The pharmacology of indole and indazole synthetic cannabinoid designer drugs AB-FUBINACA, ADB-FUBINACA, AB-PINACA, ADB-PINACA, 5F-AB-PINACA, 5F-ADB-PINACA, ADBICA and 5F-ADBICA

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Keywords: cannabinoid, THC, JWH-018, FUBINACA, PINACA

Abstract: Synthetic cannabinoid (SC) designer drugs based on indole and indazole scaffolds and featuring L-valinamide or L-*tert*-leucinamide side-chains are encountered with increasing frequency by forensic researchers and law enforcement, and are associated with serious adverse health effects. However, many of these novel SCs are unprecedented in the scientific literature at the time of their discovery, and little is known of their pharmacology. Here we report the synthesis and pharmacological characterization of AB-FUBINACA, ADB-FUBINACA, AB-PINACA, ADB-PINACA, 5F-AB-PINACA, 5F-ADB-PINACA, ADBICA, 5F-ADBICA, and several analogues. All synthesized SCs acted as high potency agonists of CB_1 (EC_{50} = 0.24–21 nM) and CB_2 (EC_{50} = 0.88–15 nM) receptors in a fluorometric assay of membrane potential, with 5F-ADB-PINACA showing the greatest potency at CB_1 receptors. The cannabimimetic activities of AB-FUBINACA and AB-PINACA *in vivo* were evaluated in rats using biotelemetry. AB-FUBINACA and AB-PINACA dose-dependently induced hypothermia and bradycardia at doses of 0.3–3 mg/kg, and hypothermia was reversed by pretreatment with a CB_1 (but not CB_2) antagonist, indicating that these SCs are cannabimimetic *in vivo*, consistent with anecdotal reports of psychoactivity in humans.

Introduction: Synthetic cannabinoids (SCs) are the most rapidly growing class of recreational designer drugs. Since the identification of the first SC designer drugs in 2008, more than 130 SCs have been reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).[1] Of the 101 new psychoactive substances notified by the EMCDDA during 2014, 30 were SCs.[1] Although these products are often mislabeled as 'research chemicals' or 'incense', and include disclaimers stating that the products are not for human consumption, SCs are recreational designer drugs intended to mimic the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**, Fig. 1) while circumventing international law.

The phytocannabinoid Δ^9-THC is the principal bioactive component of marijuana (*Cannabis sativa*), the most widely used illicit substance in the world. Δ^9-THC exerts its psychoactive effects by acting as a partial agonist at cannabinoid type-1 (CB_1) receptors,[2] although it is also a partial agonist at type-2 (CB_2) receptors. CB_1 and CB_2 receptors are classical G protein-coupled receptors (GPCRs). While CB_1 receptors are found primarily at the terminals of central and peripheral neurons where they inhibit neurotransmitter release, CB_2 receptors are mainly located in immune cells within and outside the central nervous system (CNS).[3,4] Due to the role of the CB receptor system in numerous diseases, early pharmaceutical drug discovery programs explored many phytocannabinoid analogues, such as CP 47,497 (**2**) and CP 55,940 (**3**), disclosed by Pfizer in the 1970s and 1980s.[5,6] Following structural leads from the pharmaceutical industry, such as pravadoline, Huffman and coworkers at Clemson University have discovered many indole SCs with potent cannabimimetic activity, including JWH-018 (**4**).

[FIGURE 1]

In 2008, a recreational herbal blend was found to contain the C8 homologue of CP 47,497 and JWH-018.[7] Following the prohibition of CP 47,497-C8 and JWH-018 by many governments, other structurally diverse indole SCs began to appear.[8–11] Recently, numerous SCs with clandestine design origins and no precedent in the scientific literature have been detected in forensic samples. For example, indole-3-carboxamide SC 2NE1 (APICA, SDB-001, **5**) was identified along with its indazole analogue AKB48 (APINACA, **6**).[12] Presumably intended to mimic the alphanumeric format of compound codes used throughout the pharmaceutical industry, 2NE1 and AKB48 were named after Japanese and Korean female pop music groups, respectively, by their clandestine designers. Current popular design trends for modification of the *N*-pentyl group include terminal fluorination and replacement with cyclohexylmethyl or 4-fluorobenzylic moieties.[13,14]

In 2013, novel indazole SCs AB-FUBINACA (**7**, Fig. 2) and ADB-FUBINACA (**8**) were identified in recreational products by Japanese forensic scientists.[10,15,16] Although many recent SCs have no precedent in the scientific literature prior to their identification as designer drugs, **7** and **8** were both described by Pfizer in a 2009 patent claiming CB_1 ligands as potential therapeutic agents.[17] The binding affinity and functional activity in a GTPγS binding assay of **7** (K_i = 0.9 nM, EC_{50} = 23.2 nM) and **8** (K_i = 0.36 nM, EC_{50} = 0.98 nM) at hCB_1 receptors was reported, indicating that both compounds are potent CB_1 agonists, but no further pharmacology was described. The stereochemistry of the isopropyl and *tert*-butyl side-chains of illicit **7** and **8** respectively is unresolved. However, the Pfizer patent reports activity exclusively for the (*S*)-enantiomers, and it is likely that the Pfizer compounds and the illicit SCs are (*S*)-enantiomers derived from the abundant and inexpensive L-amino acids L-valine and L-*tert*-leucine.

AB-PINACA (**9**) was identified alongside **7**, representing a hybrid of **7** and *N*-pentyl SCs like **4** and **6**.[10,15] Although previously unreported in the scientific literature, ADB-PINACA (**10**) exposure was associated with severe adverse reactions, including neurotoxicity and cardiotoxicity in the USA in late 2013,[18–20] and was recently linked to a cluster of cases of severe delirium.[21] The 5-fluorinated analogues of **9** and **10**, 5F-AB-PINACA (**11**) and 5F-ADB-PINACA (**12**) respectively, have also been identified on the Japanese market.[22,23] By 2014, **7**–**11** and **16** had been formally notified by the EMCDDA as a result of seizures in Belgium, Germany, Turkey, the UK, and Sweden.[24]

AB-FUBICA (**13**), ADB-FUBICA (**14**), AB-PICA (**15**), and 5F-AB-PICA (**17**), represent the indole analogues of indazoles **7**, **8**, **9**, and **11**, and have not appeared in the scientific literature. However, the indole analogue of **10**, ADBICA (**16**) was identified in Japan,[16] and its 5-fluoro analogue, 5F-ADBICA (**18**), was notified by the EMCDDA after law enforcement agencies in the US implicated **18** in a series of non-fatal intoxications.[24]

Despite their widespread prevalence of use, and frequent adverse reactions requiring hospitalization, very little is known about the activity of indole and indazole SCs comprising an L-valinamide or L-*tert*-leucinamide subunit. In addition to reports of the detection of SCs **7**–**11**, **16**, and **18** by forensic researchers, the metabolic profiles of **7**–**9** and **11** were recently published.[25–28]

[FIGURE 2]

The aim of the present study was to address the paucity of data regarding the pharmacology of indole and indazole SCs by synthesizing **7**–**18**, evaluating their activity at human CB_1 and

CB_2 receptors, and assessing the behavioral pharmacology of these novel SCs in rats using biotelemetry.

Results and Discussion: The original patent by Pfizer describing AB-FUBINACA and ADB-FUBINACA utilized enantiopure amino acids L-valinamide (**19**, Fig. 3) and L-*tert*-leucinamide (**20**), to give products with (*S*)-stereocentres.

[FIGURE 3]

While L-valinamide is available from numerous commercial sources as its hydrochloride salt, L-*tert*-leucinamide is derived from a non-natural amino acid, and the synthesis of **20** is shown in Scheme 1. Treatment of L-*tert*-leucine (**21)** with benzyl chloroformate gave Cbz-protected amine **22**. The free acid of **22** was converted to the corresponding amide (**23**) using the coupling reagents EDCI and HOBt, and subsequent deprotection by catalytic hydrogenation afforded **20**. The three step procedure proved to be operationally convenient and the analytically pure L-*tert*-leucinamide was obtained on a multigram scale following recrystallization.

[SCHEME 1]

The synthesis of indazole SCs **7**–**12** is shown in Scheme 2. Fischer esterification of indazole-3-carboxylic acid (**24**) gave **25**, which was deprotonated with potassium *tert*-butoxide and alkylated with either 4-fluorobenzyl bromide, 1-bromopentane, or 1-bromo-5-fluoropentane to afford the corresponding *N*-alkylindazole-3-carboxylic acid methyl esters **26**–**28**. Alkylation proceeded regioselectively to give 1-substituted 1*H*-indazoles as the major

products, however, small quantities of 2-alkylated indazoles were obtained as minor products and separated by flash chromatography. Saponification of the methyl ester of **26**–**28** to give free acids **29**–**31** was followed by amide coupling with EDC-HOBt and either L-valinamide or L-*tert*-leucinamide to give **7**–**12**.

[SCHEME 2]

Access to the corresponding indole SCs (**13**–**18**) required an alternative synthetic route, shown in Scheme 3. Excess sodium hydride was added to indole (**33**), which was subsequently alkylated with the appropriate bromoalkane, and then treated with trifluoroacetic anhydride to give the corresponding *N*-alkyl-3-(trifluoroacetyl)indole (**34**–**36**) in a one-pot process. Alkaline hydrolysis induced fluoroform elimination,[29] and furnished, upon work-up and recrystallization, the corresponding *N*-alkylindole-3-carboxylic acids (**37**–**39**) of analytical purity. Coupling of **37**–**39** with **19** or **20** using EDC-HOBt yielded **13**–**18**. Indole SCs derived from L-valinamide (**13**, **15**, **17**) were recrystallized from isopropanol to analytical purity, while those comprising L-*tert*-leucinamide (**14**, **16**, **18**) were purified by flash chromatography owing to their superior solubility in a range of alcoholic solvents.

[SCHEME 3]

The activity of synthesized indazole (**7**–**12**) and corresponding indole (**13**–**18**) SCs at CB_1 and CB_2 receptors was evaluated using a fluorometric imaging plate reader (FLIPR) assay to provide structure-activity relationship (SAR) data regarding the choice of heteroaromatic core, amino acid side-chain, and alkyl substituent in this class of SCs. Additionally, the *in*

vivo activity of **7** and **9** was compared using biotelemetry in rats to provide information regarding the increasingly common 4-fluorobenzyl motif in SCs.

The cannabimimetic activities of **7**–**18** were compared to phytocannabinoid Δ^9-THC (a partial agonist at CB_1 and CB_2), and indole SC JWH-018 (a full agonist at CB_1 and CB_2), and the data is presented in Table 1. Murine AtT-20 neuroblastoma cells were stably transfected with human CB_1 or CB_2 receptors, and activities of Δ^9-THC, JWH-018, and **7**–**18** 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 expressed CB_1 or CB_2 receptors. The maximum effects of Δ^9-THC, JWH-018, and **7**–**18** were compared to high efficacy CB_1/CB_2 receptor agonist CP 55,490, which produced a maximal decrease in fluorescence, corresponding to cellular hyperpolarization, at a concentration of 1 μM in AtT-20-CB_1 and AtT-20-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 CB_1 or CB_2 receptors.

