profile. In contrast, AB-001, and its homologue, AB-002, did not produce significant hypothermic and bradycardic effects, even at relatively higher doses (up to 30 mg/kg), indicating greatly reduced potency compared to Δ^9-THC, JWH-018, and SDB-001.



KEYWORDS: *indole, AB-001, Δ^9-tetrahydrocannabinol, cannabinoid receptor, drug abuse*

Cannabinoid (CB) receptors are G protein-coupled receptors (GPCRs) comprising two subtypes; CB_1 and CB_2.[1,2] CB_1 receptors are primarily found in the central nervous system (CNS), although they are also expressed to a lesser extent in some peripheral organs. CB_2 receptors occur mainly in T cells of the immune system, macrophages and B cells, and in hematopoietic cells. Activation of CB receptors is achieved endogenously in mammals by endocannabinoids such as anandamide and 2-arachidonoylglycerol. However, a myriad of exogenous compounds are known to act as cannabinoid agonists, including both natural products and synthetic chemicals.

The most prominent naturally occurring CB ligand is Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**, Figure 1),[3] the principal bioactive component of marijuana, which acts as a partial agonist at CB_1 and CB_2 receptors.[4] The abuse liability of the psychoactive resin, flowers, and leaves of *Cannabis sativa*, collectively termed marijuana, has resulted in near-universal proscription of the plant. Despite this, The United Nations

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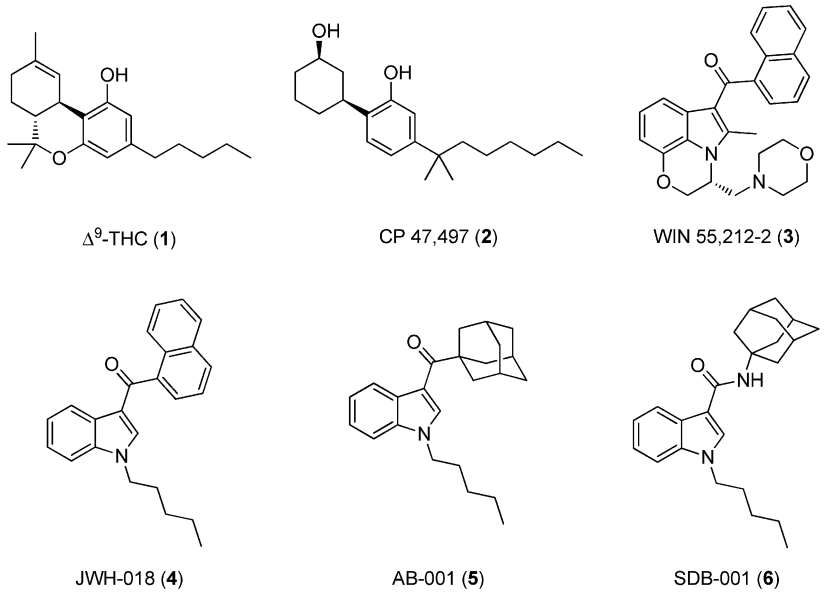


Figure 1. Selected natural and synthetic cannabimimetics.

Office on Drugs and Crime (UNODC) estimated that between 125 and 203 million people used cannabis at least once in 2009, making it the most widely produced and consumed illicit substance worldwide.[5]

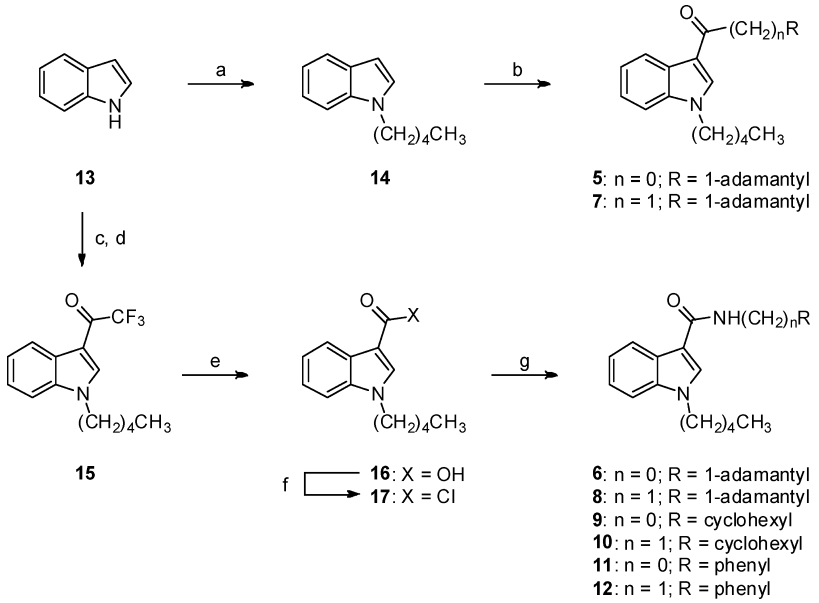
Human use of marijuana for its psychoactive and medicinal properties has been recorded since antiquity,[6] and the diversity of cannabinoid pharmacology has driven interest in CB receptors as therapeutic targets for the potential treatment of numerous diseases, including cancer, obesity, inflammation, and pain.[7−14] Most early synthetic cannabimimetics were simplified analogues of Δ^9-THC based on the cyclohexylphenol core, such as CP 47,497 (**2**), disclosed by Pfizer in 1982.[15] However, in 1992, Sterling Research Group reported WIN 55,212-2 (**3**) as the first member of a structurally novel aminoalkylindole (AAI) class of cannabimimetics.[16] Since this time, many simplified AAIs with CB receptor activity have been described. Huffman and colleagues have extensively explored the structure−activity relationships (SARs) of cannabinergic indoles such as representative member JWH-018 (**4**), resulting in a plethora of analogues.[17−19]

Herbal blends containing synthetic cannabimimetic additives have been available in many countries from retailers and Internet-based vendors since about 2004.[20−23] Although these products are often declared as incense, they are intended for human consumption as unscheduled, and therefore legal, alternatives to marijuana. One of the earliest such products, "Spice", was found in 2008 to contain two synthetic cannabinoids, the octyl homologue of CP 47,497 and JWH-018, consistent with anecdotal reports of cannabis-like effects when the material was smoked.[20] Although Δ^9-THC acts as a partial agonist at CB_1 receptors, many synthetic cannabinoids, including JWH-018, act as full agonists at CB_1 sites.[4] Differences in the CB_1 receptor efficacy of synthetic cannabimimetics, as well as unidentified off-target activity, may explain the cardiotoxicity and seizures sometimes observed following the ingestion of synthetic cannabinoids,[24−30] an outcome not generally observed with the use of marijuana or synthetic Δ^9-THC.[31] Psychotic episodes with paranoia, delusion, and agitation necessitating emergency ward admission have been reported following synthetic cannabinoid intoxication,[26,30,32] and it is likely that synthetic cannabinoids can precipitate psychoses in vulnerable individuals.[33−35] As of August 2010, more than 1000 cases of synthetic cannabinoid-induced toxicity had been reported across the United States.[36] The toxicological assessment of synthetic cannabinoids requires the development of methodologies for the identification of metabolites of clinical or forensic relevance, and there is a paucity of pertinent literature for the most recent emergent synthetic cannabimimetics.[37] In the case of JWH-018, several hydroxylated potential metabolites retained potent agonist activity at CB_1 receptors, while a major glucuronidated metabolite showed neutral antagonist activity at the same receptors.[38,39] JWH-073, an analogue of JWH-018, also produces metabolites with similarly diverse functional activities,[40] further confounding the attribution of adverse effects to specific chemical entities within herbal blends.

