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Effects of Bioisosteric Fluorine in Synthetic Cannabinoid Designer Drugs JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135

Samuel D. Banister,[†,‡] Jordyn Stuart,[§] Richard C. Kevin,[∥] Amelia Edington,[§] Mitchell Longworth,[‡] Shane M. Wilkinson,[‡] Corinne Beinat,[†,‡] Alexandra S. Buchanan,[⊥,#] David E. Hibbs,[▽] Michelle Glass,[¶] Mark Connor,[§] Iain S. McGregor,[∥] and Michael Kassiou*[,‡,◆]

[†]Department of Radiology, Stanford University School of Medicine, Stanford, California 94305, United States

[‡]School of Chemistry, The University of Sydney, Sydney, New South Wales 2006, Australia

[§]Faculty of Medicine and Health Sciences, Macquarie University, Sydney, New South Wales 2109, Australia

[∥]School of Psychology, The University of Sydney, Sydney, New South Wales 2006, Australia

[⊥]Center for Immersive and Simulation-based Learning, Stanford University School of Medicine, Stanford, California 94305, United States

[#]Department of Anaesthesia, Prince of Wales Hospital, Randwick, New South Wales 2031, Australia

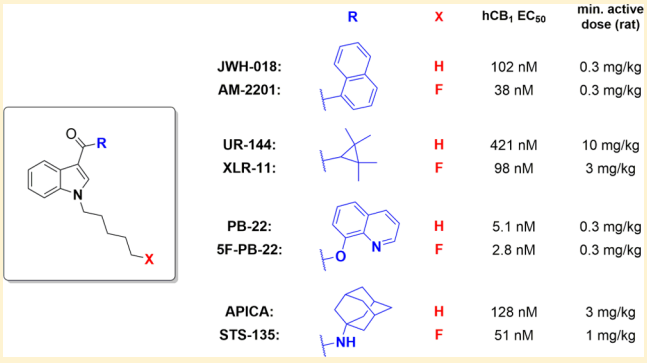
[▽]Faculty of Pharmacy, The University of Sydney, Sydney, New South Wales 2006, Australia

[¶]School of Medical Sciences, The University of Auckland, Auckland 1142, New Zealand

[◆]Discipline of Medical Radiation Sciences, The University of Sydney, Sydney, New South Wales 2006, Australia

Supporting Information

ABSTRACT: Synthetic cannabinoid (SC) designer drugs featuring bioisosteric fluorine substitution are identified by forensic chemists and toxicologists with increasing frequency. Although terminal fluorination of *N*-pentyl indole SCs is sometimes known to improve cannabinoid type 1 (CB_1) receptor binding affinity, little is known of the effects of fluorination on functional activity of SCs. This study explores the *in vitro* functional activities of SC designer drugs JWH-018, UR-144, PB-22, and APICA, and their respective terminally fluorinated analogues AM-2201, XLR-11, 5F-PB-22, and STS-135 at human CB_1 and CB_2 receptors using a FLIPR membrane potential assay. All compounds demonstrated agonist activity at CB_1 (EC_{50} = 2.8−1959 nM) and CB_2 (EC_{50} = 6.5−206 nM) receptors, with the fluorinated analogues generally showing increased CB_1 receptor potency (~2−5 times). Additionally, the cannabimimetic activities and relative potencies of JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135 *in vivo* were evaluated in rats using biotelemetry. All SCs dose-dependently induced hypothermia and reduced heart rate at doses of 0.3−10 mg/kg. There was no consistent trend for increased potency of fluorinated SCs over the corresponding des-fluoro SCs *in vivo*. Based on magnitude and duration of hypothermia, the SCs were ranked for potency (PB-22 > 5F-PB-22 = JWH-018 > AM-2201 > APICA = STS-135 = XLR-11 > UR-144).



KEYWORDS: *Cannabinoid, THC, JWH-018, AM-2201, XLR-11, PB-22*

Synthetic cannabinoids (SCs) are the most rapidly growing class of recreational "designer drugs". The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reports that, as of March 2015, 134 new SCs have been identified in the European Union (EU) since 2008, with 30 novel SCs formally notified in 2014 alone.[1] In the United States (US) in 2010, the Drug Enforcement Administration's National Forensic Laboratory Information System (NFLIS) reported 19 distinct SCs across 3286 samples, but by 2012, there were 61 SC variants identified in 41 458 cases.[2] In the EU in 2013, there were over 21 000 seizures of SCs, a more than 200-fold increase since 2008.[1] Many SCs have no precedent in the scientific literature yet bear hallmarks of rational design.

Like Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**; Figure 1), the principal bioactive component of cannabis, SCs typically exert agonist activity at both cannabinoid receptor subtypes, namely,

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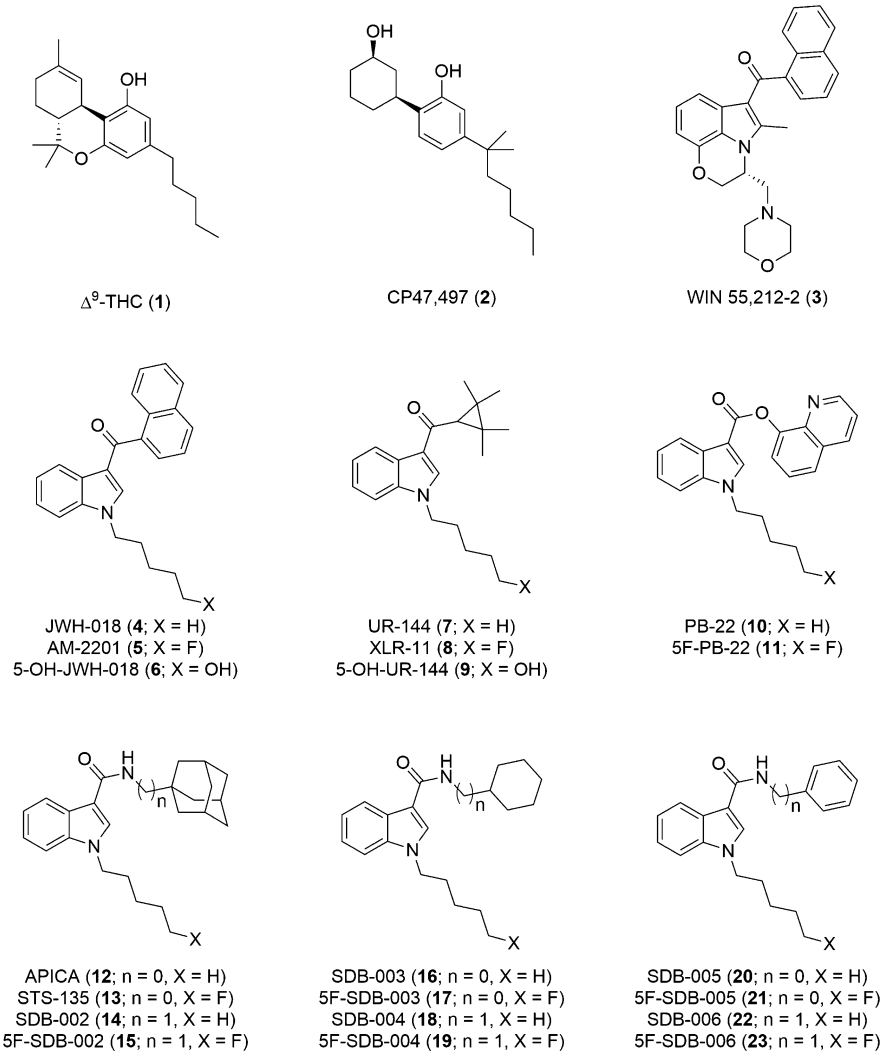

Figure 1. Selected natural and synthetic cannabinoids.

CB_1 and CB_2 receptors, with psychoactivity attributed to activation of the former.[3] Generally, SCs are found as adulterants in smoking mixtures of otherwise nonpsychoactive herbal blends and are intended to substitute for the intoxicating effects of Δ^9-THC. Although these products are disingenuously marketed as incense and labeled "not for human consumption", consumers are aware of the psychoactivity of such products and use them as technically legal cannabis substitutes. One of the earliest SC products, branded "Spice", was analyzed in 2008 and found to contain the C8-homologue of CP 47,497 (CP 47,497-C8, **2**) and an (aminoalkyl)indole analogue of WIN 55,212-2 (**3**)[4] known as JWH-018 (**4**), thereby accounting for the anecdotal cannabimimetic effects of this product.[5] After the active ingredients of Spice were identified, many governments prohibited CP 47,497-C8 and JWH-018, forcing manufacturers of Spice to circumvent restriction by substituting the active constituents with other unregulated SCs. The iterative cycle of SC identification, prohibition, and substitution has produced hundreds of differently branded products, with names like "Kronic", and "K2", containing one or more SCs.

A popular design trend in the SC market currently is the incorporation of a terminal fluorine atom in variously substituted *N*-pentylindoles.[6] The terminally fluorinated analogue of JWH-018, AM-2201 (**5**), was one of several nanomolar affinity SCs (CB_1 K_i = 1.0 nM; CB_2 K_i = 2.6 nM) reported by Makriyannis and colleagues in 2001,[7] and was identified in consumer products by forensic researchers in several countries in 2011.[8,9] Anecdotal reports that AM-2201 possesses psychoactivity at submilligram doses in humans likely instigated the trend of bioisosteric fluorine substitution in other structurally related SC designer drugs. For example, South Korea's National Forensic Service reported no fluorinated SCs in 2010, but 90% of all seized SCs were fluorinated by 2013.[10] Several dozen terminally fluorinated SCs have been reported by forensic laboratories worldwide, and the rate of emergence appears to be increasing.[6]

The SC sold as UR-144 (**7**, CB_1 K_i = 150 nM; CB_2 K_i = 1.8 nM) was first reported by Abbott Laboratories in 2010 during their exploration of CB_2-selective ligands[11,12] and has since been identified in numerous forensic samples.[9,13−15] The 5-fluoro analogue of UR-144, sold as XLR-11 (**8**), has also been identified in consumer products, despite no prior reports of its structure in the scientific literature.[16−19] In Korea, XLR-11 first appeared in 2012 and was the most frequently encountered SC by 2013.[10] XLR-11 use is associated with adverse health effects, including acute kidney injury (AKI)[20,21] and cerebral ischemia.[22] Wiley and colleagues recently showed that XLR-11 (CB_1 K_i = 24 nM; CB_2 K_i = 2.1 nM) has binding affinities