[TABLE 1]

All indole and indazole SCs activated CB_1 and CB_2 receptors. All compounds had greater potency (0.24–21 nM) than Δ^9-THC (172 nM) for 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 AtT-20-CB_2 its effects at 30 μM were only 32 ± 1 % of that mediated by CP 55,940. CP 55,940 was more potent at stimulating a cellular hyperpolarization in AtT-20-CB_2 cells than AtT-20-CB_1 cells, displaying an approximately 2-fold CB_2 preference. All indazole and indole SCs had a similar maximal effect to CP 55,940 at CB_1 and CB_2 receptors, suggesting that these SCs are also high efficacy agonists. With the exception of **13**, all novel SCs showed a mild preference

for CB_1 receptors, and it is activation of CB1 receptors that is associated with the psychoactive effects of cannabinoids.[2]

The least potent compound in the series (indole **13**) was only 11-fold more potent than Δ^9-THC at CB_1 receptors, the most potent compound (indazole **12**) showed more than 1000 times the potency of Δ^9-THC, making 5F-ADB-PINACA one of the most potent SC designer drugs reported to date. Excluding **7** and **8**, indazoles and indoles containing the L-*tert*-leucinamide group were more potent at both CB_1 and CB_2 receptors than the corresponding SC featuring the L-valinamide substituent. In the most dramatic example, the additional methyl group of **16** (EC_{50} = 0.68 nM) conferred a 17-fold increase in potency over **15** (EC_{50} = 12 nM) at CB_1 receptors. The same trend was observed for CB_2 receptors, but potency enhancement was more moderate, with **18** (EC_{50} = 1.2 nM) showing a 7-fold improvement over **17** (EC_{50} = 1.2 nM).

Surprisingly, there were no clear trends for differences of potency or efficacy between indazole SCs **7**–**12** and the corresponding indoles **13**–**18**. Similarly, choice of *N*-alkyl group had little effect on potency. However, indoles containing the L-valinamide group (**13**, **15**, and **17**), the least potent SCs identified in this series, were each less potent than the corresponding indazoles (**7**, **9**, **11**). Taken together, these results suggest that the heteroaromatic core and indole nitrogen substituent of these SCs contribute less to the activity of these compounds than the pendant amide group. The difference in CB_1 activity between L-valinamide and L-*tert*-leucinamide derivatives featuring a 1-pentyl group and containing an indazole core (**9** and **10**, respectively) or an indole core (**15** and **16**, respectively) is depicted in Figure 4.

[FIGURE 4]

Very little is known about the potency and psychoactivity of newer SCs in humans. Having demonstrated that **7**–**18** are potent and efficacious cannabimimetic agents *in vitro*, we sought to demonstrate activity of some of these SCs *in vivo*. Cross-substitution of older SCs, like JWH-018, with Δ^9-THC has been demonstrated.[30–32] Cannabinoids are known to induce hypothermia and bradycardia in rats, effects that are common to phytocannabinoids like Δ^9-THC and heteroaromatic SCs such as JWH-018.[33–35] We have previously evaluated the hypothermic and bradycardic potencies of Δ^9-THC and numerous structurally diverse SCs including JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135.[14,36] The cannabimimetic activities AB-FUBINACA and AB-PINACA were evaluated using radiotelemetry in male Wistar rats, and the effects of these SCs on body temperature (Fig. 5) and heart rate (Fig. 6) are presented below.

Rat body temperature 1 hour prior to intraperitoneal (i.p.) injection and 6 hours post injection of AB-FUBINACA and AB-PINACA are presented in 15 minute bins in Figure 5. For each drug, these data are presented for 1 hour before (baseline) and 6 hours after injection of various doses. The dashed line on the figures represents the time of SC injection. Each SC was investigated using a cohort of 3–4 rats, with a different cohort used for the two compounds. Doses were escalated from 0 mg/kg (baseline) to 0.1, 0.3, 1 and 3 mg/kg for each compound with at least 2 washout doses between each dose. The 0.1 mg/kg doses of each compound were without significant effects on body temperature and heart rate and so data for these doses are not presented.

[FIGURE 5]

A substantial hypothermic effect was evoked by 0.3–3 mg/kg of both drugs, with the peak reduction in body temperature generally greater with AB-FUBINACA (>2 °C) than AB-PINACA (>1.5 °C). As Fig. 5 shows, the 4-fluorobenzyl-substituted AB-FUBINACA appeared to confer a hypothermic effect of greater magnitude and duration (~4 h) than that observed for the pentyl-substituted AB-PINACA (~2 h) at the same dose (3 mg/kg). This was verified by a statistical analysis showing a significantly greater area under the curve for body temperature (relative to vehicle baseline) for AB-FUBINACA doses compared to AB-PINACA at 3 mg/kg ($P < 0.05$).

Results for heart rate are presented in 30 minute bins in Figure 6 with the dashed line on the figures again representing the time of SC injection. 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, $P < 0.05$).

[FIGURE 6]

To confirm that the observed effects were mediated through CB_1 or CB_2 receptors, the reversibility of the effects of AB-PINACA and AB-FUBINACA on body temperature and heart rate in rats following pretreatment with either CB_1 receptor antagonist rimonabant (SR141176, **40**, Fig. 7) or CB_2 receptor antagonist SR144528 (**41**) was assessed. Rimonabant is a potent, selective, CB_1 receptor neutral antagonist, and reverses CB_1-mediated

cannabinoid agonist effects in rodents and humans,[2,37,38] while SR144528 is selective CB_2 antagonist/inverse agonist.[39,40]

Rat body temperatures after injection (i.p.) with vehicle, CB_1 antagonist (rimonabant, 3 mg/kg), or CB_2 antagonist (SR144528, 3 mg/kg) 30 minutes prior to treatment with either AB-FUBINACA (3 mg/kg) or AB-PINACA (3 mg/kg) are presented in 15 minute bins in Fig. 8. For each treatment condition, these data are presented for 1 hour before (baseline) and 6 hours after injection of various doses. The first dashed line on the figure represents the time of vehicle/antagonist injection, and the second dashed line represents time of SC injection. Each SC was investigated using a cohort of 3–4 rats, with a different cohort used for the two compounds. The dose of each antagonist was 3 mg/kg and the dose of each SC was also 3 mg/kg.

[FIGURE 7]

Pretreatment with rimonabant was able to completely reverse the hypothermic effects of AB-FUBINACA, while pretreatment with SR144528 had no effect on the body temperature decrease induced by AB-FUBINACA (Fig. 8A). Similarly, rimonabant partially reversed the decreased body temperature effected by AB-PINACA, but SR144528 had negligible effect on AB-PINACA-induced hypothermia (Fig. 8B). These interpretations are confirmed by a statistical analysis of the areas between each drug treatment and baseline (Fig. S13 of the SI). This suggests a CB_1-mediated hypothermic mechanism. Similar trends were observed for the reversal of AB-FUBINACA- or AB-PINACA-induced bradycardia by rimonabant but not SR144528, however, these differences did not reach significance (data not shown). This is

likely due to a combination of the relatively smaller magnitude of SC-induced bradycardic effects and high variability of the heart rate data.

Conclusion: This study is the first to pharmacologically characterize the emergent class of recreational SC designer drugs based on indole and indazole scaffolds and featuring L-valinamide or L-*tert*-leucinamide side-chains. Synthetic routes to identified SCs of forensic interest (AB-FUBINACA, ADB-FUBINACA, AB-PINACA, ADB-PINACA, 5F-AB-PINACA, 5F-ADB-PINACA, ADBICA, 5F-ADBICA), as well as several undetected analogues, was developed. These synthetic routes are general for 1-alkyl-1*H*-indazole-3-carboxamides and 1-alkyl-1*H*-indole-3-carboxamides, and enable forensic chemists to proactively develop reference standards for structurally related SCs expected to appear in future. All synthesized SCs acted as agonists of CB_1 and CB_2 receptors in a FLIPR membrane potential assay, and thus are functional cannabinoids. Preliminary SARs suggest that L-*tert*-leucinamide derivatives possess greater potency at CB_1 receptors *in vitro* than corresponding L-valinamide analogues. The most potent of these was 5F-ADB-PINACA. Additionally, a 2-alkylated indazole-3-carboxamide, presumed to be a manufacturing impurity or confounding additive, was confirmed to possess cannabimimetic activity. In rats, AB-FUBINACA and AB-PINACA were able to dose-dependently decrease body temperature and heart rate at doses of 0.3–3 mg/kg, indicating that these SCs are also cannabimimetic *in vivo*. AB-FUBINACA had more potent effects on body temperature than AB-PINACA. The hypothermic effects of AB-FUBINACA and AB-PINACA appear to be mediated through CB_1 receptors and could be reversed by pretreatment with CB_1 antagonist rimonabant, but not CB_2 antagonist SR144528. Both *in vitro* and *in vivo* results confirm that all of the SCs explored have cannabimimetic effects that parallel those of Δ^9-THC, but with greater potency.

Materials and methods. General chemical synthesis details. All reactions were performed under an atmosphere of nitrogen unless otherwise specified. Commercially available chemicals were used as purchased. Flash column chromatography was performed using Merck Kieselgel 60 (230–400 mesh) silica gel. Melting points were measured in open capillaries using a Gallenkamp 5A 6797 Melting Point Apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded at 300 K using a Bruker 300, 400 or 500 MHz spectrometer. The data are reported as chemical shift (δ ppm) relative to the residual protonated solvent resonance, relative integral, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, quin. = quintet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, qd = quartet of doublets), coupling constants (*J* Hz) and assignment. Low resolution mass spectra (LRMS) were recorded using electrospray ionisation (ESI) recorded on a Finnigan LCQ ion trap spectrometer. HPLC analysis of the organic purity of the compounds submitted for *in vivo* testing (**4**-**7**) was conducted on a Waters e2695 Separations module using a Waters Sunfire C18 5 μm, 2.1 x 150 mm column and detected using a Waters 2489 UV/Vis detector set at 254 nm. Separation was achieved using water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) at a flowrate of 0.2 mL/min and a gradient of 5% B for 1 min, then 5-100% B over 30 min. Elemental analysis was obtained from the Chemical Analysis Facility in the Department of Chemistry and Biomolecular Sciences, Macquarie University, Australia.

General procedure A: amidation of 1-alkyl-1*H*-indazole-3-carboxylic acids and 1-alkylindole-3-carboxylic acids. A solution of the appropriate carboxylic acid **29**, **30**, **31**, **37**, **38**, or **39** (7.5 mmol, 1.5 equiv.) in DMF (50 mL) was treated with EDC (7.5 mmol, 1.5 equiv.), HOBt (7.5 mmol, 1.5 equiv.), DIPEA (25.5 mmol, 5.1 equiv.), **19**·HCl or **20** (5

mmol), and stirred for 24 h. The mixture was partitioned between and H_2O (100 mL) and EtOAc (50 mL), the layers separated, and the aqueous layer extracted with EtOAc (2 × 50 mL). The combined organic phases were dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The crude products were purified by flash chromatography and/or recrystallization.