There is understandable concern by health agencies worldwide about the adverse effects of synthetic cannabinoids and other "synthetic legal intoxicating drugs" (SLIDs) with no history of human use, and, in many cases, little pharmacological evaluation of any kind.[41] Indeed, around 2008, many countries moved to place JWH-018 in the most restrictive regulatory categories; however, manufacturers simply circumvented these control measures by substituting this component with unscheduled congeneric compounds possessing similar effects.[42] Delay in the conclusive identification and pharmacological characterization of emerging synthetic cannabinoids presents a potential danger to the intended consumers, and the preemptive identification and thorough assessment of novel "designer drugs" remains an important aspect of proactive public health policy. The implementation of appropriate legal restriction of synthetic cannabinoids is hampered by a scarcity of reference materials and analytical data, a problem which is compounded by the sheer rate of emergence of such compounds.[21]

Dozens of indole cannabinoids have been identified as components of Spice-like products from around the world, and in some cases the structures were previously unprecedented in the scientific literature.[43−57] In 2010, adamantan-1-yl(1-pentyl-1*H*-indol-3-yl)methanone (**5**, AB-001) was identified in

Scheme 1. Synthesis of Cannabimimetic Indoles 5−12[a]



[a]Reagents and conditions: (a) NaH, $Br(CH_2)_4CH_3$, DMF, 0 °C−rt, 1 h, 80%; (b) Me_2AlCl, $R(CH_2)_nC(O)Cl$, CH_2Cl_2, 0 °C, 2 h, 35−86%; (c) NaH (2 equiv), $Br(CH_2)_4CH_3$, DMF, 0 °C−rt, 1 h; (d) $(CF_3CO)_2O$, DMF, 0 °C−rt, 1 h; (e) KOH, MeOH, PhMe, reflux, 2 h, 75% over 3 steps; (f) $(COCl)_2$, DMF (cat.), CH_2Cl_2, rt, 1 h, quant; (g) $R(CH_2)_n NH_2$, Et_3N, CH_2Cl_2, rt, 14 h, 73−86%.

products originating from Ireland, and subsequently reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) via the Early Warning System (EWS).[58] AB-001 was contemporaneously identified in bulk powders seized by the Hungary Customs and Finance Guard, and spectroscopic characterization and human metabolites were reported, although no synthetic details or pharmacological evaluation of this compound exist in the scientific literature.[53,59,60] In 2012, *N*-(adamtan-1-yl)-1-pentyl-1*H*-indole-3-carboxamide (**6**, SDB-001), was detected in products obtained by the National Institute of Health Sciences in Japan and, like AB-001, no synthetic preparation or pharmacological assessment of this compound has been published to date.[61,62]

Despite their novel structures, AB-001 and SDB-001 appear to exploit SARs previously described for the class of cannabimimetic indoles, and are likely products of clandestine rational drug design. Systematic exploration of JWH-018 revealed that a saturated four to six carbon chain at the 1-indole position was optimal for CB_1 binding and in vivo activity in rats.[18,19] Additionally, the naphthalene group of JWH-018 is not mandatory for CB_1 binding, and many 1-alkylindoles containing alicyclic 3-acyl groups have been reported as cannabinoid ligands, including adamantane derivatives.[63] Indeed, analogues containing the *N*-(adamtan-1-yl)-1-alkyl-1*H*-indole-3-carboxamide core of SDB-001 are listed as prophetic structures in several patents by Makriyannis and colleagues, although SDB-001 itself is not described.[64−66] Pasquini and coworkers recently prepared several 5-arylated *N*-(adamtan-1-yl)-1-pentyl-1*H*-indole-3-carboxamides, however, these compounds generally functioned as selective CB_2 receptor inverse agonists rather than CB_1 receptor agonists.[67,68]

The aim of the current work was to prepare and characterize AB-001, SDB-001, and several structurally related analogues, and to provide preliminary evaluation of their pharmacological profiles in vitro and in vivo. All synthesized compounds were screened against CB_1 and CB_2 receptors in a FLIPR membrane potential assay to provide basic SARs for selective agonist activity at CB_1 receptors. Additionally, selected compounds were compared to Δ^9-THC and JWH-018 in vivo, prompted by inconsistent reports of the psychoactivity of AB-001 in humans,[58] and a complete lack of information about the psychotropic activity of SDB-001 in any animal.

RESULTS AND DISCUSSION

In order to evaluate the cannabimimetic activity of AB-001, SDB-001, and structurally related analogues **7**−**12**, a synthetic route to each class of indole was developed (Scheme 1). Indole (**13**) was treated with sodium hydride and alkylated with bromopentane, affording *N*-pentylindole (**14**). *N*-pentylindole was acylated according to the method of Okauchi and coworkers[69] by treatment with dimethylaluminum chloride, followed by 1-adamantanoyl chloride, to give AB-001 in good yield. Subjecting *N*-pentylindole to the same procedure with (1-adamatane)acetyl chloride gave homologous AB-002 (**7**), which contains an indole-adamantane distance approximating that of SDB-001.

The synthesis of SDB-001 was achieved by alkylating indole as described above, followed by treatment with trifluoroacetic anhydride in one-pot procedure, to give *N*-pentyl-3-trifluoroacetylindole (**15**). Alkaline hydrolysis of **15** induced fluoroform elimination[70] to yield, upon acidic workup and recrystallization from isopropanol, *N*-pentylindole-3-carboxylic acid (**16**) of analytical purity. The three-step procedure proved operationally convenient and common precursor **16** could be obtained on multigram scale without chromatography. An alternative synthesis of **16** involving esterification of indole-3-carboxylic acid, *N*-alkylation, and ester hydrolysis proceeded in lower yield, with requisite chromatographic purification at each step making this route less attractive. Treatment of **16** with oxalyl chloride generated the corresponding acid chloride (**17**), which was reacted with the appropriate amine to give **6** and **8**−**12** in 73−86% unoptimized yield following recrystallization. To

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explore preliminary SARs for the *N*-pentylindole-3-carboxamide scaffold at CB receptors the synthesis of **9**−**12**, containing variously spaced alicyclic or aromatic groups in place of the bulky, polycyclic adamantane moiety was undertaken. It should be noted that **11** was generated solely to probe SARs within this series, and is likely toxic to living systems due to potential enzymatic amidolysis to aniline metabolites.

Indole-3-carboxamides **6** and **8**−**12** were easily recrystallized to analytical purity from isopropanol-water; however, attempts to grow suitable crystals for X-ray diffraction were uniformly unsuccessful. By contrast, AB-001 produced large prismatic crystals by slow-evaporation from isopropanol-water and an X-ray crystal structure was obtained (Figure 2). All bond lengths and angles were as expected, with full details of X-ray data collection and tables of bond lengths and angles available in the Supporting Information.

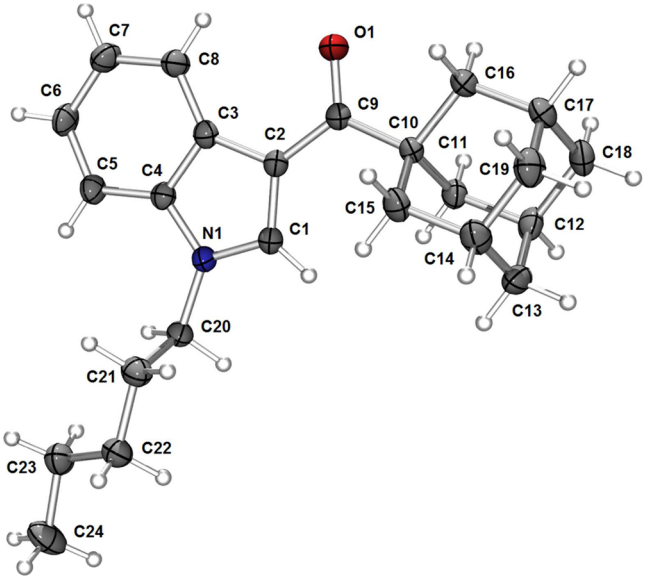

Figure 2. ORTEP diagram of the crystal structure of **5** with thermal ellipsoids at the 50% probability level.

The cannabimimetic activity of indoles **5**−**12** at CB_1 and CB_2 receptors was compared to exogenous agonist Δ^9-THC, and the results are shown in Table 1. Mouse AtT20 neuroblastoma cells were stably transfected with either rat CB_1 or human CB_2 receptors, and agonist activities of Δ^9-THC and **5**−**12** were assessed using a fluorometric imaging plate reader (FLIPR) membrane potential assay.[71] AtT20 cells endogenously express G protein-gated inwardly rectifying K^+ channels (GIRK), and these are activated by coexpressed CB_1 and CB_2 receptors (vide infra).[72] The maximum effect of the novel compounds was compared with that of the nonselective CB receptor agonist WIN 55,212-2, which produced a maximal decrease in fluorescence, corresponding to cellular hyperpolarization, of 35 ± 2% in AtT20-CB_1 cells and 28 ± 2% in AtT20-CB_2 cells.