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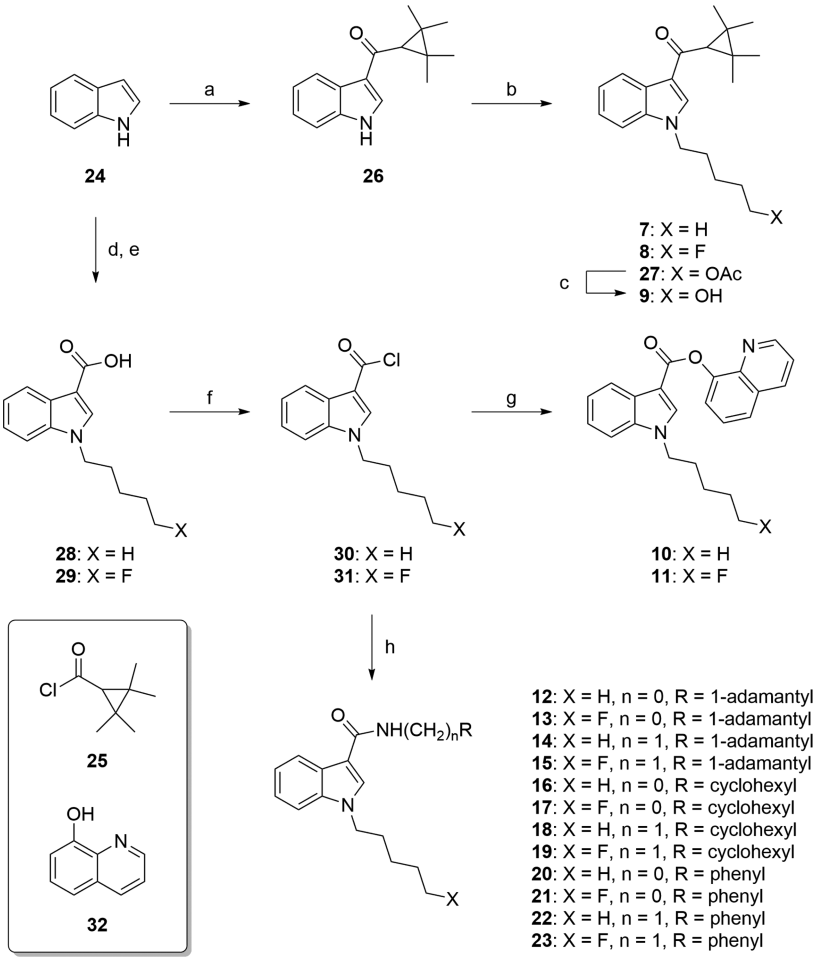
Scheme 1. Synthesis of Synthetic Cannabinoids 7−23[a]



[a]Reagents and conditions: (a) **25**, Me_2AlCl, CH_2Cl_2, 0 °C to rt, 3 h, 82%; (b) NaH, $Br(CH_2)_4X$, DMF, 0 °C to rt, 1 h, 67−91%; (c) aq. NaOH, MeOH,-THF, rt, 16 h, 94%; (d) NaH (2.0 equiv), $Br(CH_2)_4X$, DMF, 0 °C to rt, then $(CF_3CO)_2O$, 0 °Cto rt, 1 h; (e) KOH, MeOH, PhMe, reflux, 2 h, 79−88% (over 2 steps); (f) $(COCl)_2$, DMF (cat.), CH_2Cl_2, rt, 1 h, quant.; (g) **32**, Et_3N, CH_2Cl_2, rt, 24 h, 78−86%; (h) $R(CH_2)_nNH_2$, Et_3N, CH_2Cl_2, rt, 14 h, 73−90%.

and functional activities at cannabinoid receptors that are comparable to UR-144 (CB_1 K_i = 29 nM; CB_2 K_i = 4.5 nM) and that both compounds show a preference for CB_2 receptors.[23] UR-144 and XLR-11 also showed similar cannabimimetic potencies, greater than Δ^9-THC, in mice.[23]

The indole-3-carboxylate derivative PB-22 (QUPIC, **10**) and its 5-fluoropentyl analogue, 5F-PB-22 (**11**), were similarly unprecedented when discovered by forensic scientists in 2013.[24−26] Like AM-2201,[27] PB-22 and 5F-PB-22 were implicated in clinical reports of seizure,[28,29] and the latter was detected in several fatal intoxications in the USA.[30] The metabolism of PB-22 and 5F-PB-22 has been investigated, but little else is known about the effects of these SCs.[31−33] The adamantane-derived indole-3-carboxamide APICA (2NE1, SDB-001, **12**) was also unprecedented when discovered in SC products,[34] and 5-fluoro-APICA (sold as STS-135, **13**) was identified shortly thereafter.[26] The phase I metabolism of APICA and STS-135 was recently published,[35] and the pharmacology of APICA was explored (CB_1 IC_{50} = 175 nM; CB_2 IC_{50} = 176 nM),[17,36] but like PB-22 and 5F-PB-22, there are no scientific reports regarding the activity of STS-135.

The increasing popularity of *N*-(5-fluoropentyl)indole SCs is concerning because of the limited information regarding their pharmacology and toxicity, as well as those of their metabolites. Oxidation of JWH-018 produces several bioactive hydroxylated metabolites, some of which exhibit cannabinoid activity as potent as the parent compound, raising concerns about their toxicity and ultimate fate in the human body.[37−40] Many terminally fluorinated *N*-pentylindole SCs undergo thermolytic defluorination due to the route of administration (smoking), as well as metabolic oxidative defluorination *in vivo*.[33,41−44] For example, the 5-hydroxylated metabolite **6** is common to both JWH-018 and AM-2201.[9,38,43,45] Similarly, UR-144 and XLR-11 share a common 5-hydroxylated metabolite (**9**).[9,42] There is also justifiable concern regarding the fate of N-dealkylated metabolites of fluorinated SCs, given their potential for metabolism to toxic fluorinated metabolites like fluoroacetic acid.

RESULTS AND DISCUSSION

The aim of the present study was to address the paucity of data in the scientific literature regarding the pharmacology of fluorinated SCs. To this end, JWH-018, UR-144, PB-22, and APICA were compared to the corresponding 5-fluoropentyl analogues AM-2201, XLR-11, 5F-PB-22, and STS-135, respectively. The cannabinoid activity of 5-OH-UR-144, a

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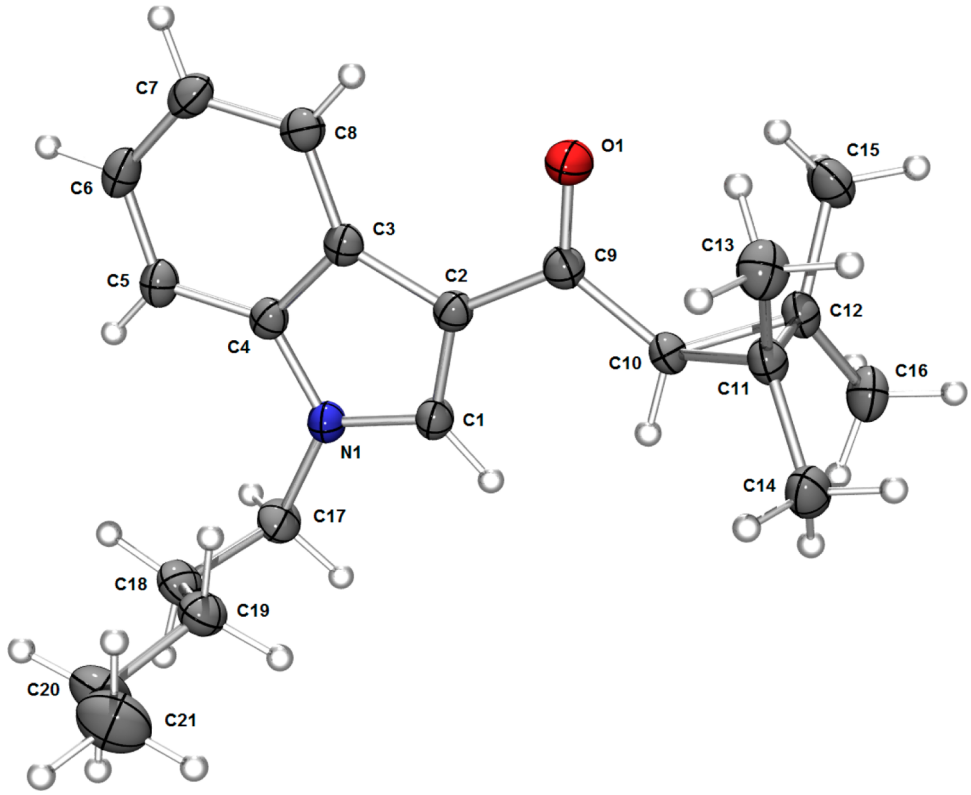

Figure 2. ORTEP diagram of the crystal structure of UR-144 (**7**) with thermal ellipsoids at the 50% probability level.

Table 1. Functional Activity of Δ^9-THC and Indole SCs 4, 5, and 7−23 at CB_1 and CB_2 Receptors

	hCB$_1$		hCB$_2$		
compound	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)	6.60 ± 0.11 (250)	51 ± 3	5.94 ± 0.57 (1157)	13 (at 10 μM)	4.6
3 (WIN 55,212-2)	6.55 ± 0.06 (284)		7.21 ± 0.09 (62)		0.2
4 (JWH-018)	6.99 ± 0.09 (102)	107 ± 6	6.88 ± 0.06 (133)	95 ± 5	1.3
5 (AM-2201)	7.43 ± 0.09 (38)	111 ± 6	7.23 ± 0.10 (58)	102 ± 7	1.5
7 (UR-144)	6.38 ± 0.06 (421)	94 ± 4	7.15 ± 0.05 (72)	104 ± 3	0.2
8 (XLR-11)	7.01 ± 0.07 (98)	110 ± 4	7.08 ± 0.15 (83)	117 ± 10	0.8
9 (5-OH-UR-144)	5.71 ± 0.12 (1959)	159 ± 11	8.18 ± 0.11 (6.5)	102 ± 5	0.003
10 (PB-22)	8.30 ± 0.06 (5.1)	114 ± 3	7.43 ± 0.08 (37)	101 ± 5	7.3
11 (5F-PB-22)	8.55 ± 0.10 (2.8)	108 ± 5	7.97 ± 0.07 (11)	101 ± 3	3.9
12 (APICA)	6.89 ± 0.11 (128)	100 ± 6	7.54 ± 0.11 (29)	91 ± 5	0.2
13 (STS-135)	7.29 ± 0.12 (51)	123 ± 8	7.88 ± 0.26 (13)	114 ± 13	0.3
14 (SDB-002)	6.58 ± 0.08 (264)	53 ± 3	7.24 ± 0.26 (57)	23 ± 4	0.2
15 (5F-SDB-002)	6.56 ± 0.16 (273)	87 ± 8	6.69 ± 0.12 (206)	39 ± 3	0.8
16 (SDB-003)	6.78 ± 0.06 (166)	82 ± 3	6.99 ± 0.08 (102)	95 ± 5	0.6
17 (5F-SDB-003)	7.13 ± 0.12 (75)	104 ± 7	7.53 ± 0.06 (29)	84 ± 3	0.4
18 (SDB-004)	6.68 ± 0.05 (207)	104 ± 3	6.67 ± 0.09 (216)	71 ± 5	1.0
19 (5F-SDB-004)	7.39 ± 0.06 (41)	107 ± 4	7.20 ± 0.12 (63)	62 ± 4	1.5
20 (SDB-005)	6.94 ± 0.07 (116)	99 ± 4	6.86 ± 0.12 (140)	74 ± 6	1.2
21 (5F-SDB-005)	6.83 ± 0.13 (148)	92 ± 7	6.87 ± 0.09 (136)	69 ± 4	0.9
22, SDB-006)	6.94 ± 0.09 (115)	96 ± 5	6.88 ± 0.22 (134)	68 ± 9	1.2
23, 5F-SDB-006	7.30 ± 0.09 (50)	87 ± 4	6.91 ± 0.11 (123)	61 ± 4	2.5