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (AB-FUBINACA, 7). Treating **29** (1.20 g, 4.4 mmol) with **19** (1.04 g, 6.8 mmol) according to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 10:90), **7** (0.94 g, 57%) as a white solid. Recrystallization from *i*-PrOH-H2O yielded material of analytical purity. m.p. 151–152 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.33 (1H, d), 7.54 (1H, d, J = 8.9 Hz), 7.37 (1H, m), 7.32 (1H, m), 7.27 (1H, m), 6.39 (1H, bs), 5.69 (1H, bs), 5.57 (2H, s), 4.58 (1H, dd, J = 8.9 Hz, 6.8 Hz), 2.35 (1H, dq., J = 13.6 Hz, 6.8 Hz), 1.09 (6H, dd, J = 7.2 Hz, 5.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.7 (CO), 162.9 (CO), 162.6 (d, $^1J_{C\text{-}F}$ = 249.1 Hz, quat.), 140.9 (quat.), 137.3 (quat.), 131.7 (d, $^4J_{C\text{-}F}$ = 3.4 Hz, quat.), 129.2 (d, $^3J_{C\text{-}F}$ = 8.3 Hz, CH), 127.3 (CH), 123.4 (quat.), 123.1 (CH), 122.8 (CH), 116.0 (d, $^2J_{C\text{-}F}$ = 21.6 Hz, CH), 109.7 (CH), 58.0 (CH), 53.1 (CH_2), 30.8 (CH), 19.6 (CH_3), 18.4 (CH_3); ^{19}F NMR (470 MHz, $CDCl_3$): δ –113.9 ppm; LRMS (+ESI): *m/z* 323.9 ($[M–CONH_3]^+$, 100%), 351.8 ($[M–NH_3]^+$, 50%), 368.8 ($[M+H]^+$, 20%) ; Anal. ($C_{20}H_{21}N_4O_2F$) calcd: C 65.20, H 5.75, N 15.21; found: C 65.28, H 5.73, N 15.21; HPLC purity: 99.2%.

(*S*)-*N*-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (ADB-FUBINACA, 8). Treating **29** (0.51 g, 1.9 mmol) with **20** (0.37 g, 2.8 mmol) according to general procedure A gave, following purification by flash

chromatography (hexane-EtOAc, 10:90), **8** (0.22 g, 31%) as a white solid. m.p. 135–137 °C; NMR (500 MHz, $CDCl_3$): δ 8.21 (1H, d, J = 8.0 Hz), 7.72 (1H, d, J = 9.7 Hz), 7.27 (1H, m), 7.19 (1H, m), 7.14 (2H, dd, J = 8.3 Hz, 5.4 Hz), 6.99 (1H, bs), 6.93 (2H, t, J = 8.6 Hz), 6.16 (1H, bs), 5.52 (2H, s), 4.74 (1H, d, 9.6 Hz), 1.15 (1H, s), 1.11 (9H, s); ^{13}C NMR (125 Hz, $CDCl_3$): δ 173.5 (CO), 162.6 (CO), 162.6 (d, $^1J_{C\text{-}F}$ = 245.8 Hz, quat.), 140.9 (quat.), 137.3 (quat.), 131.8 (d, $^4J_{C\text{-}F}$ = 3.1 Hz, quat.), 129.1 (d, $^3J_{C\text{-}F}$ = 8.3 Hz, CH), 127.1 (CH), 123.4 (quat.), 123.0 (CH), 122.6 (CH), 115.9 (d, $^2J_{C\text{-}F}$ = 21.6 Hz, CH), 109.7 (CH), 59.7 (CH), 53.1 (CH_2), 34.8 (quat.), 26.9 (CH_3); ^{19}F NMR (470 MHz, $CDCl_3$): δ –113.9; LRMS (+ESI): *m/z* 337.9 ($[M–CONH_3]^+$, 100%), 365.8 ($[M–NH_3]^+$, 50%), 382.7 ($[M+H]^+$, 21%); Anal. ($C_{21}H_{23}N_4O_2F$) calcd: C 65.95, H 6.06, N 14.65; found: C 65.38, H 6.08, N 14.38.

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1*H*-indazole-3-carboxamide (AB-PINACA, 9). Treating **30** (0.50 g, 2.2 mmol) with **19** (0.50 g, 3.3 mmol), according to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 50:50), **9** (0.44 g, 62%) as a white solid. Recrystallization from EtOAc-hexane yielded material of analytical purity. m.p. 125–126 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30 (1H, d, J = 8.3 Hz), 7.51 (1H, d, J = 8.8 Hz), 7.45-7.37 (2H, m), 7.26 (1H, m), 6.51 (1H, bs), 5.75 (1H, bs), 4.86 (1H, m), 4.38 (2H, t, J = 7.2 Hz), 2.36 (1H, dq., J = 13.6 Hz, 6.9 Hz), 1.94 (2H, quin., J = 7.4 Hz), 1.43-1.25 (4H, m), 1.08 (6H, m), 0.89 (3H, t, J = 7.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.9 (CO), 163.2 (CO), 141.0 (quat.), 136.5 (quat.), 126.8 (CH), 123.0 (quat.), 122.8 (CH), 122.7 (CH), 109.5 (CH), 57.9 (CH), 49.7 (CH_2), 30.7 (CH), 29.5 (CH_2), 29.1 (CH_2), 22.4 (CH_2), 19.6 (CH_3), 18.3 (CH_3), 14.0 (CH_3); LRMS (+ESI): *m/z* 258.9 ($[M–CONH_3]^+$, 100%), 313.9 ($[M–NH_3]^+$, 88%), 330.8 ($[M+H]^+$, 46%) ; Anal. ($C_{18}H_{26}N_4O_2$) calcd: C 65.43, H 7.93, N 16.96; found: C 65.75, H 8.11, N 16.98; HPLC purity: 97.6%.

(*S*)-*N*-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1*H*-indazole-3-carboxamide (ADB-PINACA, 10). Treating **30** (0.50 g, 2.2 mmol) with **20** (0.43 g, 3.3 mmol) according to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 50:50), **10** (0.46 g, 63%) as a white solid. mp 135–137 °C; NMR (400 MHz, $CDCl_3$): δ 8.27 (1H, m), 7.71 (1H, d, J = 9.5 Hz), 7.45-7.36 (2H, m), 7.29-7.22 (1H, m), 6.65 (1H, bs), 5.80 (1H, bs), 4.69 (1H, d, J = 9.5 Hz), 4.38 (2H, t, J = 7.2 Hz), 1.95 (2H, quin., J = 7.2 Hz), 1.45-1.25 (4H, m), 1.16 (9H, s), 0.89 (3H, t, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.2 (CO), 162.8 (CO), 141.0 (quat.), 136.5 (quat.), 126.7 (CH), 123.0 (quat.), 122.7 (CH), 122.5 (CH), 109.5 (CH), 59.7 (CH), 49.6 (CH_2), 34.8 (quat.), 29.5 (CH_2), 29.0 (CH_2), 26.9 (CH_3), 22.3 (CH_2), 14.0 (CH_3); LRMS (+ESI): *m/z* 299.9 ($[M–CONH_3]^+$, 100%), 327.9 ($[M–NH_3]^+$, 59%), 344.8 ($[M+H]^+$, 19%) ; Anal. ($C_{19}H_{28}N_4O_2$) calcd: C 66.25, H 8.19, N 16.27; found: C 66.45, H 8.40, N 16.29.

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide (5F-AB-PINACA, 11). Treating **31** (1.10 g, 4.4 mmol) with **19** (1.00 g, 6.7 mmol) according to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 10:90), **11** (0.56 g, 37%). Recrystallization from EtOAc-hexane yielded material of analytical purity. m.p. 110–111 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30 (1H, m), 7.51 (1H, d, J = 8.9 Hz), 7.45-7.39 (2H, m), 7.27 (1H, m), 6.48 (1H, bs), 5.74 (1H, bs), 4.58 (1H, dd, J = 9.1 Hz, 6.7 Hz), 4.47 (1H, t, J = 6.0 Hz), 4.43-4.36 (3H, m), 2.35 (1H, dq, J = 13.6 Hz, 6.8 Hz), 2.00 (2H, m), 1.80-1.66 (2H, m), 1.51-1.41 (2H, m), 1.08 (6H, dd, J = 7.1 Hz, 5.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.8 (CO), 163.0 (CO), 141.0 (quat.), 136.6 (quat.), 126.9 (CH), 122.99 (quat.), 122.91 (CH), 122.7 (CH), 109.4 (CH), 83.8 (CH_2, d, $^1J_{C\text{-}F}$ = 164.0 Hz), 57.9 (CH), 49.3 (CH_2), 30.7 (CH), 30.0 (CH_2, d, $^2J_{C\text{-}F}$ = 20.1 Hz), 29.4 (CH_2), 22.8 (CH_2, d, $^3J_{C\text{-}F}$ = 5.1 Hz), 19.6 (CH_3), 18.3 (CH_3); ^{19}F NMR (470 MHz,

$CDCl_3$): δ –218.6; LRMS (ESI): *m/z* 303.9 ($[M–CONH_3]^+$, 100%), 331.9 ($[M–NH_3]^+$, 59%), 348.9 ($[M+H]^+$, 48%); Anal. ($C_{18}H_{25}N_4O_2F$) calcd: C 62.05, H 7.23, N 16.08; found: C 61.96, H 7.26, N 15.83.

(*S*)-*N*-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide (5F-ADB-PINACA, 12). Treating **31** (0.40 g, 1.6 mmol) with **20** (0.32 g, 2.4 mmol) according to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 10:90), **12** (0.27 g, 47%) as a white solid. m.p. 135–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (1H, d, J = 8.2 Hz), 7.70 (1H, 9.4 Hz), 7.45-7.38 (2H, m), 7.30-7.23 (1H, m), 6.53 (1H, bs), 5.75 (1H, bs), 4.66 (1H, d, J = 9.6 Hz), 4.45-4.33 (3H, m), 2.18 (1H, bs), 2.00 (2H, quin., J = 7.7 Hz), 1.84-1.64 (2H, m), 1.48 (2H, m), 1.16 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1 (CO), 162.8 (CO), 141.0 (quat.), 136.8 (quat.), 126.9 (CH), 123.0 (quat.), 122.8 (CH), 122.7 (CH), 109.4 (CH), 83.9 (d, $^1J_{C\text{-}F}$ = 164.6, CH_2), 59.8 (CH), 49.4 (CH_2), 34.8 (quat.), 30.0 (d, $^2J_{C\text{-}F}$ = 19.7 Hz, CH_2), 29.4 (CH_2), 26.9 (CH_3), 22.8 (d, $^3J_{C\text{-}F}$ = 5.1 Hz, CH_2); ^{19}F NMR (376 MHz, $CDCl_3$): δ –218.3; LRMS (+ESI): *m/z* 317.9 ($[M–CONH_3]^+$, 100%), 345.9 ($[M–NH_3]^+$, 50%), 362.8 ($[M+H]^+$, 17%); Anal. ($C_{19}H_{27}N_4O_2F$) calcd: C 62.96, H 7.51, N 15.46; found: C 63.12, H 7.57, N 15.29.