All novel indoles had measurable agonist activity at CB_1 and CB_2 receptors, with EC_{50} values for CB_1 receptor-mediated activation of GIRK generally comparable to Δ^9-THC. Interestingly, in AtT20-CB2 cells, Δ^9-THC had low efficacy, with a maximum effect at 10 μM (13% of that of WIN55212−2) that precluded calculation of an EC_{50}. All of the novel indoles had a greater efficacy than Δ^9-THC, with SDB-002 being the least efficacious of the series. AB-001 (CB_1 EC_{50} = 35 nM, CB_2 EC_{50} = 48 nM) showed a remarkably similar profile to SDB-001 (CB_1 EC_{50} = 34 nM, CB_2 EC_{50} = 29 nM). The respective homologues of AB-001 and SDB-001, AB-002 (CB_1 EC_{50} = 37 nM, CB_2 EC_{50} = 89 nM), and SDB-002 (CB_1 EC_{50} = 43 nM, CB_2 EC_{50} = 57 nM) had similar potencies at each receptor. However, at CB_2 receptors, SDB-002 had a maximal effect about a third of AB-002, indicating a partial agonist profile for the former. None of the compounds affected membrane potential in wildtype AtT-20 cells.

The hyperpolarization of CB_1 receptors induced by AB-001, AB-002, SDB-001, and SDB-002 is shown in Figure 3A. All four ligands act as full functional agonists at CB_1 receptors, with similar potency to the classical agonist WIN 55,212−2, whereas Δ^9-THC is a partial agonist at CB_1 receptors. The hyperpolarization of CB_2 receptors induced by AB-001, AB-002, and SDB-001, is also of similar potency to WIN 55,212−2, as shown in Figure 3B. However, SDB-002 was a partial agonist at CB2 receptors in this assay, with an apparently greater potency than Δ^9-THC.

Taken together, these profiles indicate that a bulky adamantane substituent at a distance of two to four bond lengths from the 3-indole position is tolerated by both CB receptor subtypes. Additionally, the nature of the linker appears to be unimportant for activation of CB_1 receptors, with amide and ketone linkers equally well tolerated. There was little effect on CB_1 activity when the steric bulk of the adamantane cage of SDB-001 was reduced to a cyclohexane ring (SDB-003, CB_1

Table 1. Functional Activity of Δ^9-THC, WIN 55,212-2, and 5−12 at Rat CB_1 and Human CB_2 Receptors

	rCB$_1$		hCB$_2$		
compd	pEC$_{50}$ ± SEM (EC$_{50}$, nM)	max ± SEM (% WIN 55,212-2)	pEC$_{50}$ ± SEM (EC$_{50}$, nM)	max ± SEM (% WIN 55,212-2)	CB$_1$ selectivity[a]
1 (Δ^9-THC)	7.24 ± 0.12 (58)	78 ± 5	n.d.	13 (at 10 μM)	
3 (WIN 55,212−2)	7.57 ± 0.14 (27)		6.93 ± 0.1 (117)		4.3
5 (AB-001)	7.46 ± 0.16 (35)	105 ± 8	7.32 ± 0.12 (48)	86 ± 6	1.4
6 (SDB-001)	7.47 ± 0.12 (34)	98 ± 6	7.54 ± 0.11 (29)	91 ± 5	0.9
7 (AB-002)	7.43 ± 0.13 (37)	90 ± 6	7.05 ± 0.13 (89)	82 ± 7	2.4
8 (SDB-002)	7.37 ± 0.12 (43)	84 ± 6	7.24 ± 0.26 (57)	23 ± 4	1.3
9 (SDB-003)	7.43 ± 0.16 (37)	93 ± 7	6.99 ± 0.08 (102)	95 ± 5	2.7
10 (SDB-004)	7.79 ± 0.19 (16)	94 ± 8	6.67 ± 0.09 (216)	71 ± 5	13.1
11 (SDB-005)	7.68 ± 0.11 (21)	99 ± 6	6.86 ± 0.12 (140)	74 ± 6	6.7
12 (SDB-006)	7.73 ± 0.11 (19)	85 ± 5	6.88 ± 0.22 (134)	68 ± 9	7.2

[a]CB_1 selectivity expressed as the ratio of CB_2 EC_{50} to CB_1 EC_{50}. n.d. = not determined.

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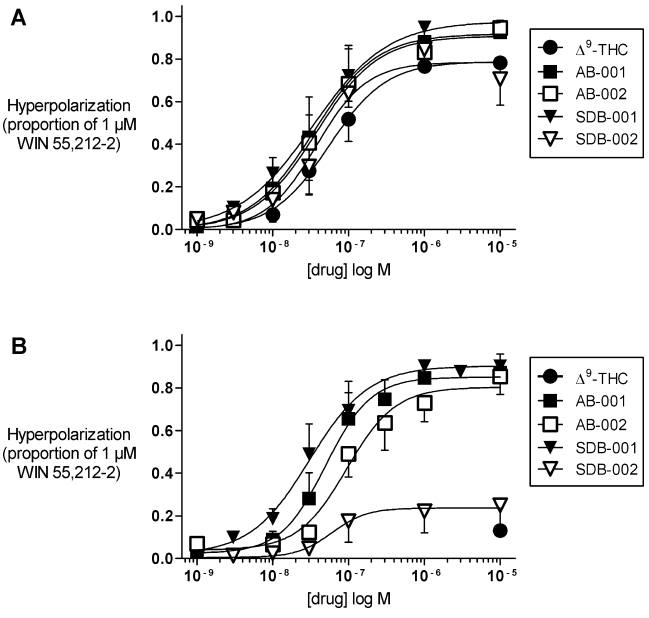

Figure 3. Hyperpolarization of (A) CB_1 and (B) CB_2 receptors induced by Δ^9-THC, AB-001, AB-002, SDB-001, and SDB-002 as a proportion of that produced by 1 μM of WIN 55,212-2. Membrane potential was measured using a fluorescent dye, as outlined in the Methods. Each point represents the mean ± SEM of at least three independent determinations, each performed in triplicate. Data was fitted with a 4 parameter logistic equation in Graphpad Prism.

EC_{50} = 37 nM), but CB_2 activity was reduced approximately 3-fold (CB_2 EC_{50} = 102 nM). The same structural simplification of SDB-002 gave homologue SDB-004, which showed roughly double the potency at CB_1 and similarly reduced CB_2 potency (CB_1 EC_{50} = 16 nM, CB_2 EC_{50} = 216 nM). Replacement of the cyclohexane ring of SDB-003 and SDB-004 with a phenyl ring gave aniline derivative SDB-005 (CB_1 EC_{50} = 21 nM, CB_2 EC_{50} = 140 nM) and benzylic analogue SDB-006 (CB_1 EC_{50} = 19 nM, CB_2 EC_{50} = 134 nM) respectively, which showed a similar preference for CB_1 receptors.

Due to conflicting anecdotal reports regarding the psychoactivity of AB-001, and a complete lack of information regarding the biological activity of SDB-001, several indoles from the current series were assessed using radiotelemetry in male Wistar rats to confirm in vivo cannabimimetic activity. The well-established ability of cannabinoids to induce hypothermia and decrease heart rate in rats is common to both classical cannabinoids like Δ^9-THC as well as synthetic cannabimimetics.[73−78] AB-002 was also included in these studies since it contains the ketone linker of AB-001 but the indole-adamantane spacing of SDB-001, representing a molecular hybrid of the two structures. It was theorized that differences in the behavioral pharmacology of AB-001, AB-002, and SDB-001 would allow delineation of the relative contributions of the indole-adamantane distance versus the nature of the linker separating these groups. For comparison, Δ^9-THC and the well-characterized synthetic cannabinoid JWH-018 were also assessed in biotelemetry assays.