[a]CB$_1$ selectivity expressed as CB$_2$ EC$_{50}$ divided by CB$_1$ EC$_{50}$.

common metabolite of UR-144 and XLR-11, was also assessed. To more fully examine the effects of terminal fluorination on the cannabinoid activity of SCs, the 5-fluoropentyl congeners of previously described APICA analogues SDB-002 (**14**), -003 (**16**), -004 (**18**), -005 (**20**), and -006 (**22**) were also synthesized and subjected to pharmacological evaluation. Although there are no literature reports of the identification of 5F-SDB-002 (**15**), -003 (**17**), -004 (**19**), or -005 (**21**), both SDB-006 and 5F-SDB-006 (**23**) were recently identified in Finland.[46]

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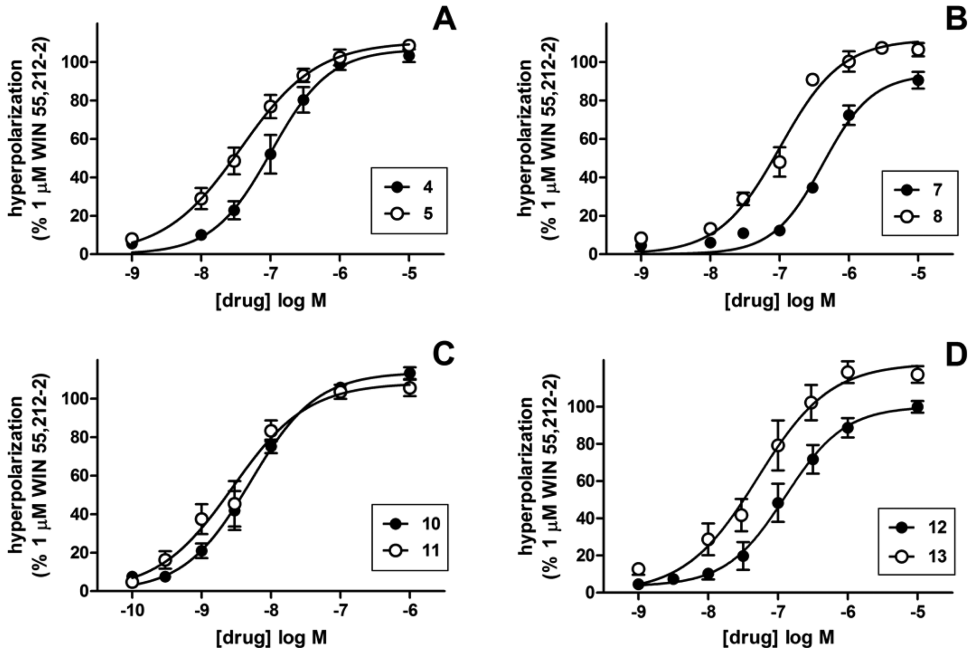

Figure 3. Hyperpolarization of CB_1 receptors induced by (A) JWH-018 (**4**) and AM-2201 (**5**), (B) UR-144 (**7**) and XLR-11 (**8**), (C) PB-22 (**10**) and 5F-PB-22 (**11**), and (D) APICA (**12**) and STS-135 (**13**) as a proportion of that produced by 1 μM WIN 55,212-2. Membrane potential was measured using a fluorescent dye, as outlined in the Methods. Each point represents the mean ± SEM of at least five independent determinations, each performed in duplicate. Data was fitted with a four parameter logistic equation in Graphpad Prism.

JWH-018 and AM-2201 were purchased from the National Measurement Institute (NMI), Australia. The synthesis of **7** to **23** is shown in Scheme 1. The synthesis of **7**−**9** started from indole (**24**), which was acylated with 2,2,3,3-tetramethylcyclopropyl-1-carbonyl chloride (**25**, freshly prepared from the corresponding carboxylic acid) under Okauchi conditions[47] to give **26** in 82% yield. Alkylation of **26** with 1-bromopentane or 1-bromo-5-fluoropentane gave **7** or **8**, respectively. Attempts to reverse the order of operations by performing the alkylation and then acylation were successful for the preparation of **7** but not **8**, due to incompatibility of the fluoroalkyl group with Lewis acids. Alkylation of **26** with the 5-bromopentyl acetate under the same conditions gave **27**, and saponification of the ester cleanly furnished **9**.

The synthesis of PB-22 and 5F-PB-22 started from indole, which was alkylated with either 1-bromopentane or 5-fluoro-1-bromopentane in the presence of excess sodium hydride and treated with trifluoroacetic anhydride to generate the intermediate N-alkylated 3-(trifluoroacetyl)indole in one pot, the hydrolysis of which provided either carboxylic acid **28** or **29**, respectively. Treating **28** or **29** with oxalyl chloride gave acid chlorides **30** and **31**, respectively, each of which was treated with 8-hydroxyquinoline (**32**) to yield esters **10** and **11**. Alternative, treating **30** and **31** with the appropriate amines gave the desired carboxamides **12**−**23** in yields of 73−90%.

Several of these novel SCs formed large prismatic crystals during recrystallization, especially the 2,2,3,3-tetramethylcyclopropanone derivatives **7** and **8**. A single crystal of **7** was obtained by slow evaporation of an isopropanol−water mixture, and an X-ray crystal structure was obtained. An ORTEP diagram of the crystal structure of **7** is shown in Figure 2. All bond lengths and angles were as expected, with the pentyl chain in a fully extended conformation. Full details of X-ray data collection and tables of bond lengths and angles are available in the Supporting Information.

All synthesized SCs were screened against CB_1 and CB_2 receptors in a fluorometric imaging plate reader (FLIPR) membrane potential assay to provide basic structure−activity relationships for agonist activity at each CB receptor subtype (see Table S8 of the Supporting Information for comparisons to available binding affinity data). Additionally, selected fluoro/des-fluoro-SC pairs were evaluated *in vivo* to allow direct comparison of the relative potency of JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135.

The cannabimimetic activity of indoles **4**, **5**, and **7**−**23** at CB_1 and CB_2 receptors was compared with the activity of established agonist Δ^9-THC, and the results are shown in Table 1. Mouse AtT20 neuroblastoma cells were stably transfected with human CB_1 or CB_2 receptors, and activities of Δ^9-THC and **7**−**23** were evaluated using a FLIPR membrane potential assay whereby endogenously expressed G protein-gated inwardly rectifying K^+ channels (GIRKs) are activated by agonists at the coexpressed CB_1 or CB_2 receptors.[48,49] The maximum effects of **4**, **5**, and **7**−**23** were compared with the high efficacy CB_1/CB_2 receptor full agonist WIN 55,212-2, which produced a maximal decrease in fluorescence, corresponding to cellular hyperpolarization, of 29% ± 2% in AtT20-CB_1 cells and 31% ± 3% in AtT20-CB_2 cells. None of the compounds produced a significant change in the membrane potential of wild-type AtT-20 cells, which do not express CB1 or CB2 receptors.

All SCs activated CB_1 and CB_2 receptors and, with few exceptions, did so with greater potency than Δ^9-THC (250 nM) for CB_1 receptor-mediated activation of GIRK (Table 1). The psychoactivity of cannabinoid ligands is largely attributed to activation of the CB_1 receptor,[3] focusing our attention to structure−activity relationships (SAR) for this series of SCs around the CB_1 receptor-mediated activation of GIRK. Δ^9-THC is a low efficacy CB_2 agonist, and in the assay of GIRK activation in AtT20-CB_2, its effects at 10 μM were only 13% of