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indole-3-carboxamide (AB-FUBICA, 13). Treating **37** (1.20 g, 4.5 mmol) with **19** (1.03 g, 6.7 mmol) according to general procedure A gave, following recrystallization from *i*-PrOH, **13** (1.28 g, 82%) as a white solid. mp 212−214 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.53 (1H, bs), 8.11 (1H, m), 7.64-7.50 (2H, m), 7.48 (1H, bs), 7.41-7.27 (2H, m), 7.24-7.10 (4H, m), 7.07 (1H, bs), 5.45 (2H, m), 4.36 (1H, m), 2.09 (1H, m), 0.94 (6H, dd, J = 6.7 Hz, 2.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.5 (CO), 164.0 (CO), 161.6 (quat., d, $^1J_{C\text{-}F}$ = 243.18 Hz), 136.0 (CH), 133.7

(quat., d, $^{4}J_{C\text{-}F}$ = 2.87 Hz), 131.6 (quat.), 129.3 (d, $^{3}J_{C\text{-}F}$ = 8.1 Hz, CH), 126.7 (CH), 122.1 (CH), 121.1 (CH), 120.8 (CH), 115.5 (d, $^{2}J_{C\text{-}F}$ = 21.4 Hz, CH), 110.6 (quat.), 110.0 (CH), 57.4 (CH), 48.7 (CH_2), 30.4 (CH), 19.5 (CH_3), 18.5 (CH_3); ^{19}F NMR (282 MHz, DMSO-d_6): δ −114.9 (m); LRMS (+ESI): *m/z* 350.9 ($[M-NH_3]^+$, 100%), 367.8 ($[M+H]^+$, 70%); Anal. ($C_{21}H_{22}N_3O_2F$) calcd: C 68.65, H 6.04, N 11.44; found: C 68.88, H 6.15, N 11.37; HPLC purity: 99.4 %.

(*S*)-*N*-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indole-3-carboxamide (ADB-FUBICA, 14). Treating **37** (0.63 g, 2.3 mmol) with **20** (0.46 g, 3.5 mmol) according to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 10:90), **14** (0.76 g, 86%) as a white solid. mp 107−108 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (1H, d, *J* = 7.7 Hz), 7.73 (1H, s), 7.31-7.20 (4H, m), 7.14-7.06 (2H, m), 6.99 (2H, t, *J* = 8.6 Hz), 6.77 (1H, d, *J* = 9.2 Hz), 6.54 (1H, bs), 5.71 (1H, bs), 5.27 (2H, s), 4.71 (1H, d, *J* = 9.2 Hz), 1.14 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.4 (CO), 165.0 (CO), 162.6 (d, $^{1}J_{C\text{-}F}$ = 247.2 Hz, quat.), 136.8 (CH), 132.0 (d, $^{4}J_{C\text{-}F}$ = 3.2 Hz, quat.), 131.8 (quat.), 128.8 (d, $^{3}J_{C\text{-}F}$ = 8.1 Hz, CH), 125.8 (quat.), 123.1 (CH), 122.1 (CH), 120.5 (CH), 116.6 (d, $^{2}J_{C\text{-}F}$= 21.6 Hz, CH), 111.4 (quat.), 110.7 (CH), 59.9 (CH), 50.1 (CH_2), 34.9 (quat.), 27.0 (CH_3); ^{19}F NMR (376 MHz, $CDCl_3$): δ −113.9; LRMS (+ESI): *m/z* 363.9 ($[M-NH_3]^+$, 100%), 381.8 ($[M+H]^+$, 42%); Anal. ($C_{22}H_{24}N_3O_2F$) calcd: C 69.27, H 6.34, N 11.02; found: C 69.68, H 6.02, N 10.95.

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1*H*-indole-3-carboxamide (AB-PICA, 15). Treating **38** (0.50 g, 2.2 mmol) with **19** (0.50, 3.3 mmol) according to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 10:90), **15** (0.58 g, 81%) as a white solid. mp 214−215 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.25

(1H, s), 8.11 (1H, d, *J* = 7.7 Hz), 7.61-7.39 (3H, m), 7.20 (1H, m), 7.13 (1H, t, *J* = 7.6 Hz), 7.06 (1H, bs), 4.35 (1H, m), 4.19 (2H, m), 2.09 (1H, dq, *J* = 13.5 Hz, 6.8 Hz), 1.08 (2H, quin., *J* = 7.3 Hz), 1.39-1.19 (4H, m), 0.94 (6H, dd, *J* = 6.6 Hz, 3.1 Hz), 0.85 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.6 (CO), 164.0 (CO), 136.1 (CH), 131.2 (quat.), 126.5 (quat.), 121.8 (CH), 121.0 (CH), 120.6 (CH), 110.3 (quat.), 109.3 (CH), 57.3 (CH), 45.8 (CH_2), 30.3 (CH), 29.3 (CH_2), 28.4 (CH_2), 21.7 (CH_2), 19.5 (CH_2), 18.5 (CH_3), 13.8 (CH_3); LRMS (+ESI): *m/z* 312.87 ($[M-NH_2]^+$, 100%), 329.80 ($[M+H]^+$, 60%); Anal. ($C_{19}H_{27}N_3O_2$) calcd: C 69.27, H 8.26, N 12.76; found: C 69.21, H 8.66, N 12.55; HPLC purity: 99.1%.

(*S*)-*N*-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1*H*-indole-3-carboxamide (ADBICA, 16). Treating **38** (0.57 g, 2.5 mmol) with **20** (0.49 g, 3.7 mmol) according to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 10:90), **16** (0.64 g, 75%) as a white solid. mp 138−139 °C; ^{1}H NMR (400 MHz,$CDCl_3$): δ 8.01 (1H, m), 7.72 (1H, s), 7.38 (1H, m), 7.33-7.22 (2H, m), 6.74 (1H, d, *J* = 8.9 Hz), 6.60 (1H, bs), 5.71 (1H, bs), 4.73 (1H, d, *J* = 8.9 Hz), 4.11 (2H, t, *J* = 7.2 Hz), 1.85 (2H, m), 1.42-1.25 (4H, m), 1,15 (9H, s), 0.89 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5 (CO), 165.2 (CO), 136.8 (CH), 131.6 (quat.), 125.6 (quat.), 122.6 (CH), 121.8 (CH), 120.4 (CH), 110.6 (quat.), 110.4 (CH), 59.9 (CH), 47.1 (CH_2), 34.9 (quat.), 29.8 (CH_2), 29.1 (CH_2), 27.0 (CH_2), 22.4 (CH_3), 14.0 (CH_3); LRMS (+ESI): *m/z* 326.9 ($[M-NH_3]^+$, 100%), 343.9 ($[M+H]^+$, 31%); Anal. ($C_{20}H_{29}N_3O_2$) calcd: C 69.94, H 8.51, N 12.23; found: C 70.23, H 8.65, N 12.17.

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1*H*-indole-3-carboxamide (5F-AB-PICA, 17). Treating **39** (1.53 g, 6.5 mmol) with **19** (1.50 g, 9.8 mmol) according to

general procedure A gave, following recrystallization from *i*-PrOH, **17** (1.56 g, 69%) as a white solid. mp 210−211 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.25, (1H, s), 8.11, (1H, d, J = 7.8 Hz), 7.61-7.39 (3H, m), 7.25-7.10 (2H, m), 7.07 (1H, bs), 4.47 (1H, t, J = 6.0 Hz), 4.35 (2H, m), 4.21 (2H, m), 2.09 (1H, dq, J = 13.5 Hz, 6.8 Hz), 1.84 (2H, m), 1.67 (2H, m), 1.35 (2H, m), 0.93 (6H, dd, J = 6.5 Hz, 2.9 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.7 (CO), 164.1 (CO), 136.1 (CH), 131.3 (quat.), 126.5 (quat.), 121.9 (CH), 121.0 (CH), 120.7 (CH), 110.4 (quat.), 109.4 (CH), 83.7 (d, $^{1}J_{C\text{-}F}$ = 161.7 Hz, CH_2), 57.4 (CH), 45.78 (CH_2), 30.5 (CH), 29.5 (CH_2), 29.3 (d, $^{3}J_{C\text{-}F}$ = 3.8 Hz, CH_2), 22.2 (d, $^{2}J_{C\text{-}F}$ = 5.3 Hz, CH_2), 19.5 (CH_3), 18.5 (CH_3); ^{19}F NMR (376 MHz, DMSO-d_6): δ −216.86; LRMS (+ESI): *m/z* 330.9 ([M−NH_3]$^{+}$, 100%), 347.8 ([M+H]$^{+}$, 67%); Anal. ($C_{19}H_{26}N_3O_2F$) calcd: C 65.68, H 7.54, N 12.09; found: C 65.83, H 7.66, N 11.99.

(*S*)-*N*-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1*H*-indole-3-carboxamide (5F-ADBICA, 18). Treating **39** (1.14 g, 4.9 mmol) with **20** (0.95 g, 7.3 mmol) according to general procedure A gave, following purification by flash chromatography (hexane-EtOAc, 90:10), **18** (1.14 g, 65%) as a white solid. mp 130−131 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (1H, m), 7.71 (1H, s), 7.37 (1H, m), 7.32-7.23 (2H, m), 6.76 (1H, d, J = 9.2 Hz), 6.67 (1H, bs), 5.74 (1H, bs), 4.74 (1H, d, J = 9.2 Hz), 4.47 (2H, dt, $^{2}J_{H\text{-}F}$ = 48, $^{3}J_{H\text{-}H}$ = 6.0 Hz), 4.13 (2H, t, J = 7.1 Hz), 1.90 (2H, m), 1.70 (2H, m), 1.45 (2H, m), 1.15 (9H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.5 (CO), 165.1 (CO), 136.7 (CH), 131.5 (quat.), 125.7 (quat.), 122.7 (CH), 121.8 (CH), 120.5 (CH), 110.8 (quat.), 110.3 (CH), 83.8 (d, $^{1}J_{C\text{-}F}$ = 164.8 Hz, CH_2), 59.9 (CH), 46.9 (CH_2), 34.9 (quat.), 30.1 (d, $^{2}J_{C\text{-}F}$ = 19.9 Hz, CH_2), 29.8 (CH_2), 27.0 (CH_3), 23.0 (d, $^{3}J_{C\text{-}F}$ = 5.1 Hz, CH_2); ^{19}F NMR (376 MHz, $CDCl_3$): δ −218.53; LRMS (+ESI): *m/z* 344.9 ([M−NH_3]$^{+}$, 100%), 361.8 ([M+H]$^{+}$, 26%); Anal. ($C_{20}H_{28}N_3O_2F$) calcd: C 66.46, H 7.81, N 11.63; found: C 66.20, H 7.94, N 11.19.

L-*tert*-leucinamide (20). To a solution of **23** (15.6 g, 59 mmol) in THF (150 mL) was added 10% Pd/C (3.0 g), and the mixture was stirred under an atmosphere of H_2 for 12 h. The suspension was filtered through a pad of Celite, and the filtrate evaporated under reduced pressure. The resulting solid was recrystallized from EtOAc-hexane to yield **20** (4.74 g, 48%) as a white solid. m.p. 105–106 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.17 (1H, bs), 6.81 (1H, bs), 3.35 (1H, bs), 2.79 (1H, s), 1.5 (2H, bs), 0.88 (9H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 176.17 (CO), 62.83 (CH), 33.56 (quat.), 26.53 (CH_3); LRMS (+ESI): *m/z* 130.9 ($[M+H]^+$, 100%).