The effects of various doses of Δ^9-THC, JWH-018, SDB-001, AB-001, and AB-002 on body temperature are shown in Figure 4. Results for 1 h prior to intraperitoneal (i.p.) injection and 5 h postinjection are presented in 15 min bins. The gray vertical bar on the figures (immediately prior to TIME = 0) represents the time of drug injection. Dose-dependent hypothermia was obtained with Δ^9-THC, JWH-018, and SDB-001 with statistically significant treatment or treatment by time effects of all 3 doses of each drug (ANOVA, planned contrasts, $P < 0.05$). A maximum decrease in body temperature was evoked by 10 mg/kg Δ^9-THC (~2 °C) within 30 min of injection. JWH-018 achieved a similar magnitude of hypothermia at 3 mg/kg, indicating the relatively greater potency of this compound in rats. The novel indole-3-carboxamide SDB-001 caused a slightly shallower hypothermia (1.6 °C at a dose of 10 mg/kg),. However, all doses of SDB-001 (1, 3, and 10 mg/kg) were similarly effective and appeared longer lasting than Δ^9-THC or JWH-018, suggesting a different pharmacokinetic profile for this compound relative to JWH-018. Although the Δ^9-THC and JWH-018 treatment groups generally returned to the same body temperature as the vehicle group within 5 h postadministration, rats treated with SDB-001 remained hypothermic for 7−11 h, depending on dose, following drug injection (data not shown). AB-001 did not produce significant hypothermia at a dose of 3 mg/kg, in contrast to the clear reduction in body temperature produced by an equivalent dose of Δ^9-THC, JWH-018, and SDB-001. Further evaluation of AB-001 at doses of 10 mg/kg and 30 mg/kg showed only modest hypothermic trends, indicating that AB-001 possesses reduced cannabimimetic activity compared to Δ^9-THC, JWH-018, and SDB-001. Similarly, AB-002 showed only trends toward hypothermia at doses of 3 mg/kg and 30 mg/kg. Taken together, these findings suggest that the nature of indole-adamantane tether, rather than the distance between these two groups, is important for in vivo activity; the carboxamide group confers cannabimimetic activity while the ketone does not.

Figure 5 shows the effects of Δ^9-THC, JWH-018, SDB-001, AB-001, and AB-002 on heart rate. As with the body temperature data, results for a baseline 1 h prior to i.p. injection and for 5 h postdrug are presented in 15 min bins in this figure. Dose-dependent decreases in heart rate were obtained with Δ^9-THC, JWH-018, and SDB-001, with statistically significant treatment or treatment by time effects of most doses tested (ANOVA, planned contrasts, $P < 0.05$). Maximal effects were obtained with 10 mg/kg Δ^9-THC and 3 mg/kg JWH-018, with intermediate effects seen with the lower doses of these two compounds. As with body temperature results, SDB-001 produced a slightly shallower, although significant, effect on heart rate that did not differ greatly between the 1, 3, and 10 mg/kg doses assessed. At doses up to 30 mg/kg, AB-001 and AB-002 produced only modest, and relatively short-lived, effects on heart rate compared to lower doses Δ^9-THC, JWH-018, and SDB-001.

Decreases in heart rate occur with cannabinoids as a result of inhibition of ongoing locomotor activity, and possibly also as a result of direct cardiac effects of these drugs. Accordingly, pronounced inhibition of ongoing locomotor activity was observed in the home cages with all three doses of Δ^9-THC and JWH-018, and SDB-001, while AB-001 and AB-002 produced only modest and nonsignificant effects even at the highest doses tested (data not shown).

■ CONCLUSION

This study represents the first attempt to characterize and quantify the activity of two novel synthetic cannabimimetics of abuse, AB-001 and SDB-001, and several analogues. The synthesis of **5−12** provides an efficient and general route to variously substituted analogues of AB-001 and SDB-001, and should assist the rapid generation of reference standards for

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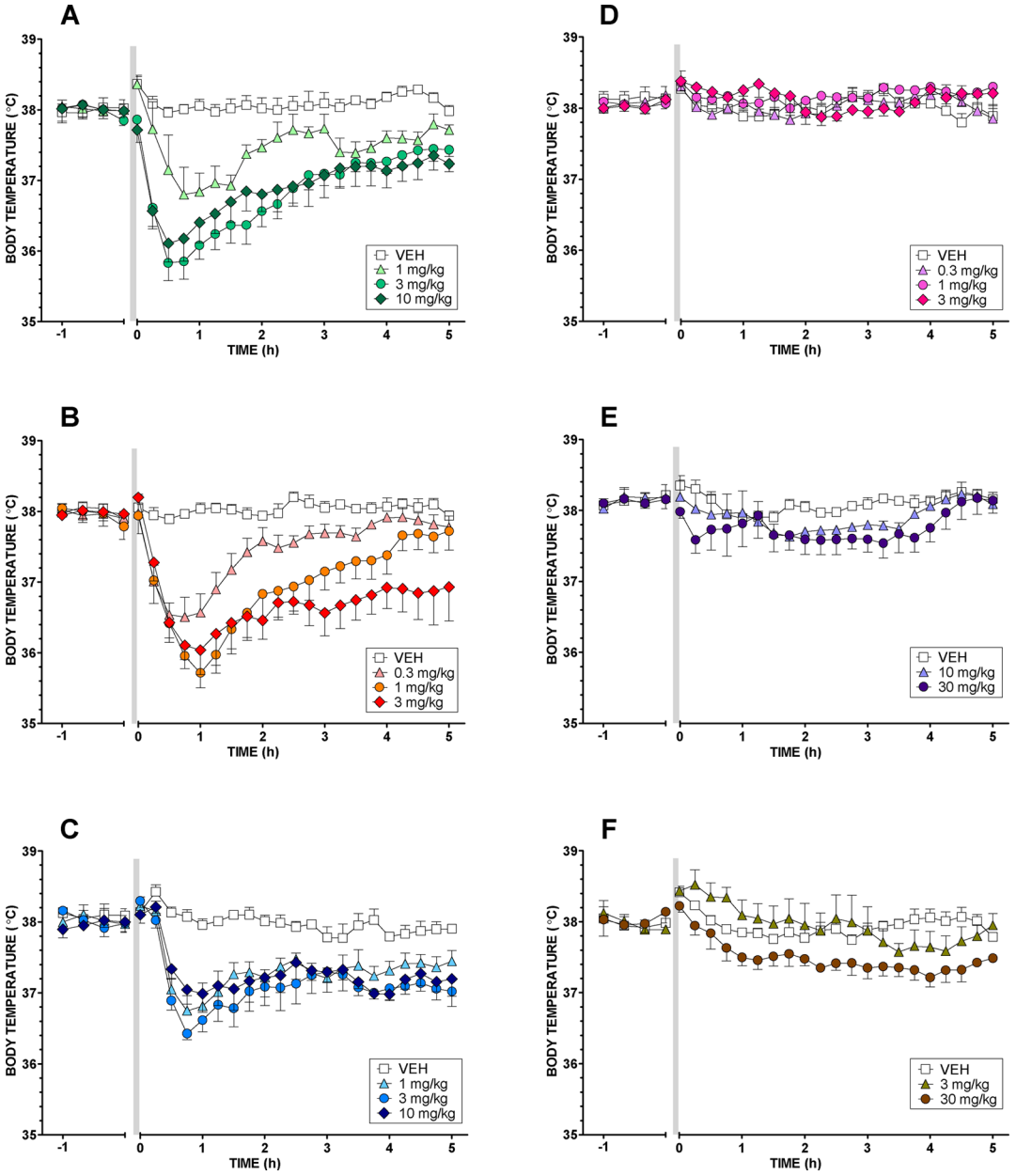

Figure 4. Effects of (A) Δ^9-THC, (B) JWH-018, (C) SDB-001, (D) low and (E) high doses of AB-001, and (F) AB-002 on rat body temperature. Gray bar denotes time of intraperitoneal injection.

emergent cannabimimetics based on these chemotypes. Preliminary SARs for **5**–**12** suggest that structural heterogeneity is tolerated at the 3-indole position, with various polycyclic, alicyclic, and aromatic groups tolerated by CB_1 and CB_2 receptors. All AB and SDB compounds were found to act as approximately equipotent full agonists at CB_1 and CB_2 receptors in vitro (with the exception of SDB-002), and displayed more potent CB_1 agonist activity than Δ^9-THC. However, despite the remarkable similarity of the functional activity profiles of AB-001, AB-002, and SDB-001, only SDB-001 proved potently cannabimimetic in vivo. SDB-001 dose-dependently induced hypothermia in rats with a potency similar to Δ^9-THC, and roughly a third as potently as JWH-018. By contrast, AB-001 and AB-002 showed only hypothermic trends at a dose three times greater than the dose of SDB-001 eliciting maximal hypothermia. Additionally, the hypothermic profile of SDB-001 differed from Δ^9-THC and JWH-018, showing minimal regression to the pretreatment body temperature after 5 h, suggesting that the effects of SDB-001 may be relatively long-lived. Similarly, Δ^9-THC, JWH-018, and SDB-001 all dose-dependently reduced heart rate in rats, while AB-001 and AB-002 did not. Taken together, it appears that the nature of the functional group linking the adamantane and indole units, while unimportant for in vitro activity at CB_1 and CB_2 receptors, is crucial for cannabimimetic activity. The potent cannabimimetic of SDB-001, but not AB-001 or AB-002, confirms that in vivo activity is attributable to the carboxamide core and not the indole–adamantane distance.