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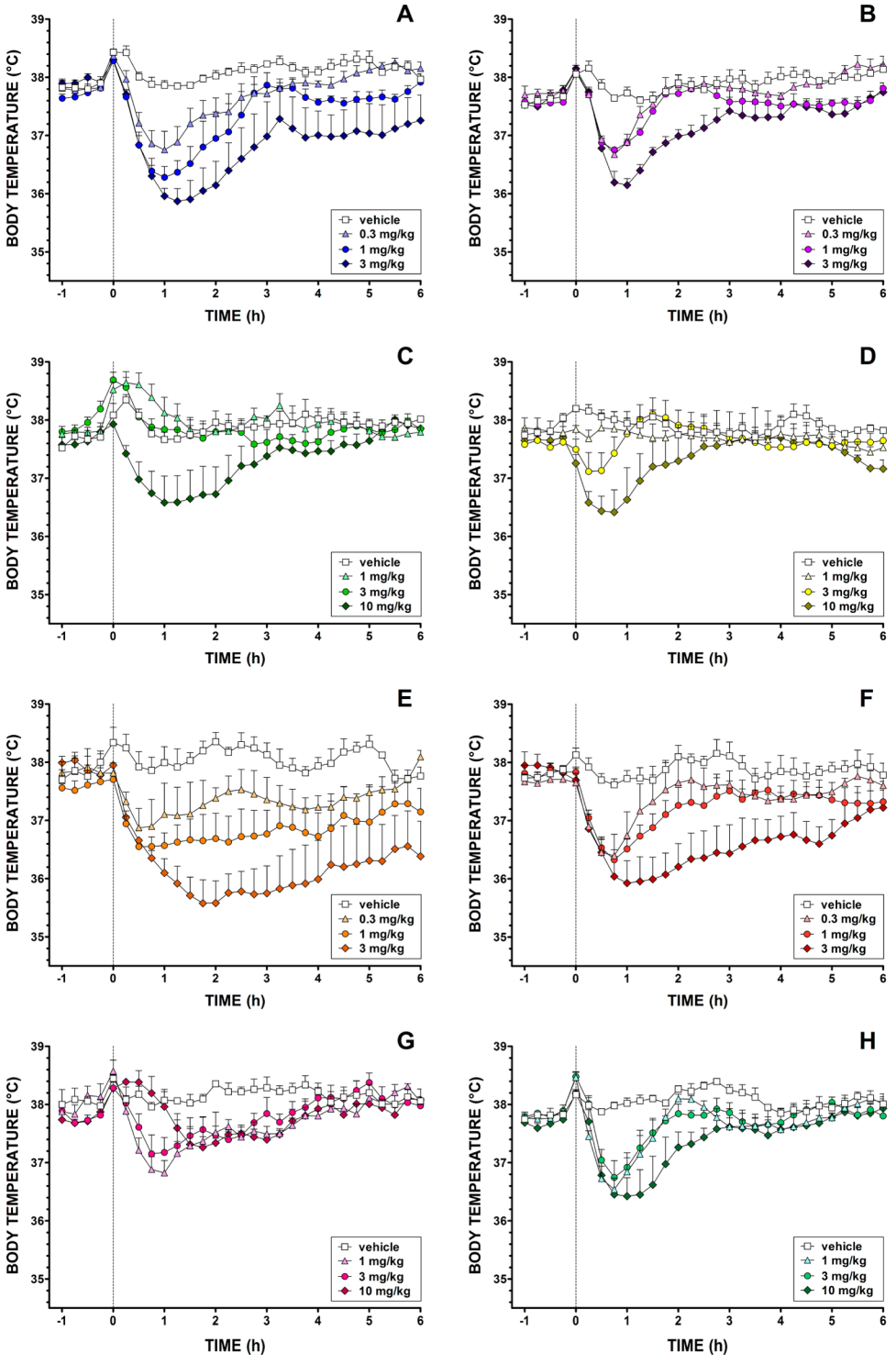

Figure 4. Effects of (A) JWH-018, (B) AM-2201, (C) UR-144, (D) XLR-11, (E) PB-22, (F) 5F-PB-22, (G) APICA, and (H) STS-135 on rat body temperature. Dashed line denotes time of intraperitoneal injection. Each point represents the mean ± SEM for three animals.

CB_1/CB_2 agonist WIN 55,212-2. WIN 55,212-2 showed an approximately 4-fold preference for stimulating a cellular hyperpolarization in AtT-20-CB2 compared with AtT-20-CB1 cells.

The most potent compounds in the series were PB-22 (CB_1 EC_{50} = 5.1 nM; CB_2 EC_{50} = 37 nM) and 5F-PB-22 (CB_1 EC_{50} = 2.8 nM; CB_2 EC_{50} = 11 nM), both possessing nanomolar potency at CB_1 receptors. PB-22 and 5F-PB-22 were an order of magnitude more potent at CB_1 receptors than the next most potent SCs AM-2201 (CB_1 EC_{50} = 38 nM), STS-135 (CB_1 EC_{50} = 51 nM), 5F-SDB-004 (CB_1 EC_{50} = 41 nM), and 5F-SDB-006 (CB_1 EC_{50} = 50 nM). Most SCs in the series demonstrated little selectivity for either CB receptor subtype, with the exception of 5-OH-UR-144, a UR-144 metabolite, which was a potent and selective CB_2 receptor agonist (EC_{50} = 6.5 nM, 300-fold selectivity).

Excluding two des-fluoro/fluoro analogue pairs (**14**/**15** and **20**/**21**), for which there was little change, terminal fluorination

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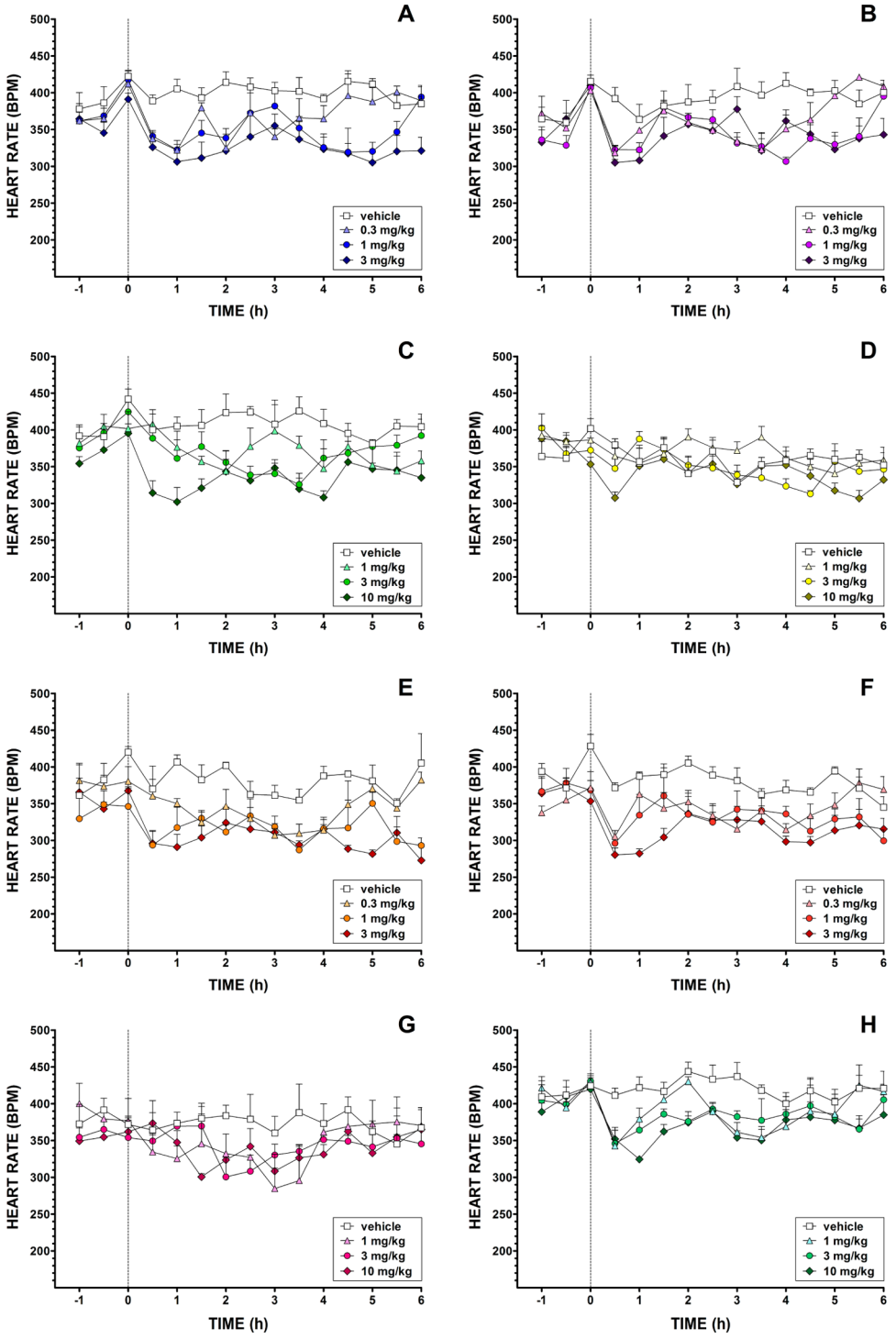

Figure 5. Effects of (A) JWH-018, (B) AM-2201, (C) UR-144, (D) XLR-11, (E) PB-22, (F) 5F-PB-22, (G) APICA, and (H) STS-135 on rat heart rate. Dashed line denotes time of intraperitoneal injection. Each point represents the mean ± SEM for three animals.

produced a roughly 2−5-fold increase in CB_1 receptor potency. This was most pronounced moving from UR-144 (EC_{50} = 421 nM) to XLR-11 (EC_{50} = 98 nM) and from **18** (EC_{50} = 207 nM) to **19** (EC_{50} = 41 nM). Interestingly, **14** possessed the lowest efficacy at CB_1 and CB_2 receptors in the series, and while fluorination to give **15** did not improve potency, it did increase efficacy at CB_1 and CB_2 receptors. The relative potency change for des-fluoro/fluoro pairs JWH-018/AM-2201, UR-144/XLR-11, PB-22/5F-PB-22, and APICA/STS-135 is depicted in Figure 3.

Our results with UR-144, XLR-11, and common metabolite 5-OH-UR-144 are broadly consistent with previous studies. In an assay of GTPγS binding in HEK 293 cell membranes, Wiley and colleagues found that UR-144 showed a preference for CB_1, in contrast to our findings of a CB_2 preference for UR-144.[23] Wiley and colleagues found that XLR-11 had a similar potency at CB_1 and CB_2 receptors, consistent with XLR-11 in our assay.[23] A definitive explanation for these differences is not possible, but it should be noted that HEK 293 cells are thought to express a significantly different complement of G proteins

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from AtT-20 cells,[50] and any signaling differences between ligands might be magnified by the cumulative nature of the GTPγS binding assay when compared with the acute, peak effect measurements made for GIRK activation. Consistent with our data from the GIRK assay, UR-144/XLR-11 common metabolite 5-OH-UR-144 was previously reported to be highly CB_2 preferring in binding assays, significantly more so than UR-144 and XLR-11.[12,23]

Although the potency of any drug effect depends on the number of receptors expressed in the system under study, and we have not directly measured the number of receptors in our AtT-20-CB_1 and -CB_2 cells at the time of these assays, WIN 55,212-2 is recognized as having a higher affinity for CB_2 receptors than CB_1 receptors in binding assays,[11,12] so it is likely that CB_2 preference of the SCs is genuine and not an artifact of our expression systems.

Various Internet drug forums contain anecdotal reports by SC consumers regarding the potency and psychoactivity of these compounds. For more well-studied SCs, like JWH-018, cross-substitution with Δ^9-THC has been demonstrated.[51−53] However, for the majority of SCs, there are little to no pharmacological data or formal *in vivo* evaluations available. The ability to induce hypothermia and bradycardia in rats is common to phytocannabinoid Δ^9-THC and newer, non-classical synthetic cannabinoids such as JWH-018.[23,36,54,55] The cannabimimetic activities of des-fluoro/fluoro SC pairs JWH-018/AM-2201, UR-144/XLR-11, PB-22/5F-PB-22, and APICA/STS-135 were therefore evaluated using biotelemetry in male Wistar rats, and the effects of various doses of JWH-018/AM-2201, UR-144/XLR-11, PB-22/5F-PB-22, and APICA/STS-135 on body temperature (Figure 4) and heart rate (Figure 5) are presented.