***N*-Cbz-L-*tert*-leucine (22).** A cooled (0 °C) solution of L-*tert*-leucine (**21**, 10.0 g, 76 mmol) and 5 M aq. NaOH (15 mL, 75 mmol, 1.0 equiv.) in H_2O (25 mL) was treated dropwise with benzyl chloroformate (12 mL, 84 mmol, 1.1 equiv.) and 2 M aq. NaOH (42 mL, 84 mmol, 1.1 equiv.), simultaneously. The mixture was warmed to rt, stirred for 2 h, and the pH adjusted to 10 by the addition of sat. aq. $NaHCO_3$. The aqueous layer was washed with Et_2O (3 × 50 mL), acidified to pH 3 with 2 M aq. HCl, and extracted with Et_2O (4 × 50 mL). The combined organic phases were dried ($MgSO_4$) and the solvent evaporated under reduced pressure to give a **22** (20 g, 99%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43-7.29 (5H, m), 5.78 (1H, bs), 5.36 (1H, d, J = 9.5 Hz), 5.12 (2H, m), 4.21 (1H, d, J = 9.5 Hz), 1.02 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.0 (CO), 156.4 (CO), 136.3 (quat.), 128.7 (CH), 128.4 (CH), 128.3 (CH), 67.4 (CH), 62.3 (CH_2), 34.7 (quat.), 26.6 (CH_3); LRMS (–ESI): *m/z* 528.9 ($[2M–H]^-$, 100%), 263.9 ($[M–H]^-$, 76%).

***N*-Cbz-L-*tert*-leucinamide (23).** To a solution of **22** (20.2 g, 76 mmol) in DMF (400 mL) was added NH_4Cl (4.95 g, 93 mmol, 1.2 equiv.), Et_3N (32 mL, 229 mmol, 3.0 equiv.), HOBt

(13.2 g, 98 mmol, 1.3 equiv.), and EDC·HCl (18.8 g, 98 mmol, 1.3 equiv.). After stirring for 16 h, the reaction was quenched with sat. aq. $NaHCO_3$ (100 mL), and the aqueous phase was extracted with EtOAc (3 × 200 mL). The combined organic phases were washed with H_2O (3 × 200 mL), brine (100 mL), dried ($MgSO_4$), and the solvent removed under reduced pressure. The obtained crude solid was recrystallized from EtOAc-hexane to give **23** (16.9 g, 84%) as a white solid. m.p. 138–140 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40-7.29 (5H, m), 6.11 (1H, bs), 5.75 (1H, bs), 5.62 (1H, d, J = 9.5 Hz), 5.08 (2H, m), 4.03 (1H, d, J = 9.5 Hz), 1.01 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.1 (CO), 156.7 (CO), 136.4 (quat.), 128.7 (CH), 128.3 (CH), 128.1 (CH), 67.2 (CH), 62.4 (CH_2), 34.4 (quat.), 26.6 (CH_3); LRMS (+ESI): *m/z* 264.8 ($[M+H]^+$, 100%).

Methyl 1*H*-indazole-3-carboxylate (25). A solution of indazole-3-carboxylic acid (**24**, 2.00 g, 12.3 mmol) in MeOH (30 mL) was treated with conc. H_2SO_4 (2 mL) and heated at reflux for 4 h. The mixture was concentrated *in vacuo* and dissolved in EtOAc (50 mL). The organic phase was washed with sat. aq. $NaHCO_3$ (20 mL), H_2O (20 mL), brine (20 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The crude solid was recrystallized from EtOAc-hexane to give 25 (1.65 g, 76%) as a white solid. m.p. 168–170 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (1H, m), 7.77 (1H, m), 7.49 (1H, m), 7.35 (1H, m), 4.08 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.41 (CO), 141.42 (quat.), 136.27 (quat.), 127.73 (CH), 123.60 (quat,), 122.50 (CH), 121.92 (CH), 111.35 (CH), 52.31 (CH_3); LRMS (ESI): *m/z* 176.8 ($[M+H]^+$, 100%).

General procedure B: alkylation of methyl 1*H*-indazole-3-carboxylate. To a cooled (0 °C) solution of **25** (2.17 g, 12.3 mmol) in THF (60 mL) was added *t*-BuOK (1.52 g, 13.5 mmol, 1.1 equiv.). The mixture was warmed to rt, stirred for 1 h, cooled (0 °C), and the

appropriate bromoalkane (19.7 mmol, 1.6 equiv.) was added dropwise. The mixture was warmed to rt, stirred for 48 h, and H_2O (60 mL) was added. The layers were separated, the aqueous layer was extracted with EtOAc (2 × 50 mL), and the combined organic phases were washed with H_2O (3 × 50 mL), brine (20 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure.

Methyl 1-(4-fluorobenzyl)-1*H*-indazole-3-carboxylate (26). Treating **25** (2.30 g, 13.1 mmol) with 4-fluorobenzyl bromide (1.78 mL, 14.3 mmol) according to general procedure B gave, following purification by flash chromatography (hexane-EtOAc 70:30), **26** (2.50 g, 67%) as a clear glass-like solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (1H, dt, J = 8.1 Hz, 1.1 Hz), 7.44-7.27 (3H, m), 7.25-7.16 (2H, m), 7.03-6.93 (2H, m), 5.67 (2H, s), 4.05 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.1 (CO), 162.6 (d, $^1J_{C\text{-}F}$ = 247.3 Hz, quat.), 140.6 (quat.), 135.3 (quat.), 131.6 (d, $^4J_{C\text{-}F}$ = 3.0 Hz, quat.), 129.2 (d, $^3J_{C\text{-}F}$ = 8.1 Hz, CH), 127.3 (CH), 124.3 (CH), 123.5 (quat.), 122.5 (CH), 116.0 (d, $^2J_{C\text{-}F}$ = 22.1 Hz, CH), 110.0 (CH), 53.5 (CH_2), 52.3 (CH_3); ^{19}F NMR (282 MHz, $CDCl_3$): δ –113.8 (m); LRMS (+ESI): *m/z* 284.8 ($[M+H]^+$, 100%).

Methyl 1-pentyl-1*H*-indazole-3-carboxylate (27). Treating **25** (1.57 g, 8.9 mmol) with 1-bromopentane (1.75 mL, 14.1 mmol) according to general procedure B gave, following purification by flash chromatography (hexane-EtOAc 70:30), **26** (1.46 g, 77%) as a clear glass-like solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (1H, m), 7.50-7.39 (2H, m), 7.30 (1H, m), 4.47 (2H, t, J = 7.4 Hz), 4.04 (3H, s), 1.97 (2H, quin., J = 7.2 Hz), 1.40-1.25 (4H, m), 0.88 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.0 (CO), 140.7 (quat.), 134.9 (quat.), 126.8 (CH), 123.9 (quat.), 123.1 (CH), 122.5 (CH),109.8 (CH), 61.1 (CH_2), 50.1 (CH_3), 29.7 (CH_2), 29.1 (CH_2), 22.4 (CH_2), 14.0 (CH_3); LRMS (+ESI): *m/z* 246.9 ($[M+H]^+$, 100%).

Methyl 1-(5-fluoropentyl)-1*H*-indazole-3-carboxylate (28). Treating **25** (2.30 g, 13.1 mmol) with 1-bromo-5-fluoropentane (2.42 g, 14.3 mmol) according to general procedure B gave, following purification by flash chromatography (hexane-EtOAc 70:30), **28** (2.39 g, 69%) as a colorless glass-like solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (1H, m), 7.50-7.41 (2H, m), 7.32 (1H, m), 4.54-4.43 (3H, m), 4.35 (1H, t, J = 5.9 Hz), 4.04 (3H, s), 2.03 (2H, quin., J = 7.6 Hz), 1.80- 1.64 (2H, m), 1.52-1.41 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.2 (CO), 140.7 (quat.), 134.8 (quat.), 127.0 (CH), 123.9 (CH), 123.3 (quat.), 122.5 (CH), 109.7 (CH), 83.8 (d, $^{1}J_{C\text{-}F}$ = 165.1 Hz, quat.), 52.2 (CH_2), 49.9 (CH_3), 30.1 (d, $^{2}J_{C\text{-}F}$ = 19.7, CH_2), 29.6 (CH_2), 22.9 (d, $^{3}J_{C\text{-}F}$ = 5.1 Hz, CH_2); ^{19}F NMR (376 MHz, $CDCl_3$): δ –218.7; LRMS (+ESI): *m/z* 264.8 ($[M+H]^+$, 100%).

General procedure C: hydrolysis of methyl 1-alkyl-1*H*-indazole-3-carboxylates. A solution of the appropriate methyl 1-alkyl-1*H*-indazole-3-carboxylate (12.3 mmol) in MeOH (100 mL) was treated with 1 M aq. NaOH (18.5 mL, 18.5 mmol, 1.5 equiv.) and stirred for 24 h. The solvent was reduced *in vacuo*, the residue dissolved in H_2O, acidified with 1 M aq. HCl, and extracted with EtOAc (2 × 50 mL). The organic phase was dried ($MgSO_4$) and the solvent evaporated under reduced pressure to afford the free acid, which was used in the subsequent coupling step without further purification.

1-(4-Fluorobenzyl)-1*H*-indazole-3-carboxylic acid (29). Subjecting **26** (2.30 g, 8.1 mmol) to general procedure C gave **29** (2.10 g, 96%) as a white solid. m.p. 200–203 °C; ^{1}H NMR (300 MHz, d_6-DMSO): δ 13.02 (1H, bs), 8.09 (1H, m), 8.47 (1H, m), 7.47 (1H, m), 7.39-7.27 (3H, m), 7.16 (2H, m), 5.76 (2H, s); ^{13}C NMR (75 MHz, d_6-DMSO): δ 163.4 (CO), 161.7 (d, $^{1}J_{C\text{-}F}$ = 244.8 Hz, quat.), 140.4 (quat.), 135.1 (quat.), 132.9 (d, $^{4}J_{C\text{-}F}$ = 2.8 Hz, quat.), 129.7 (d,

$^{3}J_{C\text{-}F}$ = 8.3 Hz, CH), 126.9, 123.2, 123.0, 121.6, 115.5 (d, $^{2}J_{C\text{-}F}$ = 21.3 Hz, CH), 110.7 (CH), 51.8 (CH_2); ^{19}F NMR (282 MHz, $CDCl_3$): δ –114.6; LRMS (+ESI): *m/z* 270.9 ($[M+H]^+$, 100%).

1-pentyl-1*H*-indazole-3-carboxylic acid (30). Subjecting **27** (0.96 g, 3.9 mmol) to general procedure C gave **30** (0.65 g, 72%) as a white solid. m.p. 81–82 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.85 (1H, bs), 8.26 (1H, m), 7.56-7.41 (2H, m), 7.34 (1H, m), 4.49 (2H, t, *J* = 7.3 Hz), 1.99 (2H, m), 1.45-1.25 (4H, m), 0.88 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.3 (CO), 140.9 (quat.), 134.0 (quat.), 127.0 (CH), 124.0 (quat.), 123.6 (CH), 122.5 (CH), 109.9 (CH), 50.2 (CH_2), 29.6 (CH_2), 29.0 (CH_2), 22.4 (CH_2), 14.0 (CH_3); LRMS (+ESI): *m/z* 323.9 ($[M+H]^+$, 100%).