METHODS

General Chemical Synthesis Details. All reactions were performed under an atmosphere of nitrogen or argon unless otherwise specified. Toluene was dried over sodium wire and distilled from sodium benzophenone ketyl. Dichloromethane and methanol were

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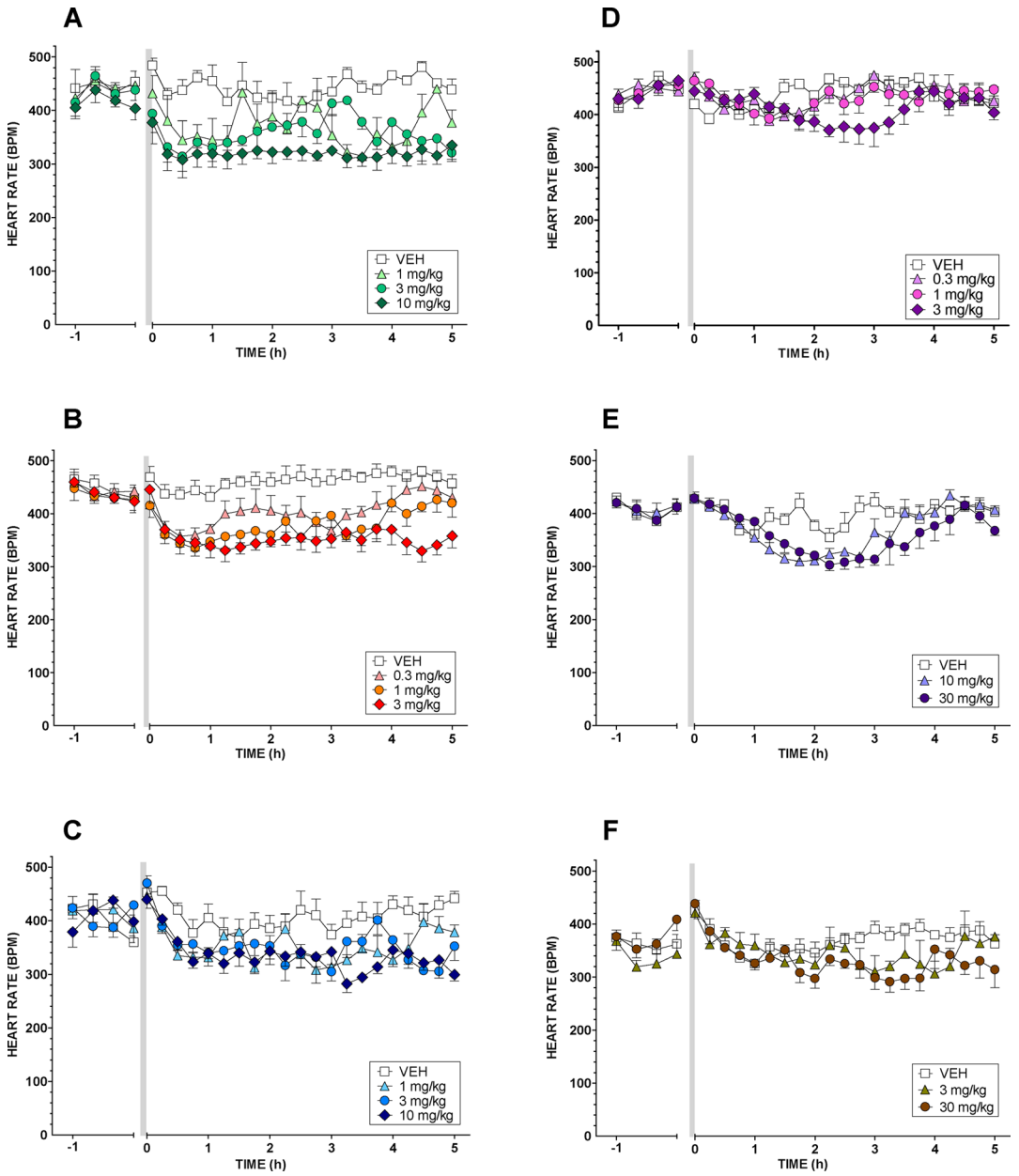

Figure 5. Effects of (A) Δ^9-THC, (B) JWH-018, (C) SDB-001, (D) low and (E) high doses of AB-001, and (F) AB-002 on rat heart rate. Gray bar denotes time of intraperitoneal injection.

distilled from calcium hydride. Anhydrous DMF (Sigma-Aldrich) was used as purchased. Commercially available chemicals (Sigma-Aldrich) were used as purchased. Analytical thin layer chromatography (TLC) was performed using Merck aluminum-backed silica gel 60 F254 (0.2 mm) plates which were visualized using shortwave (254 nm) ultraviolet fluorescence. Flash chromatography was performed using Merck Kieselgel 60 (230–400 mesh) silica gel. Melting points were measured in open capillaries using a Stuart SMP10 Melting Point Apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded at 300 K using either a Bruker AVANCE DRX400 (400.1 MHz) or AVANCE III 500 Ascend (500.1 MHz) spectrometer. The data are reported as chemical shift (δ ppm) relative to the residual protonated solvent resonance, relative integral, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, sep = septet, m = multiplet), coupling constants (J Hz), and assignment. Assignment of signals was assisted by COSY, DEPT, HSQC, and HMBC experiments where necessary. Low resolution mass spectra (LRMS) was recorded using electrospray ionization (ESI) recorded on a Finnigan LCQ ion trap spectrometer. Elemental analysis was obtained from the Chemical Analysis Facility in the Department of Chemistry and Biomolecular Sciences, Macquarie University, Australia.

1-Pentylindole (14). A cooled (0 °C) suspension of sodium hydride (60% dispersion in mineral oil, 1.50 g, 37.5 mmol, 1.5 equiv) in DMF (45 mL) was treated slowly with a solution of indole (2.93 g, 25.0 mmol) in DMF (5 mL), warmed to ambient temperature and stirred for 10 min. The mixture was cooled to 0 °C, treated portionwise with 1-bromopentane (3.25 mL, 26.25 mmol, 1.05 equiv), warmed to ambient temperature and stirred for 1 h. The reaction was poured portionwise onto ice–water (400 mL) and extracted with EtOAc (4 × 50 mL). The combined organic extracts were washed with H_2O (2 × 200 mL), brine (200 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. Purification by flash chromatography, eluting with hexane (R_f 0.32), gave **14** as a pale yellow oil (3.75 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.64 (1H, d, J = 7.9 Hz), 7.35 (1H, d, J = 8.3 Hz), 7.21 (1H, dd, J = 7.6 Hz), 7.12–7.09 (2H, m), 6.49 (1H, d, J = 3.1 Hz), 4.12 (2H, t, J = 7.2 Hz), 1.85 (2H, quin., J = 7.3 Hz), 1.40–1.26 (4H, m), 0.90 (3H, t, J = 7.1 Hz). All spectroscopic data matched those previously reported.[79]

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General Procedure for 3-Acylation of 1-Pentylindole. A solution of the appropriate carboxylic acid (1.2 mmol) in CH_2Cl_2 (4 mL) was treated with $(COCl)_2$ (240 μL, 2.8 mmol, 2.3 equiv.) followed by DMF (1 drop). After stirring for 1 h, the solution was evaporated in vacuo, and the crude acid chloride was used immediately in the following step.