Body temperature 1 h prior to intraperitoneal (ip) injection and 6 h postinjection of JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135 are presented in 15 min bins in Figure 4. The dashed line on the figures represents the time of SC injection. Doses were escalated from 0 (baseline) to 3 mg/kg for all compounds. If a large magnitude effect on body temperature was not evident at a 3 mg/kg dose (defined as an approximate mean maximal drop in body temperature of 1.5 °C) then a further 10 mg/kg dose was tested. Each SC was investigated in an ascending dose sequence using a single cohort of three or four rats, with a new cohort of rats used for each SC.

Results showed a dose-dependent hypothermia for all SCs, with statistically significant treatment or treatment by time effects at the specified doses (ANOVA, planned contrasts, SC dose versus vehicle, $P < 0.05$). A large hypothermic effect (mean >1.5 °C) was evoked by 3 mg/kg of JWH-018, AM-2201, PB-22, and 5F-PB-22. However, doses of 10 mg/kg of APICA, STS-135, UR-144, and XLR-11 were required to induce more modest hypothermia, indicating lower *in vivo* potency of these compounds. Terminal fluorination had no obvious effect on *in vivo* potency in any of the pairs of SCs examined. This can be clearly seen in Figure S5, Supporting Information, which displays the mean maximal decrease in body temperature induced by different doses of each compound, and Figure S6, Supporting Information, which shows the area under the curve (AUC) for body temperature for each dose of each compound relative to baseline. Formal statistical comparison of each pair of fluorinated versus nonfluorinated compounds showed no significant statistical difference between any of these pairs.

On the basis of the mean maximal hypothermia and AUC obtained with a 3 mg/kg dose of each compound (Figures S5 and S6, Supporting Information), it is possible to rank SCs in decreasing order of potency: PB-22 > 5F-PB-22 = JWH-018 > AM-2201 > APICA = STS-135 = XLR-11 > UR-144.

Results for heart rate are presented in 30 min bins in Figure 5 with the dashed line on the figures again representing the time of ip injection of each SC. Results were generally consistent with body temperature data, although data were generally more variable than with body temperature data, reflecting the multiple determinants of heart rate including locomotor activity, stress, and direct cardiovascular pharmacological effects. All doses shown produced a significant decrease in heart rate, with statistically significant treatment or treatment by time effects at these doses (ANOVA, planned contrasts, SC dose versus vehicle, $P < 0.05$).

There is variability in the duration of effects for each SC, as determined by hypothermic and bradycardic responses. A point of interest is the potential two-stage hypothermic and bradycardic response demonstrated by JWH-018 and AM-2201. Given their several common metabolites, it is possible that the second hypothermic and bradycardic reponse oberserved for JWH-018 and AM-2201 around 4−5 h postinjection may be due to the combination of active parent compounds and active metabolites.

CONCLUSION

This study is the first to pharmacologically characterize the effect of terminal fluorination across structurally diverse classes of 3-subsituted *N*-pentylindole SC designer drugs, exemplified by JWH-018/AM-2201, UR-144/XLR-11, PB-22/5F-PB-22, and APICA/STS-135. A synthetic route to UR-144, XLR-11, and their common metabolite 5-OH-UR-144 was established. The synthesis of PB-22, 5F-PB-22, APICA, STS-135, and several analogues of the latter was achieved. These routes may prove useful to forensic chemists and pharmacologists interested in the cannabinoid activity of novel *N*-alkyl-3-acylindoles, *N*-alkylindole-3-carboxylates, and *N*-alkylindole-3-carboxamides. All synthesized SCs acted as agonists of CB_1 and CB_2 receptors in a FLIPR membrane potential assay and thus are cannabinoids.

Preliminary structure−activity relationships suggest that terminal fluorination of the *N*-pentyl substituent of these SCs generally enhances potency of CB_1 receptor activation, consistent with previously demonstrated improvements to binding affinity conferred by such a change, as well as anecdotal reports of the potent psychoactive effects of fluorinated SCs. However, in rats, although JWH-018/AM-2201, UR-144/XLR-11, PB-22/5F-PB-22, and APICA/STS-135 were able to dose-dependently decrease body temperature and heart rate at doses of 0.3−10 mg/kg, depending on the SC, there was no obvious effect of fluorination on *in vivo* potency. The reasons for the discrepancy between *in vitro* and *in vivo* results is not entirely clear but may reflect pharmacokinetic factors, species-related differences in ligand affinity and efficacy, or an inability of body temperature and heart rate measures to detect subtle differences in overall potency. Nonetheless, the *in vivo* results confirm that all of the SCs explored have cannabimimetic effects that parallel those of Δ^9-THC, albeit with a wide range of differing potencies across compounds.

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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 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) were recorded using electrospray ionization (ESI) 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.

General Procedure A: N-Alkylation of 1H-Indole-3-yl(2,2,3,3-tetramethylcyclopropyl)methanone. A cooled (0 °C) solution of **26** (241 mg, 1.0 mmol) in DMF (2 mL) was treated portionwise with sodium hydride (60% dispersion in mineral oil, 80 mg, 2.0 mmol, 2.0 equiv), stirred for 10 min, allowed to warm to ambient temperature, and stirred for 1 h. The mixture was cooled to 0 °C and treated with the appropriate bromoalkane (1.05 mmol, 1.05 equiv) in a single portion, allowed to warm to ambient temperature, and stirred for 1 h. The mixture was poured portionwise onto cooled (0 °C) half-saturated aq. NH_4Cl (40 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL) and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give crude products. The crude products were purified using flash chromatography or recrystallization.

*(1-Pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)-methanone (**7**).* Treating **26** (241 mg, 1.0 mmol) with 1-bromopentane (130 μL, 1.05 mmol) according to general procedure A gave, following recrystallization from isopropanol, **7** (283 mg, 91%) as prismatic colorless crystals. R_f 0.41 (hexane−EtOAc, 65:35); mp (*i*-PrOH−H_2O) 72−74 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.42−8.40 (1H, m, ArH), 7.66 (1H, s, ArH), 7.35−7.33 (1H, m, ArH), 7.29−7.24 (2H, m, ArH), 4.14 (2H, t, J = 7.5 Hz, NCH_2), 1.95 (1H, s, CH), 1.89 (2H, quin., J = 7.5 Hz, CH_2), 1.40−1.33 (10H, m, 2 × CH_2, 2 × CH_3), 1.31 (6H, s, 2 × CH_3), 0.91 (3H, t, J = 7.0 Hz, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.7 (CO), 136.8 (quat.), 133.6 (CH), 126.5 (quat.), 123.0 (CH), 122.8 (CH), 122.2 (CH), 119.8 (quat.), 109.8 (CH), 47.1 (NCH_2), 41.8 (CH), 31.6 ($C_{quat.}$), 29.8 (CH_2), 29.2 (CH_2), 24.2 (CH_3), 22.4 (CH_2), 17.2 (CH_3), 14.0 (CH_3). LRMS (+ESI) m/z 644.80 ([2M + Na]$^+$, 80%), 622.40 ([2M]$^+$, 55%), 312.07 ([M + H]$^+$, 100%). Anal. ($C_{21}H_{29}NO$) Calcd: C 80.98, H 9.38, N 4.50. Found: C 80.99, H 9.54, N 4.49.

*(1-(5-Fluoropentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone (**8**).* Treating **26** (241 mg, 1.0 mmol) with 1-bromo-5-fluoropentane (177 mg, 1.05 mmol) according to general procedure A, followed by purification using flash chromatography (hexane−EtOAc, 90:10), gave **8** (287 mg, 87%) as a white crystalline solid. R_f 0.47 (hexane−EtOAc, 80:20); mp (*i*-PrOH−H_2O) 76−77 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41−8.39 (1H, m, ArH), 7.65 (1H, s, ArH), 7.33−7.32 (1H, m, ArH), 7.29−7.24 (2H, m, ArH), 4.43 (2H, dt, $^2J_{H-F}$ = 47.0 Hz, $^3J_{H-H}$ = 6.0 Hz, CH_2F), 4.17 (2H, t, J = 7.3 Hz, NCH_2), 1.97−1.91 (3H, m, $NCH_2C\underline{H}_2$, CH), 1.77−1.69 (2H, m, $C\underline{H}_2CH_2F$), 1.52−1.47 (2H, m, $NCH_2CH_2C\underline{H}_2$), 1.35 (6H, s, CH_3), 1.30 (6H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.8 (CO), 136.7 (quat.), 133.5 (CH), 126.5 (quat.), 123.1 (CH), 122.9 (CH), 122.3 (CH), 119.9 (quat.), 109.7 (CH), 83.8 (d, $^1J_{C-F}$ = 165.0 Hz, CH_2F), 47.0 (NCH_2), 41.8 (CH), 31.8 ($C_{quat.}$), 30.1 (d, $^2J_{C-F}$ = 19.7 Hz, CH_2), 29.8 (CH_2) 24.2 (CH_3), 23.0 (d, $^3J_{C-F}$ = 4.8 Hz, CH_2), 17.2 (CH_3). LRMS (+ESI) m/z 680.73 ([2M + Na]$^+$, 100%), 658.53 ([2M]$^+$, 42%), 330.20 ([M + H]$^+$, 98%). Anal. ($C_{21}H_{28}NOF$) Calcd: C 76.56, H 8.57, N 4.25. Found: C 76.65, H 8.58, N 4.31.

*(1-(5-Hydroxypentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone (**9**).* A solution of **27** (185 mg, 0.5 mmol) in THF (2.5 mL) was treated dropwise with a solution of NaOH (40 mg, 1.0 mmol, 2.0 equiv) in MeOH−H_2O (80:20, 2.5 mL), and the mixture was stirred for 16 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between half-saturated aq. $NaHCO_3$ (10 mL) and CH_2Cl_2 (10 mL). The layers were separated, and the aqueous layer was washed with CH_2Cl_2 (2 × 5 mL). The combined organic phases were washed with brine (10 mL) and dried ($MgSO_4$), and the solvent was evaporated. The crude product was purified using flash chromatography (hexane−EtOAc, 50:50, R_f 0.22) from **8** (154 mg, 94%) to give **9** as a white crystalline solid. Mp 80−81 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41−8.39 (1H, m, ArH), 7.66 (1H, s, ArH), 7.34−7.33 (1H, m, ArH), 7.28−7.24 (2H, m, ArH), 4.16 (2H, t, J = 7.5 Hz, NCH_2), 3.64 (2H, t, J = 6.5 Hz, $C\underline{H}_2OH$), 1.95 (1H, s, CH), 1.92 (2H, quin., J = 7.5 Hz, CH_2), 1.64−1.58 (2H, m, CH_2), 1.55 (1H, br s, OH), 1.47−1.43 (2H, m, CH_2), 1.35 (6H, s, 2 × CH_3), 1.31 (6H, s, 2 × CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.8 (CO), 136.7 (quat.), 133.6 (CH), 126.5 (quat.), 123.1 (CH), 122.8 (CH), 122.2 (CH), 119.8 (quat.), 109.7 (CH), 62.6 (CH_2OH), 47.1 (NCH_2), 41.8 (CH), 32.3 (CH_2), 31.8 ($C_{quat.}$), 29.9 (CH_2), 24.2 (CH_3), 23.4 (CH_2), 17.2 (CH_3). LRMS (+ESI) m/z 676.73 ([2M + Na]$^+$, 89%), 654.47 ([2M]$^+$, 79%), 350.00 ([M + Na]$^+$, 32%), 328.00 ([M + H]$^+$, 100%). Anal. ($C_{21}H_{29}NO_2$) Calcd: C 77.02, H 8.93, N 4.28. Found: C 76.64, H 9.27, N 4.32.