1-(5-fluoropentyl)-1*H*-indazole-3-carboxylic acid (31). Subjecting **28** (2.2 g, 8.3 mmol) to general procedure C gave **31** (1.9 g, 91%) as a white solid. m.p. 80–82 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.88 (1H, bs), 8.27 (1H, m), 7.55-7.43 (2H, m), 7.36 (1H, m), 4.60-4.45 (3H, m), 4.34 (1H, t, *J* = 5.9 Hz), 2.06 (2H, m), 1.86-1.62 (2H, m), 1.58-1.39 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.4 (CO), 140.2 (quat.), 134.2 (quat.), 127.2 (CH), 124.0 (CH), 123.7 (CH), 122.5 (quat.), 109.8 (CH), 83.8 (d, $^{1}J_{C\text{-}F}$ = 165.1 Hz, CH_2), 50.0 (CH_2), 30.1 (d, $^{2}J_{C\text{-}F}$ = 19.8 Hz, CH_2), 29.5 (CH_2), 22.8 (d, $^{3}J_{C\text{-}F}$ = 4.9 Hz, CH_2); ^{19}F NMR (282 MHz, $CDCl_3$): δ –218.6; LRMS (+ESI): *m/z* 187.1 (100%), 250.9 ($[M]^+$, 49%).

General procedure D: one-pot synthesis of 1-alkyl-3-(trifluoroacetyl)indoles. A cooled (0 °C) suspension of NaH (60% dispersion in mineral oil, 0.68 g, 17.1 mmol, 2.0 equiv.), in DMF (10 mL) was treated with a solution of indole (**33**, 1.00 g, 8.5 mmol) in DMF (2 mL), warmed to rt, and stirred for 10 min. The mixture was cooled to 0 °C, treated slowly with the

appropriate bromoalkane (1.05 equiv.), warmed to rt, and stirred for 1 h. The solution was cooled to 0 °C, treated with $(CF_3CO)_2O$ (3.00 mL, 21.3 mmol, 2.5 equiv.), warmed to rt, and stirred for 1 h. The mixture was poured onto ice-water (120 mL) and stirred vigorously. The mixture was filtered and the precipitate dried to give the crude product as a red solid which was used in the following step without purification.

1-(4-Fluorobenzyl)-3-(trifluoroacetyl)indole (34). Subjecting 4-fluorobenzyl bromide (1.13 mL, 9.0 mmol) to general procedure D gave **34** as a red crystalline solid (2.72 g, 100%). m.p. 83–86 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (1H, m), 7.96 (1H, m), 7.38 (1H, m), 7.43-7.29 (2H, m), 7.22-7.12 (2H, m), 7.11-7.00 (2H, m), 5.38 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.1 (q, $^2J_{C\text{-}F}$ = 34.9 Hz, CO), 162.9 (d, $^1J_{C\text{-}F}$ = 247.5 Hz, quat.), 137.5 (q, $^3J_{C\text{-}F}$ = 4.9 Hz, CH), 136.9 (quat.), 130.7 (d, $^3J_{C\text{-}F}$ = 3.4 Hz, quat.), 129.7 (d, $^2J_{C\text{-}F}$ = 8.1 Hz, CH), 127.3 (quat.), 125.0 (quat.), 124.3 (CH), 123.0 (CH), 117.1 (q, $^1J_{C\text{-}F}$ = 291.8 Hz, quat.), 116.4 (d, $^2J_{C\text{-}F}$ = 22.2 Hz, CH), 110.8 (CH), 110.2 (CH), 50.8 (CH_2); ^{19}F NMR (282 MHz, $CDCl_3$): δ –112.9 (m), –72.3; LRMS (+ESI): *m/z* 321.9 ($[M+H]^+$, 100%).

1-pentyl-3-(trifluoroacetyl)indole (35). Subjecting 1-bromopentane (1.11 mL, 8.96 mmol) to general procedure D gave **35** as a red crystalline solid (2.41 g, 100%). m.p. 56–57 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (1H, m), 7.93 (1H, d, J = 1.5 Hz), 7.47-7.33 (3H, m), 4.20 (2H, t, J = 7.21), 1.93 (2H, quin., J = 7.16 Hz), 1.46-1.31 (4H, m), 0.92 (3H, t, J = 7.02 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.9 (q, $^2J_{C\text{-}F}$ = 34.7 Hz, CO), 137.4 (q, $^3J_{C\text{-}F}$ = 4.9 Hz, CH), 136.8 (quat.), 127.3 (quat.), 124.6 (quat.), 124.0 (CH), 122.9 (CH), 117.3 (q, $^1J_{C\text{-}F}$ = 291.5 Hz, quat.), 110.5 (CH), 109.6 (CH), 47.8 (CH_2), 29.6 (CH_2), 29.0 (CH_2), 22.3 (CH_2), 14.0 (CH_3); ^{19}F NMR (376 MHz, $CDCl_3$): δ –72.2; LRMS (ESI): *m/z* 284.0 ($[M+H]^+$, 100%).

1-(5-fluoropentyl)-3-(trifluoroacetyl)indole (36). Subjecting 1-bromo-5-fluoropentane (1.51 g, 9.0 mmol) according to general procedure D gave **36** as a red solid (2.59 g, 100%). m.p. 55–57 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (1H, m), 7.93 (1H, m), 7.46-7.32 (3H, m), 4.45 (2H, dt, $^{2}J_{H\text{-}F}$ = 45, $^{3}J_{H\text{-}H}$ = 5.8 Hz), 4.23 (2H, t, J = 7.2 Hz), 1.99 (2H, m), 1.75 (2H, m), 1.52 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.9 (q, $^{2}J_{C\text{-}F}$ = 34.8 Hz, CO), 137.4 (q, $^{3}J_{C\text{-}F}$ = 5.0 Hz, CH), 136.7 (quat.), 127.3 (quat.), 124.7 (CH), 124.1 (CH), 122.9 (CH), 117.2 (q, $^{1}J_{C\text{-}F}$ = 291.3 Hz, quat.), 110.4 (CH), 109.7 (quat.), 83.7 (d, $^{1}J_{C\text{-}F}$ = 165.1 Hz, CH_2), 47.7 (CH_2), 30.0 (d, $^{2}J_{C\text{-}F}$ 19.9 Hz, CH_2), 29.5 (CH_2), 23.0 (d, $^{3}J_{C\text{-}F}$ = 4.5 Hz, CH_2); ^{19}F NMR (470 MHz, $CDCl_3$): δ –72.2, –218.9; LRMS (+ESI): *m/z* 302.0 ($[M+H]^+$, 100%).

General procedure E: synthesis of 1-alkylindole-3-carboxylic acids. To a refluxing solution of KOH (1.57 g, 28.1 mmol, 3.3 equiv.) in MeOH (3 mL) was added, portionwise, a solution of the appropriate crude 1-alkyl-3-trifluoroacetylindole (8.5 mmol) in toluene (7 mL). After heating at reflux for 2 h, the mixture was cooled to ambient temperature, and H_2O (30 mL) added. The layers were separated and the organic layer extracted with 1 M aq. NaOH (8 mL). The combined aqueous phases were acidified to pH 1 with 10 M aq. HCl, extracted with Et_2O (3 × 10 mL), dried ($MgSO_4$), and the solvent removed under reduced pressure. The crude solid was recrystallized from *i*-PrOH to give the appropriate 1-alkylindole-3-carboxylic acid as colorless crystals.

1-(4-Fluorobenzyl)indole-3-carboxylic acid (37). Subjecting **34** (2.74 g, 8.5 mmol) to general procedure E gave **37** (1.51 g, 66%) as a colorless crystalline solid. m.p. 205–208 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22 (1H, s), 8.02 (1H, m), 7.54 (1H, m), 7.40-7.31 (2H, m), 7.24-7.11 (4H, m), 5.48 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.5 (CO), 161.6 (d, $^{1}J_{C\text{-}F}$ = 243.3 Hz, quat.), 136.2 (CH), 135.4 (quat.), 133.4 (d, $^{3}J_{C\text{-}F}$ = 3.2 Hz, quat.), 129.5

(d, $^{3}J_{C\text{-}F}$ = 8.3 Hz, CH), 126.6 (quat.), 122.4 (CH), 121.4 (CH), 120.9 (CH), 115.4 (CH, d, $^{2}J_{C\text{-}F}$ = 21.7), 111.0 (CH), 107.0 (quat.), 48.7 (CH_2) ppm; ^{19}F NMR (282 MHz, DMSO-d_6) δ –114.80 (m) ppm; LRMS (+ESI): *m/z* 283.9 (100%), 269.9 ($[M+H]^+$, 10%).

1-Pentylindole-3-carboxylic acid (38). Subjecting **35** (2.00 g, 7.1 mmol) to general procedure E gave **38** (0.88 g, 54%) as a colorless crystalline solid. m.p. 101–102 °C (lit mp 106-108 °C)[28]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.87 (1H, bs), 8.26 (1H, m), 7.93 (1H, s), 7.39 (1H, m). 7.35-7.27 (2H, m), 4.17 (2H, t, J = 7.1 Hz), 1.90 (2H, quin., J = 7.1 Hz), 1.46-1.25 (4H, m), 0.91 (3H, t, J = 6.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7 (CO), 136.9 (CH), 135.6 (quat.), 127.2 (quat.), 123.0 (quat.), 122.3 (CH), 122.1 (CH), 110.2 (CH), 106.4 (CH), 47.3 (CH_2), 29.7 (CH_2), 29.1 (CH_2), 22.4 (CH_2), 14.0 (CH_3); LRMS (+ESI): *m/z* 245.9 (100%), 231.9 ($[M+H]^+$, 16%).

1-(5-Fluoropentyl)indol-3-carboxylic acid (39). Subjecting **36** (2.57 g, 8.5 mmol) to general procedure E gave **39** (1.36 g, 68%) as a colorless crystalline solid. m.p. 117–118 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (1H, m), 7.93 (1H, s), 7.38 (1H, m), 7.35-7.28 (2H, m), 4.43 (2H, dt, $^{2}J_{H\text{-}F}$ = 48, $^{3}J_{H\text{-}H}$ = 5.9 Hz), 4.19 (2H, t, J = 7.1 Hz), 1.95 (2H, m), 1.73 (2H, m), 1.48 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8 (CO), 136.8 (CH), 135.5 (quat.), 127.2 (quat.), 123.1 (CH), 122.3 (CH), 122.1 (CH), 110.1 (CH), 106.6 (quat.), 83.8 (d, $^{1}J_{C\text{-}F}$ = 164.9 Hz, CH_2), 47.2 (CH_2), 30.1 (d, $^{2}J_{C\text{-}F}$ = 20.0 Hz, CH_2), 29.7 (CH_2), 23.0 (d, $^{3}J_{C\text{-}F}$ = 5.0 Hz, CH_2); ^{19}F NMR (376 MHz, $CDCl_3$): δ –218.6; LRMS (+ESI): *m/z* 263.9 (100%), 249.9 ($[M+H]^+$, 18 %).

In vitro **pharmacological assessment of SCs.** Mouse AtT-20 neuroblastoma cells stably transfected with human CB_1 or human CB_2 have been previously described[14,36,41] and 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. 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 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.[42] 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 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 CP 55,940 was added to allow for normalization between assays.

***In vivo* pharmacological assessment of SCs.** Four cohorts of 3–4 adult male Wistar rats (Animal Resources Centre, Perth, Australia) initially weighing between 168 and 186 g were used for biotelemetric assessment of body temperature and heart rate changes following each compound or following either compound administered with a CB_1 and CB_2 antagonist. 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.[14,36] 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 one 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). The first two cohorts then received 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. At least two washout days were given between each dose.