A cooled (0 °C) solution of **14** (187 mg, 1.0 mmol) in CH_2Cl_2 (3 mL) was treated dropwise with a solution of 1 M Me_2AlCl in hexane (1.5 mL, 1.5 mmol, 1.5 equiv) and stirred for 30 min. To this solution was added dropwise a solution of the freshly prepared acid chloride in CH_2Cl_2 (3 mL), and the reaction stirred for 2 h. The reaction was quenched by dropwise addition to a solution of 1 M aq. HCl (6 mL), the layers separated, and the aqueous phase extracted with CH_2Cl_2 (2 × 8 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (2 × 12 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The crude products were purified by flash chromatography.

Adamantan-1-yl(1-pentyl-1*H*-indol-3-yl)methanone (5). Treating **14** (187 mg, 1.0 mmol) with 1-adamantanecarboxylic acid (216 mg, 1.2 mmol) according to the general procedure gave, following purification by flash chromatography (hexane-EtOAc, 95:5, R_f 0.16), **5** (302 mg, 86%) as a white crystalline solid. Recrystallization from *i*-PrOH-H_2O yielded colorless needles. mp (*i*-PrOH-H_2O) 125−126 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.52 (1H, m), 7.93 (1H, s), 7.34 (1H, m), 7.31−7.25 (2H, m), 4.17 (2H, t, *J* = 7.3 Hz), 2.15 (9H, br s), 1.90 (2H, quin., *J* = 7.3 Hz), 1.83 (6H, br s), 1.44−1.30 (4H, m), 0.92 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 202.1 (CO), 135.7 (quat.), 133.2, 128.7 (quat.), 123.7, 123.2, 122.4, 113.0 (quat.), 109.5, 47.2 (CH_2), 47.0 (quat.), 40.7 (CH_2), 37.1 (CH_2), 29.8 (CH_2), 29.1 (CH_2), 28.7, 22.4 (CH_2), 14.1; LRMS (+ESI) *m*/*z* 720.93 ([2M + Na]$^+$, 100%), 371.93 ([M + Na]$^+$, 4%), 350.00 ([M + H]$^+$, 10%); Anal. ($C_{24}H_{31}NO$) calcd: C 82.47, H 8.94, N 4.01; found: C 82.37, H 9.33, N 3.93.

2-(Adamantan-1-yl)-1-(1-pentyl-1*H*-indol-3-yl)ethanone (7). Treating **14** (936 mg, 5.0 mmol) with 1-adamantaneacetic acid (1.17 g, 6.0 mmol) according to the general procedure gave, following purification by flash chromatography (hexane-EtOAc, 90:10, R_f 0.18), **7** (636 mg, 35%) as a white crystalline solid. mp 93−94 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.49 (1H, m), 7.68 (1H, s), 7.35 (1H, m), 7.32−7.27 (2H, m), 4.16 (2H, t, *J* = 7.3 Hz), 2.56 (2H, s), 1.95 (3H, br s), 1.89 (2H, quin., *J* = 7.4 Hz), 1.74−1.60 (12H, m), 1.41−1.27 (4H, m), 0.90 (3H, t, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.1 (CO), 136.9 (quat.), 135.2, 126.7 (quat.), 123.32, 123.25, 122.6, 118.8 (quat.), 109.8, 54.0 (CH_2), 47.2 (CH_2), 43.5 (CH_2), 37.0 (CH_2), 33.9 (quat.), 29.7 (CH_2), 29.2 (CH_2), 29.0, 22.4 (CH_2), 14.1; LRMS (+ESI) *m*/*z* 111.47 ([3 M + Na]$^+$, 100%), 749.07 ([2 M + Na]$^+$, 72%); Anal. ($C_{25}H_{33}NO$) calcd: C 82.60, H 9.15, N 3.85; found: C 82.27, H 9.54, N 3.98.

1-Pentylindole-3-carboxylic Acid (16). A cooled (0 °C) suspension of sodium hydride (60% dispersion in mineral oil, 6.000 g, 150.0 mmol, 2.0 equiv) in DMF (90 mL) was treated slowly with a solution of indole (8.790 g, 75.03 mmol) in DMF (10 mL), warmed to ambient temperature, and stirred for 10 min. The mixture was cooled to 0 °C, treated portionwise with 1-bromopentane (9.75 mL, 78.6 mmol, 1.05 equiv), warmed to ambient temperature and stirred for 1 h. The solution was cooled to 0 °C, treated slowly with trifluoroacetic anhydride (26.10 mL, 187.5 mmol, 2.5 equiv), warmed to ambient temperature and stirred for 1 h. The reaction was poured portionwise onto vigorously stirred ice−water (1000 mL), and stirred (with external ice-bath cooling if necessary) until complete solidification of the product occurred. The precipitate was collected by filtration and air-dried to give crude **15** as a pink solid (21.16 g, 74.69 mmol, 100%).

A solution of crude **15** (21.00 g, 74.13 mmol) in PhMe (65 mL) was slowly added to a refluxing solution of KOH (13.26 g, 236.3 mmol, 3.3 equiv) in MeOH (25 mL), and the mixture heated at reflux for 2 h. The mixture was cooled to ambient temperature and H_2O (250 mL) was added. The layers were separated, and the organic layer was extracted with 1 M aq. NaOH (70 mL). The combined aqueous phases were acidified to pH 1 with 10 M aq. HCl, extracted with Et_2O (3 × 100 mL), dried ($MgSO_4$), and the solvent evaporated. The crude residue was recrystallized from *i*-PrOH to give **16** as colorless crystals (13.03 g, 56.2 mmol, 75%). mp (*i*-PrOH) 106−108 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.27−8.24 (1H, m), 7.93 (1H, s), 7.40−7.38 (1H, m), 7.33−7.29 (2H, m), 4.17 (2H, t, *J* = 7.2 Hz), 1.90 (2H, quin., *J* = 7.2 Hz), 1.39−1.32 (4H, m), 0.91 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7 (COOH), 136.9 (quat.), 135.6, 127.2 (quat.), 123.0, 122.3, 122.1, 110.2, 106.4 (quat.), 47.3 (CH_2), 29.7 (CH_2), 29.1 (CH_2), 22.4 (CH_2), 14.0; Anal. ($C_{14}H_{17}NO_2$) calcd: C 72.70, H 7.41, N 6.06; found: C 72.67, H 7.77, N 5.93

General Procedure for Amidation of 1-Pentylindole-3-carboxylic Acid. A solution of **16** (576 mg, 2.50 mmol) in CH_2Cl_2 (5 mL) was treated with $(COCl)_2$ (420 μL, 5.00 mmol, 2.0 equiv.) followed by DMF (1 drop). After stirring for 1 h, the solution was evaporated in vacuo. A solution of the crude acid chloride in CH_2Cl_2 (5 mL) was added dropwise to a solution of Et_3N (870 μL, 6.25 mmol, 2.5 equiv.) and the appropriate amine (3.0 mmol, 1.2 equiv.) in CH_2Cl_2 (20 mL). The mixture was stirred for 14 h, the solvent evaporated, and the residue partitioned between EtOAc (200 mL) and H_2O (25 mL). The layers were separated and the organic phase was washed with 1 M aq. HCl (3 × 25 mL), sat. aq. $NaHCO_3$ (3 × 25 mL), brine (50 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The crude products were recrystallized from *i*-PrOH-H_2O.