General Procedure B: Esterification of 1-Alkylindole-3-carboxylic Acids. A suspension of the appropriate 1-alkylindole-3-carboxylic acid (**28** or **29**, 1.0 mmol) in CH_2Cl_2 (2 mL) was treated with oxalyl chloride (170 μL, 2.0 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 and Et_3N (490 μL, 3.5 mmol, 3.5 equiv) in CH_2Cl_2 (5 mL) was slowly treated with a solution of 8-hydroxyquinoline (**32**, 174 mg, 1.2 mmol, 1.2 equiv) in CH_2Cl_2 (5 mL). The mixture was stirred for 24 h, the solvent was evaporated, and the residue was partitioned between EtOAc (75 mL) and H_2O (25 mL). The layers were separated, and the organic phase was washed with sat. aq. $NaHCO_3$ (3 × 25 mL) and brine (25 mL) and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The crude products were purified using flash chromatography.

*Quinolin-8-yl 1-Pentyl-1H-indole-3-carboxylate (**10**).* Treating **28** (230 mg, 1.0 mmol) with 8-hydroxyquinoline (174 mg, 1.2 mmol, 1.2 equiv) according to general procedure B gave, following purification by flash chromatography (hexane−EtOAc, 50:50, R_f 0.50), **10** (280 mg, 78%) as an off-white crystalline solid. Mp 111−112 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.92 (1H, dd, J = 4.1, 1.3 Hz), 8.33 (1H, dd, J = 7.7, 1.4 Hz), 8.19 (1H, dd, J = 8.4, 1.3 Hz), 8.17 (1H, s) 7.75 (1H, dd, J = 7.8, 1.3 Hz) 7.63−7.57 (2H, m), 7.43−7.40 (2H, m) 7.34−7.29 (2H, m), 4.20 (2H, t, J = 7.3 Hz), 1.94 (2H, quin., J = 7.1 Hz), 1.40−1.37 (4H, m), 0.93 (3H, t, J = 6.7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.4 (COO), 150.7 (CH), 147.8 ($C_{quat.}$), 142.2 ($C_{quat.}$), 136.9 ($C_{quat.}$), 136.1 (CH), 135.6 (CH), 129.7 ($C_{quat.}$), 127.5 ($C_{quat.}$), 126.4 (CH), 125.7 (CH), 123.0 (CH), 122.21 (CH), 122.19 (CH), 122.16 (CH), 121.7 (CH), 110.2 (CH), 106.1 ($C_{quat.}$), 47.3 (CH_2), 29.8 (CH_2), 29.2 (CH_2), 22.4 (CH_2), 14.0 (CH_3). LRMS (+ESI) m/z 738.73 ([2M + Na]$^+$, 53%), 716.73 ([2M]$^+$, 100%), 358.93 ([M + H]$^+$, 60%), 214.07 (M − C_9H_7N − OH, 17%). Anal. ($C_{23}H_{22}N_2O_2$) Calcd: C 77.07, H 6.19, N 7.82. Found: C 77.29, H 6.19, N 7.89.

*Quinolin-8-yl 1-(5-Fluoropentyl)-1H-indole-3-carboxylate (**11**).* Treating **29** (249 mg, 1.0 mmol) with 8-hydroxyquinoline (174 mg, 1.2 mmol, 1.2 equiv) according to general procedure B gave, following purification by flash chromatography (hexane−EtOAc, 50:50, R_f 0.38), **11** (324 mg, 86%) as an off-white/tan crystalline solid. Mp 116−117 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (1H, dd, J = 4.1, 1.5 Hz),

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8.33 (1H, dd, J = 6.9, 1.7 Hz), 8.19 (1H, dd, J = 8.3, 1.4 Hz), 8.17 (1H, s) 7.75 (1H, dd, J = 7.8, 1.5 Hz), 7.63−7.57 (2H, m), 7.43−7.40 (2H, m), 7.34−7.29 (2H, m), 4.45 (2H, dt, $^2J_{CF}$ = 47.3, $^3J_{HH}$ = 5.9 Hz, CH_2F), 4.22 (2H, t, J = 7.2 Hz), 1.99 (2H, quin., J = 7.5 Hz), 1.80−1.70 (2H, m), 1.56−1.49 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.4 (COO), 150.7 (CH), 147.8 ($C_{quat.}$), 142.1 ($C_{quat.}$), 136.8 ($C_{quat.}$), 136.1 (CH), 135.5 (CH), 129.7 ($C_{quat.}$), 127.5 ($C_{quat.}$), 126.4 (CH), 125.7 (CH), 123.1 (CH), 122.29 (CH), 122.25 (CH), 122.16 (CH), 122.7 (CH), 110.1 (CH), 106.3 ($C_{quat.}$), 83.8 (d, $^1J_{CF}$ = 166.0 Hz, CH_2F), 47.2 (CH_2), 30.1 (d, $^2J_{CF}$ = 18.9 Hz, $\underline{C}H_2CH_2F$), 29.8 (CH_2), 23.0 (d, $^3J_{CF}$ = 5.0 Hz, $\underline{C}H_2CH_2CH_2F$). LRMS (+ESI) m/z 774.73 ([2M + Na]$^+$, 66%), 752.60 ([2M]$^+$, 100%), 399.00 ([M + Na]$^+$, 33%), 376.87 ([M + H]$^+$, 99%), 232.00 (M − C_9H_7N − OH, 23%). Anal. ($C_{23}H_{21}N_2O_2F$) Calcd: C 73.39, H 5.62, N 7.44. Found: C 73.46, H 5.46, N 7.37.

General Procedure C: Amidation of 1-Alkylindole-3-carboxylic Acids. A suspension of the appropriate 1-alkylindole-3-carboxylic acid (**28** or **29**, 2.0 mmol) in CH_2Cl_2 (5 mL) was treated with oxalyl chloride (420 μL, 5.0 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 the appropriate amine (2.4 mmol, 1.2 equiv) and Et_3N (700 μL, 5.0 mmol, 2.5 equiv) in CH_2Cl_2 (20 mL). The mixture was stirred for 14 h, the solvent was evaporated, and the residue was partitioned between EtOAc (200 mL) and 1 M aq. HCl (50 mL). The layers were separated, and the organic phase was washed with 1 M aq. HCl (2 × 50 mL), sat. aq. $NaHCO_3$ (3 × 50 mL), and brine (50 mL) and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The crude products were recrystallized from *i*-PrOH or *i*-PrOH−H_2O. The preparation of **12**, **14**, **16**, **18**, **20**, and **22** was described previously.[36]

*N-(Adamantan-1-yl)-1-(5-fluoropentyl)-1H-indole-3-carboxamide (**13**).* Treating (5-fluoropentyl)indole-3-carboxylic acid (499 mg, 2.0 mmol, 1.0 equiv) with 1-aminoadamantane (363 mg, 2.4 mmol, 1.2 equiv) according to general procedure C gave **13** (663 mg, 87%) as fine white needles. Mp (*i*-PrOH−H_2O) 138−140 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88 (1H, d, J = 7.5 Hz), 7.64 (1H, s), 7.36 (1H, d, J = 7.5 Hz), 7.28−7.22 (2H, m), 5.69 (1H, br s, NH), 4.41 (2H, dt, $^2J_{CF}$ = 47.0, $^3J_{HH}$ = 6.0 Hz, CH_2F), 4.14 (2H, t, J = 7.2 Hz), 2.19 (6H, br s), 2.14 (3H, br s), 1.90 (2H, quin., J = 7.8 Hz), 1.78−1.62 (8H, m), 1.48−1.41 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.5 (CO), 136.7 (quat.), 131.3 (CH), 125.4 (quat.), 122.4 (CH), 121.3 (CH), 120.1 (CH), 112.6 (quat.), 110.3 (CH), 83.8 (d, $^1J_{CF}$ = 165.0 Hz, CH_2F), 52.2 (quat.), 46.7 (CH_2), 42.4 (CH_2), 36.7 (CH_2), 30.1 (d, $^2J_{CF}$ = 19.9 Hz, $\underline{C}H_2CH_2F$), 29.8 (CH_2), 29.7 (CH), 23.0 (d, $^3J_{CF}$ = 5.0 Hz, $\underline{C}H_2CH_2CH_2F$). LRMS (+ESI) m/z 764.80 ([2M]$^+$, 100%), 383.13 ([M + H]$^+$, 41%). Anal. ($C_{24}H_{31}N_2OF$) Calcd: C 75.36, H 8.17, N 7.32. Found: C 75.37, H 8.43, N 7.42.