For the antagonist studies (Fig. 8), the third and fourth cohort of drug-naïve rats were used for each compound, with a 48 hr washout period between each dose. Each cohort received injections of either vehicle, CB_1 antagonist (rimonabant, 3 mg/kg), or CB_2 antagonist (SR144528, 3 mg/kg), followed by AB-FUBINACA (3 mg/kg) or AB-PINACA (3 mg/kg). The vehicle or antagonist injections were given to rats 30 minutes prior to the AB-FUBINACA or AB-PINACA injections.

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

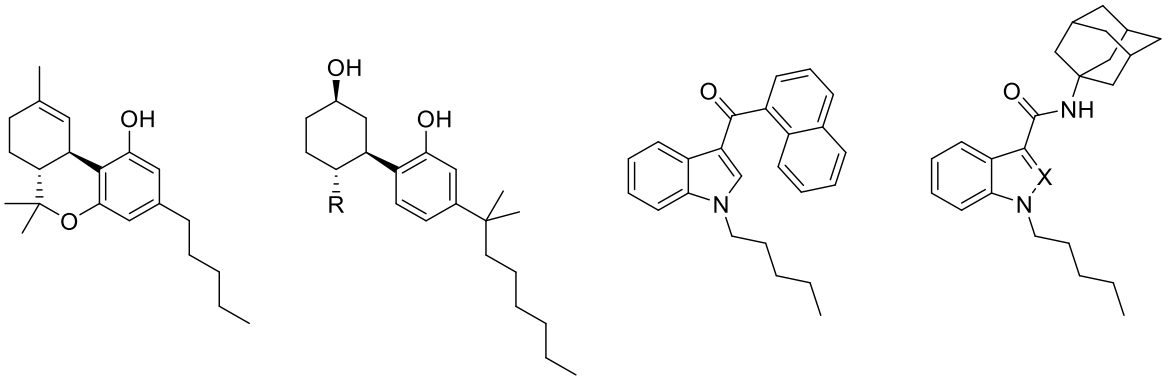
We calculated the area between baseline and drug-treatment body temperature curves for each rat as a measure of compound potency. Briefly, for any time point, the area between baseline data points (B_t) and drug-treatment data points (D_t) and the subsequent time points (B_{t+1} and D_{t+1}) forms a trapezoid, the area of which can be calculated via the formula:

$$Area = \frac{(B_t - D_t) + (B_{t+1} - D_{t+1})}{2}$$

These areas were summed from the time of injection to 6 hrs post-injection. This data was analysed using a two-way mixed model ANOVA with Bonferroni corrected contrasts comparing the compounds at each dose.

For the antagonist studies, the area between the vehicle-vehicle baseline and the vehicle-SC (i.e. vehicle-AB-FUBINACA or vehicle-AB-PINACA), rimonabant-SC and SR144528-SC treatments was calculated over a 3 hr time period post-injection of SC. These areas were analyzed using a one-way repeated measures ANOVA with planned Dunnet's contrasts comparing the antagonist areas to the vehicle-drug area.

Figure 1. Selected phytocannabinoids and synthetic cannabinoids.



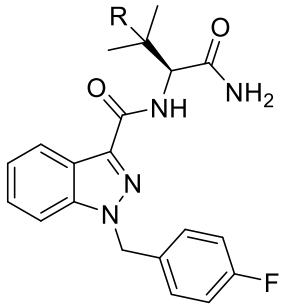
Δ9-THC (**1**)

CP 47,497 (**2**, R = H)
CP 55,940 (**3**, R = $(CH_2)_3OH$)

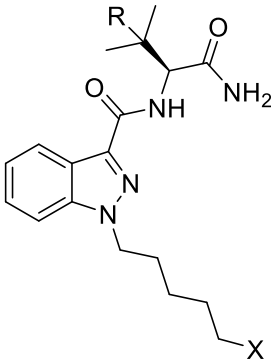
JWH-018 (**4**)

2NE1 (**5**, X = CH)
AKB48 (**6**, X = N)

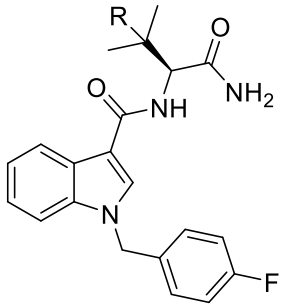
Figure 2. Indole- and indazole-3-carboxamide synthetic cannabinoid designer drugs.



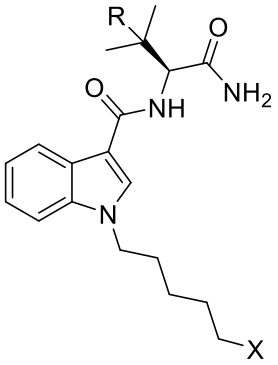
AB-FUBINACA (**7**: R = H)
ADB-FUBINACA (**8**: R = CH_3)



AB-PINACA (**9**: R = H; X = H)
ADB-PINACA (**10**: R = CH_3; X = H)
5F-AB-PINACA (**11**: R = H; X = F)
5F-ADB-PINACA (**12**: R = CH_3; X = F)

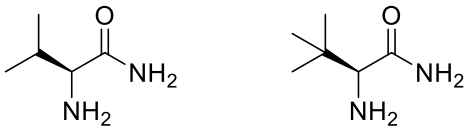


AB-FUBICA (**13**: R = H)
ADB-FUBICA (**14**: R = CH_3)



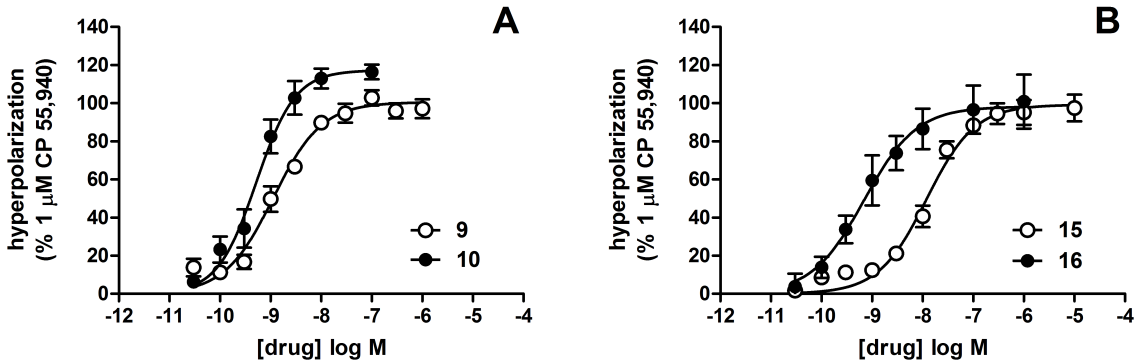
AB-PICA (**15**: R = H; X = H)
ADBICA (**16**: R = CH_3; X = H)
5F-AB-PICA (**17**: R = H; X = F)
5F-ADBICA (**18**: R = CH_3; X = F)

Figure 3. Amino acid derivatives L-valinamide (**19**) and L-*tert*-leucinamide (**20**).



19 **20**

Figure 4. Hyperpolarization of CB_1 receptors induced by (A) AB-PINACA (**9**) and ADB-PINACA (**10**) and (B) AB-PICA (**15**) and ADBICA (**16**) as a proportion of that produced by 1 μM CP 55,940.[a]



[a]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 4 parameter logistic equation in Graphpad Prism.

Figure 5. Effects of (A) AB-FUBINACA and (B) AB-PINACA on rat body temperature. Dashed line denotes time of intraperitoneal injection. Each point represents the mean ± SEM for 3 animals.

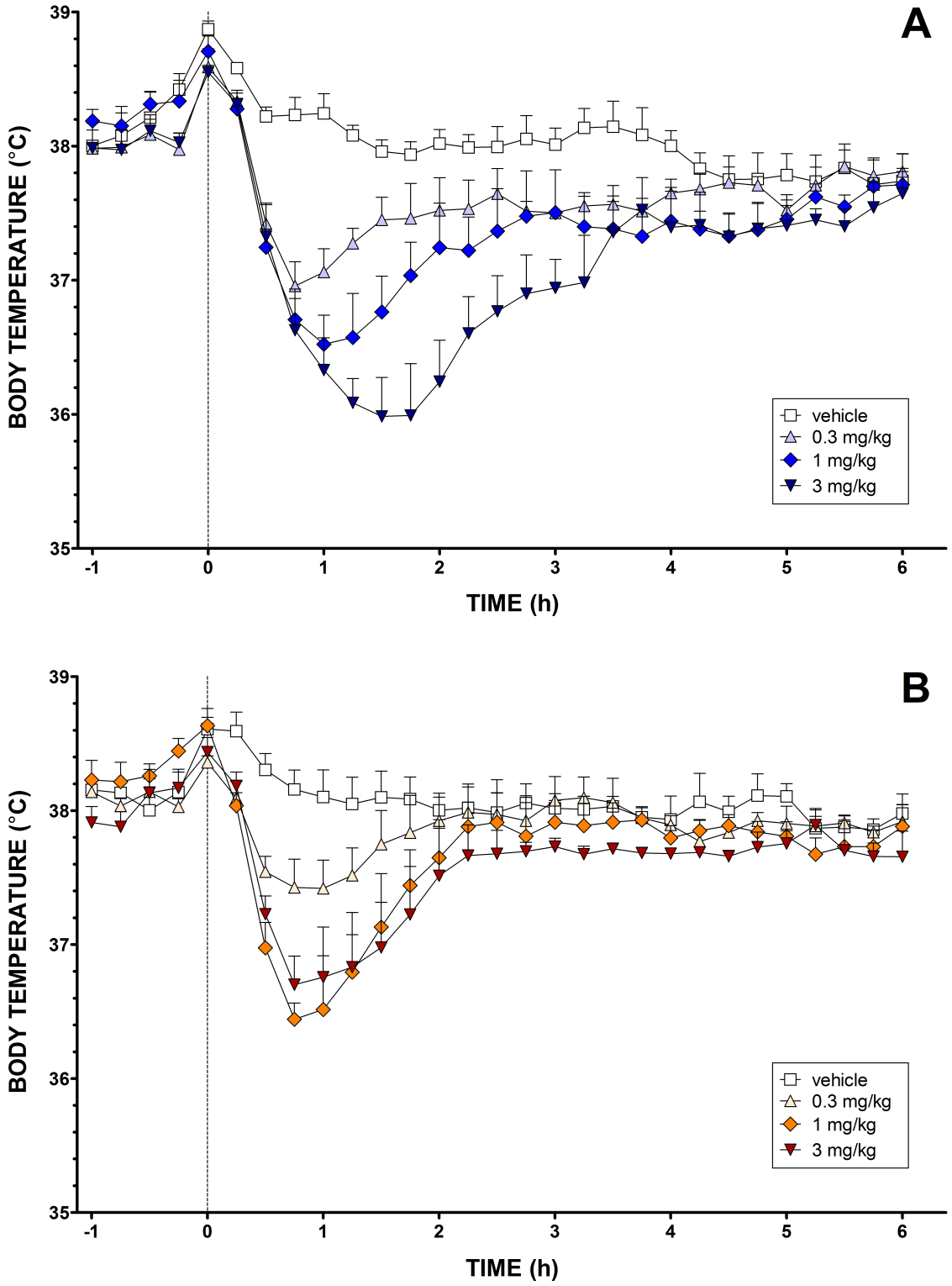


Figure 6. Effects of (A) AB-FUBINACA and (B) AB-PINACA on rat heart rate. Dashed line denotes time of intraperitoneal injection. Each point represents the mean ± SEM for 3 animals.