***N*-(Adamantan-1-yl)-1-pentyl-1*H*-indole-3-carboxamide (6).** Subjecting 1-aminoadamantane (454 mg) to the general procedure gave **6** (787 mg, 86%) as a white crystalline solid. mp (*i*-PrOH-H_2O) 140−141 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.87 (1H, d, *J* = 7.5 Hz), 7.65 (1H, s), 7.36 (1H, d, *J* = 8.0 Hz), 7.28−7.22 (2H, m), 5.71 (1H, br s, NH), 4.11 (2H, t, *J* = 7.2 Hz), 2.19 (6H, br s), 2.14 (3H, br s), 1.84 (2H, quin., *J* = 7.3 Hz), 1.76 (6H, m) 1.39−1.24 (4H, m), 0.88 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.7 (CO), 136.7 (quat.), 131.5, 125.3 (quat.), 122.3, 121.2, 120.0, 112.3 (quat.), 110.4, 52.2 (quat.), 46.9 (CH_2), 42.4 (CH_2), 36.6 (CH_2), 29.8, 29.7 (CH_2), 29.1 (CH_2), 22.4 (CH_2), 14.0; LRMS (+ESI) *m*/*z* 751.80 ([2M + Na]$^+$, 100%), 387.67 ([M + Na]$^+$, 55%); Anal. ($C_{24}H_{32}N_2O$) calcd: C 79.08, H 8.85, N 7.68; found: C 79.13, H 8.86, N 7.47.

***N*-(Adamantan-1-ylmethyl)-1-pentyl-1*H*-indole-3-carboxamide (8).** Subjecting 1-(aminomethyl)adamantane (496 mg) to the general procedure gave **8** (748 mg, 79%) as a white crystalline solid. mp (*i*-PrOH-H_2O) 160−162 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89−7.87 (1H, m), 7.75 (1H, s), 7.40−7.38 (1H, m), 7.31−7.22 (2H, m), 6.03 (1H, br s, NH), 4.12 (2H, t, *J* = 7.3 Hz), 3.22 (2H, d, *J* = 6.5 Hz), 2.01 (3H, br s), 1.86 (2H, quin., *J* = 7.0 Hz), 1.69 (6H, m), 1.61 (6H, br s), 1.39−1.27 (4H, m), 0.88 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.6 (CO), 136.8 (quat.), 132.0, 125.2 (quat.), 122.4, 121.4, 119.9, 111.3 (quat.), 110.6, 51.0 (CH_2), 47.0 (CH_2), 40.6 (CH_2), 37.6 (CH_2), 34.1 (quat.), 29.8 (CH_2), 29.1 (CH_2), 28.4, 22.4 (CH_2), 14.0; LRMS (+ESI) *m*/*z* 779.73 ([2 M + Na]$^+$, 100%), 401.67 ([M + Na]$^+$, 42%); Anal. ($C_{25}H_{34}N_2O$) calcd: C 79.32, H 9.05, N 7.40; found: C 79.15, H 9.00, N 7.19.

***N*-Cyclohexyl-1-pentyl-1*H*-indole-3-carboxamide (9).** Subjecting cyclohexylamine (340 μL) to the general procedure gave **9** (633 mg, 81%) as a white crystalline solid. mp (*i*-PrOH-H_2O) 156−157 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.87 (1H, d, *J* = 7.1 Hz), 7.79 (1H, s), 7.38 (1H, d, *J* = 7.3 Hz), 7.29−7.23 (2H, m), 5.89 (1H, br s, NH), 4.13 (2H, t, *J* = 7.2 Hz), 4.10−4.04 (1H, m), 2.10−2.07 (2H, m), 1.86 (2H, quin., *J* = 7.3 Hz), 1.77 (2H, dt, *J* = 13.7, 3.9 Hz), 1.66 (1H, dt, *J* = 13.1, 3.9 Hz), 1.51−1.43 (2H, m), 1.39−1.20 (7H, m), 0.89 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.6 (CO), 136.7 (quat.), 131.7 (quat.), 125.4 (quat.), 122.4, 121.4, 120.0, 111.2 (quat.), 110.5, 48.2, 47.0 (CH_2), 33.7 (CH_2), 29.8 (CH_2), 29.2 (CH_2), 25.8 (CH_2), 25.1 (CH_2), 22.4 (CH_2), 14.0; LRMS (+ESI) *m*/*z* 647.60 ([2M + Na]$^+$, 39%), 335.60 ([M + Na]$^+$, 100%); Anal. ($C_{20}H_{28}N_2O$) calcd: C 76.88, H 9.03, N 8.97; found: C 76.48, H 9.18, N 8.96.

***N*-(Cyclohexylmethyl)-1-pentyl-1*H*-indole-3-carboxamide (10).** Subjecting (aminomethyl)cyclohexane (390 μL) to the general procedure gave **10** (594 mg, 73%) as a white crystalline solid. mp (*i*-PrOH-H_2O) 123−125 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90−7.86 (1H, m), 7.72 (1H, s), 7.41−7.36 (1H, m), 7.30−7.23 (2H, m), 6.00 (1H, br s, NH), 4.12 (2H, t, *J* = 7.2 Hz), 3.36 (2H, t, *J* = 6.4 Hz),

1.89−1.82 (4H, m), 1.75 (2H, dt, J = 12.8, 3.4 Hz), 1.70−1.57 (4H, m), 1.39−1.13 (7H, m), 1.09−0.99 (2H, m), 0.88 (3H, t, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.5 (CO), 136.8 (quat.), 131.7 (quat.), 125.3 (quat.), 122.4, 121.4, 120.0, 111.3 (quat.), 110.5, 47.0 (CH_2), 45.9 (CH_2), 38.4, 31.2 (CH_2), 29.8 (CH_2), 29.2 (CH_2), 26.6 (CH_2), 26.1 (CH_2), 22.4 (CH_2), 14.0; LRMS (+ESI) m/z 675.67 ([2M + Na]$^+$, 61%), 349.60 ([M + Na]$^+$, 100%); Anal. ($C_{21}H_{30}N_2O$) calcd: C 77.26, H 9.26, N 8.58; found: C 77.28, H 9.62, N 8.61.

***N*-Phenyl-1-pentyl-1*H*-indole-3-carboxamide (11).** Subjecting aniline (275 μL) to the general procedure gave **11** (622 mg, 81%) as a white crystalline solid. mp (i-PrOH-H_2O) 125−128 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07−8.05 (1H, m), 7.80 (1H, s), 7.76 (1H, br s, NH) 7.67 (2H, d, J = 7.6 Hz), 7.42−7.40 (1H, m), 7.37 (2H, dd, J = 8.2, 7.7 Hz), 7.33−7.28 (2H, m), 7.12 (1H, t, J = 7.4 Hz), 4.13 (2H, t, J = 7.2 Hz), 1.87 (2H, quin., J = 7.3 Hz), 1.39−1.28 (4H, m), 0.89 (3H, t, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.4 (CO), 138.7 (quat.), 136.8 (quat.), 131.9, 129.2, 125.6 (quat.), 124.0, 122.8, 121.8, 120.3, 120.2, 111.2 (quat.), 110.6, 47.1 (CH_2), 29.8 (CH_2), 29.1 (CH_2), 22.4 (CH_2), 14.0; LRMS (+ESI) m/z 329.53 ([M + Na]$^+$, 100%); Anal. ($C_{20}H_{22}N_2O$) calcd: C 78.40, H 7.24, N 9.14; found: C 78.01, H 7.57, N 8.77.

***N*-Benzyl-1-pentyl-1*H*-indole-3-carboxamide (12).** Subjecting benzylamine (330 μL) to the general procedure gave **12** (678 mg, 84%) as a white crystalline solid. mp (i-PrOH-H_2O) 101−102 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (1H, d, J = 7.7 Hz), 7.73 (1H, s), 7.42−7.35 (4H, m), 7.31−7.21 (4H, m), 6.19 (1H, br s, NH), 4.72 (2H, d, J = 5.7 Hz), 4.13 (2H, t, J = 7.2 Hz), 1.86 (2H, quin., J = 7.3 Hz), 1.37−1.29 (4H, m), 0.89 (3H, t, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.3 (CO), 139.1 (quat.), 136.8 (quat.), 131.7, 128.9, 128.0, 127.6, 125.5 (quat.), 122.5, 121.5, 120.3, 110.8 (quat.), 110.5, 47.0 (CH_2), 43.7 (CH_2), 29.8 (CH_2), 29.2 (CH_2), 22.4 (CH_2), 14.0; LRMS (+ESI) m/z 343.60 ([M + Na]$^+$, 100%); Anal. ($C_{21}H_{24}N_2O$) calcd: C 78.71, H 7.55, N 8.74; found: C 78.87, H 7.74, N 8.77.

X-ray Data Collection. The solid was crystallized from i-PrOH-H_2O to give colorless needle crystals by slow evaporation at ambient temperature.