*N-(Adamantan-1-ylmethyl)-1-(5-fluoropentyl)-1H-indole-3-carboxamide (**15**).* Treating (5-fluoropentyl)indole-3-carboxylic acid (499 mg, 2.0 mmol, 1.0 equiv) with 1-(aminomethyl)adamantane (397 mg, 2.4 mmol, 1.2 equiv) according to general procedure C gave **15** (579 mg, 73%) as a white crystalline solid. Mp (*i*-PrOH−H_2O) 148−150 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88 (1H, d, J = 7.5 Hz), 7.74 (1H, s), 7.39 (1H, d, J = 7.5 Hz), 7.29−7.26 (2H, m), 6.01 (1H, br s, NH), 4.42 (2H, dt, $^2J_{CF}$ = 47.5, $^3J_{HH}$ = 5.5 Hz, CH_2F), 4.16 (2H, t, J = 7.0 Hz), 3.22 (2H, d, J = 6.0 Hz), 2.02 (3H, br s), 1.92 (2H, quin., J = 7.0 Hz), 1.75−1.62 (14H, m), 1.49−1.43 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.5 (CO), 136.8 (quat.), 131.8 (CH), 125.3 (quat.), 122.5 (CH), 121.5 (CH), 120.0 (CH), 111.6 (quat.), 110.5 (CH), 83.8 (d, $^1J_{CF}$ = 165.0 Hz, CH_2F), 51.0 (CH_2), 46.8 (CH_2), 40.6 (CH_2), 37.2 (CH_2), 34.1 (quat.), 30.1 (d, $^2J_{CF}$ = 19.9 Hz, $\underline{C}H_2CH_2F$), 29.8 (CH_2), 28.5 (CH), 23.0 (d, $^3J_{CF}$ = 5.0 Hz, $\underline{C}H_2CH_2CH_2F$). LRMS (+ESI) m/z 793.00 ([2M]$^+$, 100%), 397.13 ([M + H]$^+$, 60%). Anal. ($C_{25}H_{33}N_2OF$) Calcd: C 75.72, H 8.39, N 7.06. Found: C 75.46, H 8.69, N 7.09.

*N-Cyclohexyl-1-(5-fluoropentyl)-1H-indole-3-carboxamide (**17**).* Treating (5-fluoropentyl)indole-3-carboxylic acid (499 mg, 2.0 mmol, 1.0 equiv) with cyclohexylamine (275 μL, 2.4 mmol, 1.2 equiv) according to general procedure C gave **17** (488 mg, 74%) as fine white needles. Mp (*i*-PrOH) 145−147 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89 (1H, d, J = 7.0 Hz), 7.69 (1H, s), 7.37 (1H, d, J = 7.0 Hz), 7.29−7.23 (2H, m), 5.82 (1H, d, J = 7.5 Hz, NH), 4.42 (2H, dt, $^2J_{CF}$ = 47.0, $^3J_{HH}$ = 6.0 Hz, CH_2F), 4.15 (2H, t, J = 7.0 Hz), 4.10−4.02 (1H, m), 2.10−2.07 (2H, m), 1.91 (2H, quin., J = 7.6 Hz), 1.78−1.67 (5H, m), 1.50−1.42 (4H, m), 1.33−1.20 (3H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.4 (CO), 136.7 (quat.), 131.4 (CH), 125.5 (quat.), 122.5 (CH), 121.4 (CH), 120.1 (CH), 111.6 (quat.), 110.4 (CH), 83.8 (d, $^1J_{CF}$ = 165.0 Hz, CH_2F), 48.1 (CH), 46.8 (CH_2), 33.7 (CH_2), 30.1 (d, $^2J_{CF}$ = 19.7 Hz, $\underline{C}H_2CH_2F$), 29.8 (CH_2), 25.9 (CH_2), 25.1 (CH_2), 23.0 (d, $^3J_{CF}$ = 4.9 Hz, $\underline{C}H_2CH_2CH_2F$). LRMS (+ESI) m/z 660.73 ([2M]$^+$, 100%), 331.13 ([M + H]$^+$, 78%). Anal. ($C_{20}H_{27}N_2OF$) Calcd: C 72.70, H 8.24, N 8.48. Found: C 72.38, H 8.46, N 8.42.

*N-(Cyclohexylmethyl)-1-(5-fluoropentyl)-1H-indole-3-carboxamide (**19**).* Treating (5-fluoropentyl)indole-3-carboxylic acid (499 mg, 2.0 mmol, 1.0 equiv) with (aminomethyl)cyclohexane (310 μL, 2.4 mmol, 1.2 equiv) according to general procedure C gave **19** (578 mg, 84%) as fine white crystals. Mp (*i*-PrOH) 114−116 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89 (1H, d, J = 7.0 Hz), 7.71 (1H, s), 7.38 (1H, d, J = 7.0 Hz), 7.30−7.24 (2H, m), 6.00 (1H, br s, NH), 4.42 (2H, dt, $^2J_{CF}$ = 47.0, $^3J_{HH}$ = 6.0 Hz, CH_2F), 4.15 (2H, t, J = 7.0 Hz), 3.36 (2H, t, J = 6.5 Hz), 1.91 (2H, quin., J = 7.8 Hz), 1.85−1.82 (2H, m), 1.78−1.60 (6H, m), 1.48−1.42 (2H, m), 1.31−1.17 (3H, m), 1.04 (2H, ddd, J = 24.3, 12.3, 3.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.4 (CO), 136.7 (quat.), 131.6 (CH), 125.4 (quat.), 122.5 (CH), 121.5 (CH), 120.1 (CH), 111.5 (quat.), 110.4 (CH), 83.8 (d, $^1J_{CF}$ = 165.0 Hz, CH_2F), 46.8 (CH_2), 45.8 (CH_2), 38.4 (CH), 31.2 (CH_2), 30.1 (d, $^2J_{CF}$ = 19.9 Hz, $\underline{C}H_2CH_2F$), 29.8 (CH_2), 26.6 (CH_2), 26.1 (CH_2), 23.0 (d, $^3J_{CF}$ = 4.9 Hz, $\underline{C}H_2CH_2CH_2F$). LRMS (+ESI) m/z 710.93 ([2M + Na]$^+$, 95%), 688.73 ([2M]$^+$, 100%), 345.07 ([M + H]$^+$, 48). Anal. ($C_{21}H_{29}N_2OF$) Calcd: C 73.22, H 8.49, N 8.13. Found: C 72.97, H 8.62, N 8.01.

*N-Phenyl-1-(5-fluoropentyl)-1H-indole-3-carboxamide (**21**).* Treating (5-fluoropentyl)indole-3-carboxylic acid (499 mg, 2.0 mmol, 1.0 equiv) with aniline (220 μL, 2.4 mmol, 1.2 equiv) according to general procedure C gave **21** (584 mg, 90%) as a white powder. Mp (*i*-PrOH) 116−119 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07−8.05 (1H, m), 7.79 (1H, s), 7.71 (1H, br s, NH) 7.67 (2H, d, J = 8.0 Hz), 7.42−7.36 (3H, m), 7.34−7.29 (2H, m), 7.13 (1H, t, J = 7.5 Hz), 4.42 (2H, dt, $^2J_{CF}$ = 47.0, $^3J_{HH}$ = 6.0 Hz, CH_2F), 4.18 (2H, t, J = 7.0 Hz), 1.93 (2H, quin., J = 7.5 Hz), 1.76−1.68 (2H, m), 1.50−1.44 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.3 (CO), 138.6 (quat.), 136.8 (quat.), 131.8 (CH), 129.2 (CH), 125.6 (quat.), 124.0 (CH), 122.9 (CH), 121.9 (CH), 120.4 (CH), 120.2 (CH), 111.4 (quat.), 110.5 (CH), 83.8 (d, $^1J_{CF}$ = 165.0 Hz, CH_2F), 46.9 (CH_2), 30.1 (d, $^2J_{CF}$ = 19.9 Hz, $\underline{C}H_2CH_2F$), 29.7 (CH_2), 23.0 (d, $^3J_{CF}$ = 4.9 Hz, $\underline{C}H_2CH_2CH_2F$). LRMS (+ESI) m/z 670.73 ([2M + Na]$^+$, 90%), 648.67 ([2M]$^+$, 89%), 325.00 ([M + H]$^+$, 100). Anal. ($C_{20}H_{21}N_2OF$) Calcd: C 74.05, H 6.53, N 8.64. Found: C 74.20, H 6.69, N 8.65.

*N-Benzyl-1-(5-fluoropentyl)-1H-indole-3-carboxamide (**23**).* Treating (5-fluoropentyl)indole-3-carboxylic acid (499 mg, 2.0 mmol, 1.0 equiv) with benzylamine (260 μL, 2.4 mmol, 1.2 equiv) according to general procedure C gave **23** (549 mg, 81%) as fine white needles. Mp (*i*-PrOH) 128−130 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.94 (1H, d, J = 8.0 Hz), 7.72 (1H, s), 7.42−7.34 (5H, m), 7.31−7.22 (3H, m), 6.25 (1H, br s, NH), 4.71 (2H, d, J = 5.5 Hz), 4.41 (2H, dt, $^2J_{CF}$ = 47.5 Hz, $^3J_{HH}$ = 6.0 Hz, CH_2F), 4.14 (2H, t, J = 7.5 Hz), 1.91 (2H, quin., J = 8.0 Hz), 1.76−1.64 (2H, m), 1.48−1.41 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.2 (CO), 139.0 (quat.), 136.7 (quat.), 131.5 (CH), 128.9 (CH), 128.0 (CH), 127.6 (CH), 125.6 (quat.), 122.6 (CH), 121.6 (CH), 120.4 (CH), 111.0 (quat.), 110.3 (CH), 83.8 (d, $^1J_{CF}$ = 165.0 Hz, CH_2F), 46.8 (CH_2), 43.7 (CH_2), 30.1 (d, $^2J_{CF}$ = 19.9 Hz, $\underline{C}H_2CH_2F$), 29.8 (CH_2), 23.0 (d, $^3J_{CF}$ = 4.9 Hz, $\underline{C}H_2CH_2CH_2F$). LRMS (+ESI) m/z 698.73 ([2M + Na]$^+$, 100%), 676.87 ([2M]$^+$, 31%), 339.13 ([M + H]$^+$, 29). Anal. ($C_{21}H_{23}N_2OF$) Calcd: C 74.53, H 6.85, N 8.28. Found: C 74.59, H 6.94, N 8.36.