The single-crystal X-ray diffraction experiments were carried out at the Faculty of Pharmacy, University of Sydney using a Bruker APEX-II CCD-based diffractometer with an X-ray wavelength of 0.71073 Å (Mo Kα) and at an experimental temperature of 150 K. The single crystal of X was mounted on the tip of a thin glass fiber with a minimum amount of Paratone N oil, which acted as both an adhesive and a cryoprotectant, and inserted in the cold N2 stream of an Oxford Cryosystem COBRA cooler. X-ray diffraction data were collected using 0.3° $\Delta\omega$-scans, maintaining the crystal-to-detector distance at 6.0 cm. A total of 1664 frames were collected. The diffraction data were integrated using SAINT+,[80] which included corrections for Lorentz, polarization and absorption effects. Unit cell parameters for **5** at 150 K were refined from 250 reflections.

The structure was solved using Direct Methods (SHELX-S),[81] and refined using full-matrix least-squares (SHELXL).[81] All non-hydrogen atoms were treated as anisotropic, while hydrogen atoms were placed in idealized positions, with U_{eq} set at 1.5 times that of the parent atom.

empirical formula	C_{24} H_{31} NO
formula weight	349.50
temperature	150(2) K
crystal system, space group	monoclinic, $P2(1)/n$
unit cell dimensions	a = 12.022 Å, α =90°
	b = 20.718 Å, β = 101.25°
	c = 16.094 Å, γ = 90°
Z	8
goodness-of-fit on F^2	1.030
final R indices [I>2σ(I)]	R1 = 0.0584, wR2 = 0.1571
R indices (all data)	R1 = 0.0872, wR2 = 0.1764

In Vitro Pharmacological Assessment of 5−12. Mouse AtT20 neuroblastoma cells stably transfected with rat CB_1[72] or human CB_2 were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U penicillin/streptomycin, and 300 μg/mL G418. The cDNA clone for the human CB2 receptor (GenBank accession number AY242132) with 3 × N-terminal HA tags was obtained from the Missouri S&T cDNA Resource Center (www.cdna.org). Cells were passaged at 80% confluency as required. Cells for assays were grown in 75 cm^2 flasks and used at 90% confluence. The day before the assay cells were detached from the flask with trypsin/EDTA (Sigma) and resuspended in 10 mL of Leibovitz's L-15 media supplemented with 1% FBS, 100U penicillin/streptomycin and 15 mM glucose (membrane potential assay and Ca5 calcium assay). The cells were plated in volume of 90 μL in black walled, clear bottomed 96-well microplates (Corning) which had been precoated with poly-L-lysine (Sigma, Australia). Cells were incubated overnight at 37 °C in ambient CO_2.

Membrane potential was measured using a FLIPR Membrane Potential Assay kit (blue) from Molecular Devices, as described previously.[71] The dye was reconstituted with assay buffer of composition (mM): NaCl 145, HEPES 22, Na_2HPO_4 0.338, $NaHCO_3$ 4.17, KH_2PO_4 0.441, $MgSO_4$ 0.407, $MgCl_2$ 0.493, $CaCl_2$ 1.26, glucose 5.56 (pH 7.4, osmolarity 315 ± 5). Prior to the assay, cells were loaded with 90 μL/well of the dye solution without removal of the L-15, giving an initial assay volume of 180 μL/well. Plates were then incubated at 37 °C at ambient CO_2 for 45 min. Fluorescence was measured using a FlexStation 3 (Molecular Devices) microplate reader with cells excited at a wavelength of 530 nm and emission measured at 565 nm. Baseline readings were taken every 2 s for at least 2 min, at which time either drug or vehicle was added in a volume of 20 μL. The background fluorescence of cells without dye or dye without cells was negligible. Changes in fluorescence were expressed as a percentage of baseline fluorescence after subtraction of the changes produced by vehicle addition, which was less than 2% for drugs dissolved in assay buffer or DMSO. The final concentration of DMSO was not more than 0.1%.

Data was analyzed with PRISM (GraphPad Software Inc., San Diego, CA), using four-parameter nonlinear regression to fit concentration−response curves. In all plates, a maximally effective concentration of WIN 55,212-2 was added to allow for normalization between assays.

In Vivo Pharmacological Assessment of 5−12. *Subjects.* The experiments involved 16 adult male Wistar rats (obtained from Animal Resources Centre, Perth, Australia) weighing between 190 and 220 g at the start of the experiment. Throughout the period of data acquisition, the rats were singly housed in testing cages in an air-conditioned testing room (ambient temperature 22 ± 1 °C) with a 12 h reverse light/dark cycle (lights on from 7:00 to 19:00). Standard rodent chow and water were provided ad libitum. All efforts were made to minimize both the number of rats used and the distress to those involved. All experiments were approved by the University of Sydney Animal Ethics Committee.

Surgery. Biotelemetry transmitters (TA11CTA-F40, Data Sciences International, St. Paul, MN) were implanted according to manufacturers instructions. Briefly, the rats were anaesthetized with isoflurane (3% induction, 2% maintenance) and once adequate depth of anesthesia was reached, a rostro-caudal incision was made along the midline of the abdomen. A biotelemetry transmitter was placed in the peritoneal cavity according to the manufacturer's protocol and the wound sutured closed. The rats were allowed one week of recovery, during which their wellbeing was monitored daily, before data collection commenced.

Biotelemetry System. The biotelemetry system (Data Science International, D.S.I., St. Paul, MN) was used to measure heart rate, body temperature, and physical activity in freely moving rats in their home cages. Data for these three variables were gathered continuously at 1000 Hz and organized in 5 min bins. Data was acquired, processed and analyzed using the Dataquest A.R.T. software (version 4.3, D.S.I., St. Paul, MN).

Procedure. Each drug (Δ^9-THC, JWH-018, SDB-001, AB-001, AB-002) was tested in a cohort of four drug-naïve rats using a within-subjects design. To minimize the effects of injection stress on physiological parameters, rats were habituated over several days to

receiving injections of vehicle at a particular time of day (12:30 pm) and then being immediately returned to their home cages. An ascending dose response sequence was then employed in each experiment where the lowest dose of the drug was tested, followed by the next lowest dose, and so on until a maximal physiological response was seen. This ensured the safety of animals when hitherto untested compounds were being assessed. Two washout days were given in between each dose. In some cases, a further vehicle test was done after the completion of drug testing to ensure that baselines, before and after drug treatment, had not changed.

Drugs. Δ^9-THC was obtained from Sigma Aldrich and JWH-018 from the National Measurement Institute (Pymble, Sydney, NSW, Australia). All other drugs were synthesized by the authors. Drugs were initially dissolved in 5% ethanol, then 5% Tween 80 was added, and then 90% physiological saline was added. All drugs were injected intraperitoneally at a volume of 1 mL/kg.

Statistical Analysis. Activity, body temperature, and heart rate data were compared across conditions using software SPSS version 19.0. Planned contrasts (two-way ANOVA) compared each dose of the drug with vehicle treatment for (a) 1 h before treatment and (b) 5 h after treatment. The minimum level of statistical significance was set at $p < 0.05$.

■ ASSOCIATED CONTENT

Supporting Information

X-ray crystallographic data; selected ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

S.D.B., S.M.W., and M.L. performed the synthesis, purification, and chemical characterization of compounds **5**−**12** under the supervision of M.K. J.S. conducted all in vitro pharmacological evaluation with guidance from M.C. N.A. and K.E. carried out all behavioral pharmacology with direction from I.S.M. L.B. and C.G. compared synthesized SDB-001 (**6**) with a forensic sample. D.E.H. provided X-ray crystallographic analysis of AB-001 (**5**). M.G. assisted the creation of stably transfected cells expressing hCB_2R. The manuscript was prepared by M.C., I.S.M., and M.K., based on drafts written by S.D.B., S.M.W., and N.A. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

CB, cannabinoid; FLIPR, fluorometric imaging plate reader; GIRK, G-protein-gated inwardly rectifying K^+ channels; i.p., intraperitoneal; SAR, structure−activity relationship; SLID, synthetic legal intoxicating drug; Δ^9-THC, Δ^9-tetrahydrocannabinol; TLC, thin layer chromatography

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