*1H-Indole-3-yl(2,2,3,3-tetramethylcyclopropyl)methanone (**26**).* A solution of 2,2,3,3-tetramethylcyclopropanecarboxylic acid (**25**,

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1.19 g, 8.4 mmol) in CH_2Cl_2 (20 mL) was treated with $(COCl)_2$ (1.4 mL, 2.4 mmol, 2.0 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 **24** (820 mg, 7.0 mmol) in CH_2Cl_2 (15 mL) was treated dropwise with a solution of 1 M Me_2AlCl in hexane (10.5 mL, 10.5 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 (15 mL), and the reaction was stirred for 3 h. The reaction was quenched by dropwise addition to a solution of 1 M aq. HCl (30 mL), the layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (2 × 30 mL) and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$, R_f 0.26), to give **26** as a pale yellow solid (1.38 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.51 (1H, br s, NH), 8.44−8.41 (1H, m, ArH), 7.75 (1H, d, J = 2.5 Hz, ArH), 7.39−7.37 (1H, m, ArH), 7.25−7.22 (2H, m, ArH), 1.97 (1H, s, CH), 1.37 (6H, s, CH_3), 1.30 (6H, s, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.1 (CO), 136.6 (quat.), 131.2 (CH), 125.5 (quat.), 123.4 (CH), 122.4 (CH), 122.2 (CH), 120.9 (quat.), 111.7 (CH), 41.9 (CH), 32.0 (quat.), 24.1 (CH_3), 17.2 (CH_3). All spectral data were consistent with those published previously.[11,12]

*(1-(5-Acetoxypentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone (**27**).* Treating **26** (362 mg, 1.5 mmol) with 5-bromopentyl acetate (275 μL, 1.65 mmol, 1.1 equiv) according to the general procedure gave, following purification by flash chromatography (hexane−EtOAc, 65:35, R_f 0.30), **27** (371 mg, 67%) as a white crystalline solid. Mp (*i*-PrOH−H_2O) 57−59 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42−8.39 (1H, m, ArH), 7.65 (1H, s, ArH), 7.35−7.31 (1H, m, ArH), 7.29−7.24 (2H, m, ArH), 4.17 (2H, t, J = 7.2 Hz, NCH_2), 4.05 (2H, t, J = 6.6 Hz, CH_2OAc), 2.02 (3H, s, Ac), 1.97−1.89 (3H, m, CH, CH_2), 1.71-.164 (2H, m, CH_2), 1.46−1.40 (2H, m, CH_2), 1.35 (6H, s, 2 × CH_3), 1.31 (6H, s, 2 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.7 (CO), 171.2 ($\underline{C}(O)OCH_3$) 136.7 (quat.), 133.4 (CH), 126.5 (quat.), 123.1 (CH), 122.9 (CH), 122.3 (CH), 119.9 (quat.), 109.7 (CH), 64.1 ($\underline{C}H_2OAc$), 47.0 (NCH_2) 41.8 (CH), 31.8 ($C_{quat.}$), 29.8 (CH_2), 28.3 (CH_2), 24.2 (CH_3), 23.5 (CH_2), 21.1 (CH_3), 17.2 (CH_3). LRMS (+ESI) m/z 760.67 ($[2M + Na]^+$, 72%), 370.07 ($[M + H]^+$, 100%). Anal. ($C_{23}H_{31}NO_3$) Calcd: C 74.76, H 8.46, N 3.79. Found: C 74.43, H 8.58, N 3.93.

General Procedure D: Synthesis of 1-Alkylindole-3-carboxylic Acids. A cooled (0 °C) mixture of sodium hydride (60% dispersion in mineral oil, 2.00 g, 50.0 mmol, 2.0 equiv) in DMF (30 mL) was treated slowly with a solution of indole (2.93 g, 25 mmol) in DMF (3 mL), warmed to ambient temperature, and stirred for 10 min. The mixture was cooled in an ice−water bath and then treated slowly with the appropriate bromoalkane (26.3 mmol, 1.05 equiv). The mixture was warmed to ambient temperature and stirred for 1 h, at which point TLC analysis indicated complete consumption of indole. The solution was cooled to 0 °C and treated slowly with trifluoroacetic anhydride (8.70 mL, 62.5 mmol, 2.5 equiv). The resultant clear, red solution was warmed to ambient temperature and stirred for 1 h, at which point TLC analysis indicated the complete conversion of the 1-alkylindole to 1-alkyl-3-trifluoroacetylindole. The reaction was poured portionwise onto stirred ice−water (500 mL), and the mixture was vigorously stirred at 0 °C until complete solidification of the oil layer had occurred. The precipitate was collected by filtration and air-dried to give crude 1-alkyl-1*H*-3-trifluoroacetylindoles as pink solids.

A solution of crude 1-alkyl-1*H*-3-trifluoroacetylindole (25.0 mmol) in toluene (22 mL) was slowly added to a refluxing solution of KOH (4.63 g, 82.5 mmol, 3.3 equiv) in MeOH (8 mL), and the mixture was heated at reflux for 2 h. The mixture was cooled to ambient temperature, and H_2O (80 mL) was added. The layers were separated, and the organic layer was extracted with 1 M aq. NaOH (25 mL). The combined aqueous phases were acidified to pH 1 with 10 M aq. HCl, extracted with Et_2O (3 × 50 mL) or CH_2Cl_2 (3 × 50 mL), and dried ($MgSO_4$), and the solvent was evaporated. The crude products were recrystallized from isopropanol to give analytically pure materials.

*1-Pentylindole-3-carboxylic Acid (**28**).* Treating indole with 1-bromopentane according to general procedure D gave, after recrystallization from *i*-PrOH, **28** as colorless crystals (4.57 g, 19.8 mmol, 79%). Mp (i-PrOH) 106−108 °C. ^{1}H NMR (500 MHz, CDCl3): 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, CDCl3): 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 (CH2), 14.0. Anal. (C14H17NO2) Calcd: C 72.70, H 7.41, N 6.06. Found: C 72.67, H 7.77, N 5.93

*1-(5-Fluoropentyl)indole-3-carboxylic Acid (**29**).* Treating indole with 1-bromo-5-fluoropentane according to general procedure D gave, after recrystallization from *i*-PrOH (twice), **29** as colorless crystals (5.28 g, 21.2 mmol, 85%). Mp (*i*-PrOH−H_2O) 120−122 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.29−8.25 (1H, m), 7.93 (1H, s), 7.40−7.37 (1H, m), 7.34−7.30 (2H, m), 4.43 (2H, dt, $^2J_{CF}$ = 47.3, $^3J_{HH}$ = 5.9 Hz, CH_2F), 4.18 (2H, t, J = 7.1 Hz), 1.98−1.92 (2H, m, CH_2), 1.78−1.68 (2H, m, CH_2), 1.51−1.45 (2H, m, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.9 (CO), 136.8 (quat.), 135.5 (CH), 127.1 (quat.), 123.1 (CH), 122.3 (CH), 122.1 (CH), 110.1 (CH), 106.6 (quat.), 83.8 (d, $^1J_{CF}$ = 166.0 Hz, CH_2F), 47.1 (CH_2), 30.0 (d, $^2J_{CF}$ = 20.1 Hz, $\underline{C}H_2CH_2F$), 29.6 (CH_2), 22.9 (d, $^3J_{CF}$ = 5.0 Hz, $\underline{C}H_2CH_2CH_2F$). Anal. ($C_{14}H_{16}FNO_2$) Calcd: C 67.45, H 6.47, N 5.62. Found: C 67.31, H 6.60, N 5.46.

X-ray Data Collection. The solid was crystallized from *i*-PrOH−H_2O to give colorless 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 **7** 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 N_2 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 1588 frames were collected. The diffraction data were integrated using SAINT+,[56] which included corrections for Lorentz, polarization, and absorption effects. Unit cell parameters for **7** at 150 K were refined from 999 reflections.

The structure was solved using direct methods (SHELX-S)[57] and refined using full-matrix least-squares (SHELXL).[57] 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	$C_{21}H_{29}NO$
formula wt	311.45
temp (K)	150.15
cryst syst	monoclinic
space group	$P2_1/n$
unit cell dimensions	
a (Å)	12.112
α (deg)	90
b (Å)	10.799
β (deg)	93.69
c (Å)	13.920
γ (deg)	90
Z	4
GOF on F^2	1.050
final R indices [$I > 2\sigma(I)$]	
R_1	0.0398
wR_2	0.1019
R indices (all data)	
R_1	0.0478
wR_2	0.1077

***In Vitro* Pharmacological Assessment of SCs.** Mouse AtT-20 neuroblastoma cells stably transfected with human CB_1 or human CB_2

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have been previously described[36] and were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U of penicillin/streptomycin, and 300 μg/mL G418. 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, 100 U of 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.[49] The dye was reconstituted with assay buffer of composition (mM): NaCl 145, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (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 were 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 SCs.** Eight cohorts of three or four adult male Wistar rats (Animal Resources Centre, Perth, Australia) initially weighing between 200 and 230 g were used for biotelemetric assessment of each compound. The rats were singly housed in an air-conditioned testing room (22 ± 1 °C) on a 12 h reverse light/dark cycle (lights on from 21:00 to 09:00). Standard rodent chow and water were provided *ad libitum*. All experiments were approved by The University of Sydney Animal Ethics Committee.

Biotelemetry transmitters (TA11CTA-F40, Data Sciences International, St. Paul, MN) were implanted as previously described.[36] Briefly, following anaesthetization (isoflurane, 3% induction, 2% maintenance), a rostro-caudal incision was made along the midline of the abdomen, and a biotelemetry transmitter (TA11CTA-F40, Data Sciences International, St. Paul, MN) was placed in the peritoneal cavity according to the manufacturers protocol. The wound was sutured closed, and the rats were allowed 1 week of recovery before data collection.

The rats were habituated over multiple days to injections of vehicle (5% EtOH, 5% Tween 80, 90% physiological saline) at a set time of day (11:00 am). Each cohort then received intraperitoneal injections of each compound at the same time of day in an ascending dose sequence (0.1, 0.3, 1, 3 mg/kg). This ascending sequence reduces the risk posed to the animals in assessing hitherto untested compounds, and the use of multiple cohorts limits the potential development of tolerance to the compound. Two washout days were given between each dose. If only a modest or negligible hypothermic response was seen at 3 mg/kg, then a further 10 mg/kg dose of the compound was given. Two washout days were given between each dose.

Data for heart rate and body temperature was gathered continuously at 1000 Hz, organized into 15 or 30 min bins using Dataquest A.R.T. software (version 4.3, Data Sciences International, St. Paul, MN), and analyzed using PRISM (Graphpad Software Inc., San Diego, CA).

■ ASSOCIATED CONTENT

Supporting Information

X-ray crystallographic data, selected ^1H and ^{13}C NMR spectra, and area under curve (AUC) plots for biotelemetry. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00107.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: michael.kassiou@sydney.edu.au.

Author Contributions

S.D.B., M.L., S.M.W., C.B., and A.S.B. performed the synthesis, purification, and chemical characterization of compounds **7**−**23** with guidance from M.K. J.S. and A.E. conducted all *in vitro* pharmacological evaluation under the supervision of M.C., with data analysis performed by J.S., S.D.B., A.S.B., and M.C. R.C.K. carried out all behavioral pharmacology with direction from I.S.M. D.E.H. provided X-ray crystallographic analysis of UR-144 (**7**). M.G. assisted the creation of stably transfected cells expressing hCB_2R. The manuscript was prepared by S.D.B., A.S.B., M.C., I.S.M., and M.K. 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; ip, intraperitoneal; NMR, nuclear magnetic resonance; pi, post-injection; SAR, structure−activity relationship; SC, synthetic cannabinoid; Δ^9-THC, Δ^9-tetrahydrocannabinol; TLC, thin layer chromatography

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