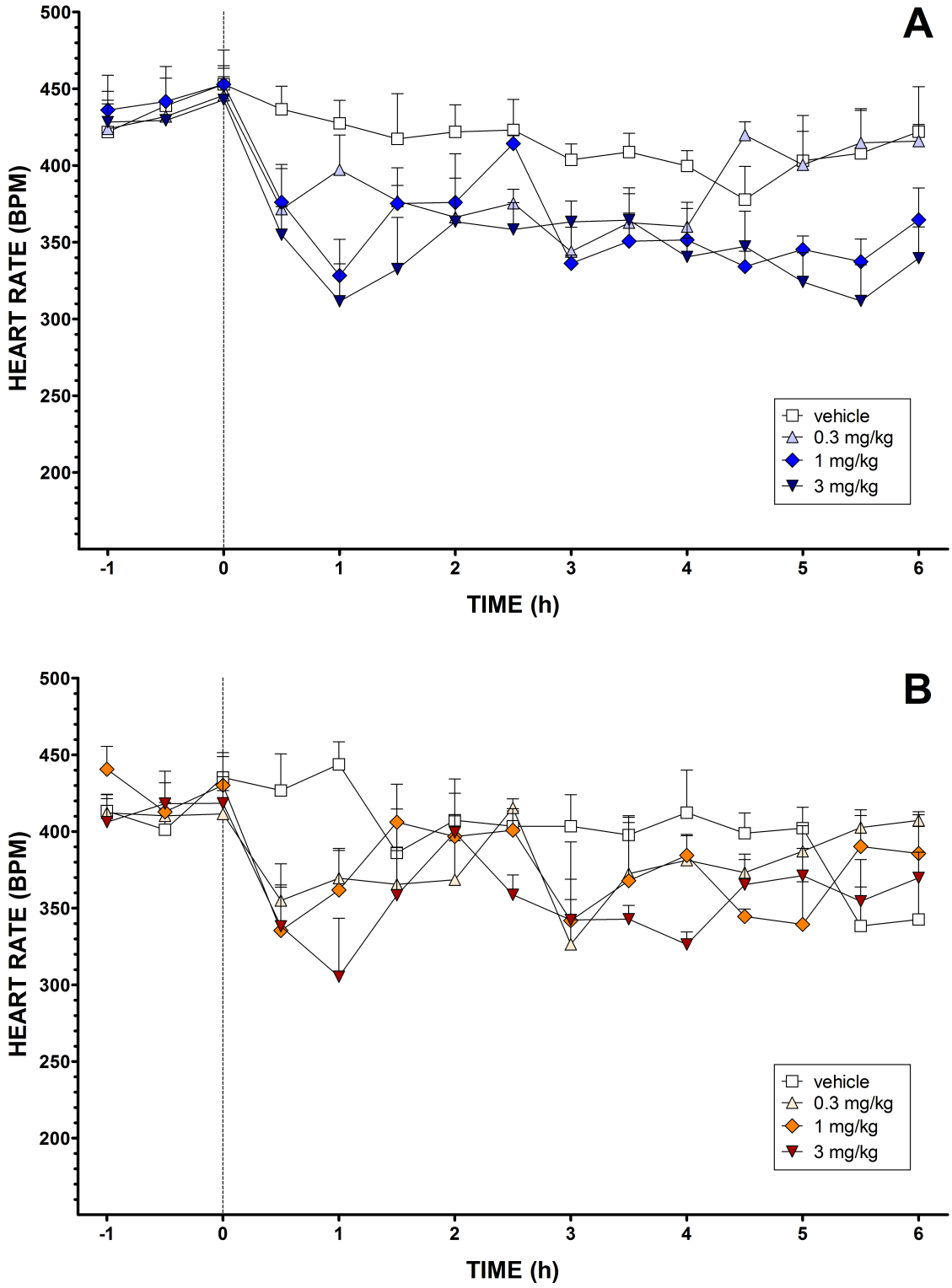
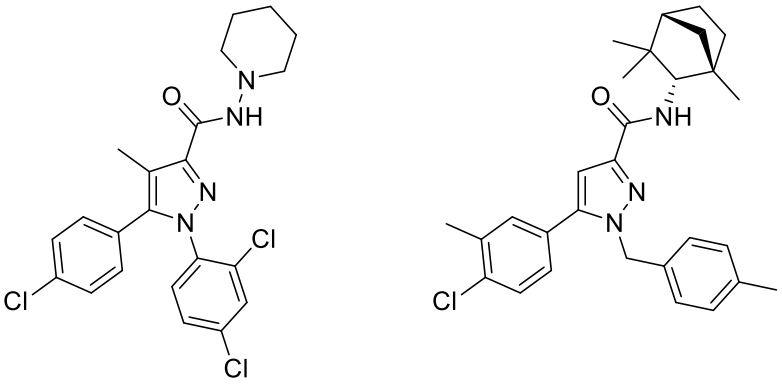
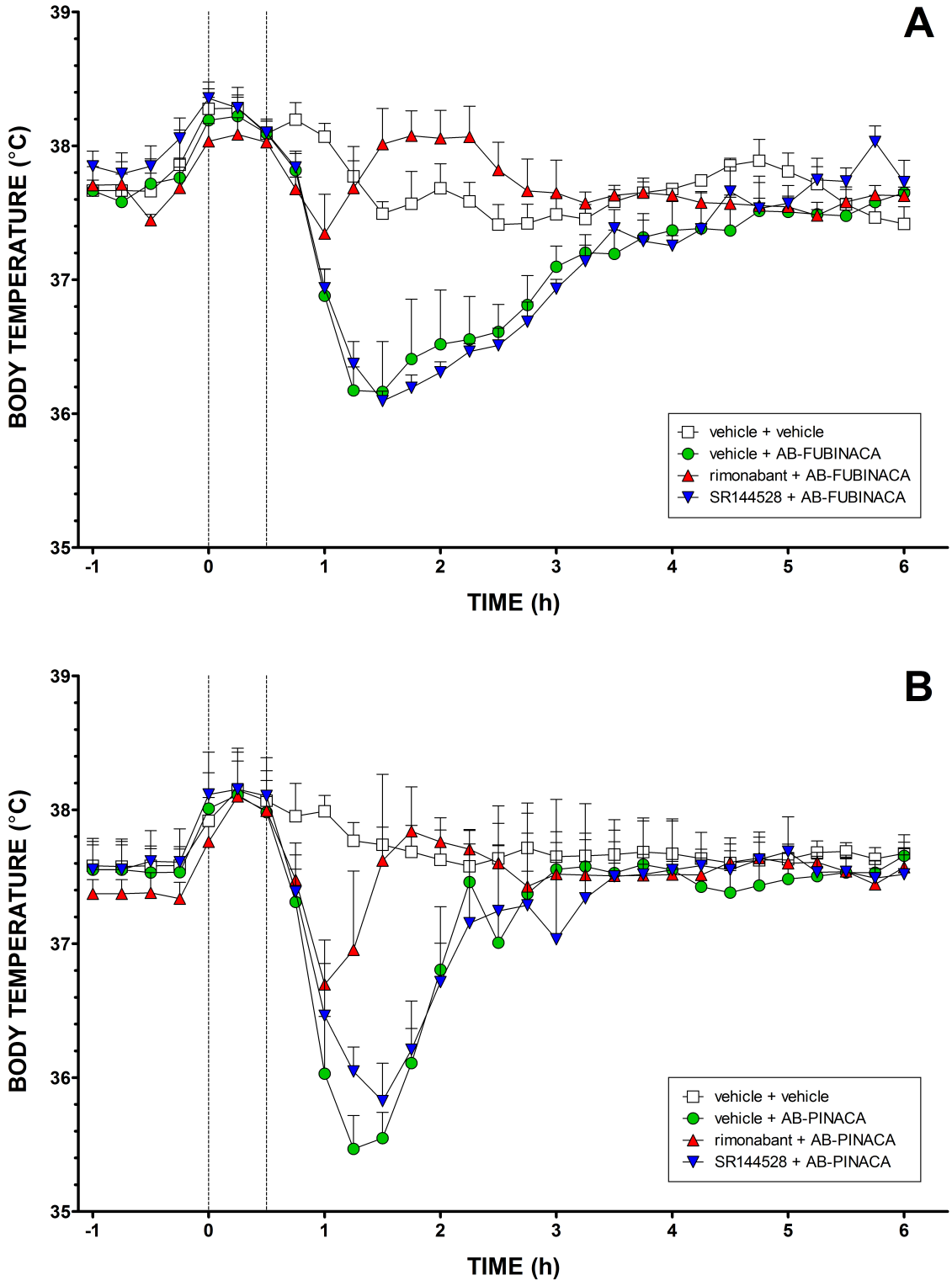


Figure 7. Structures of selective CB_1 receptor antagonist rimonabant (SR141176, **40**) and selective CB_2 receptor antagonist SR144528 (**41**).

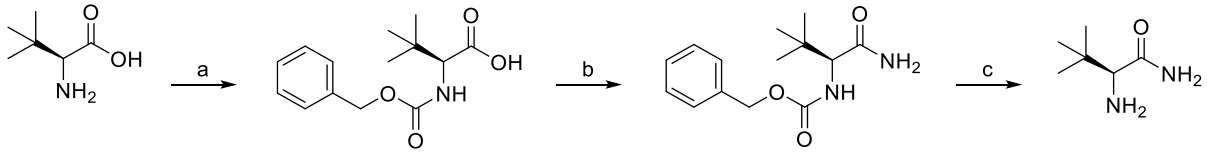


40 **41**

Figure 8. Effects of 3 mg/kg of (A) AB-FUBINACA or (B) AB-PINACA on rat body temperature following pretreatment (30 minutes prior) with vehicle, 3 mg/kg rimonabant (CB_1 antagonist), or 3 mg/kg SR144528 (CB_2 antagonist). The first dashed line denotes time of intraperitoneal injection of vehicle or antagonist. Second dashed line represents time of intraperitoneal injection of SC. Each point represents the mean ± SEM for 3 animals.



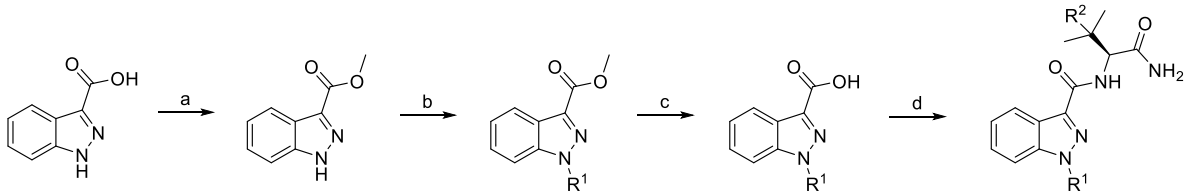
Scheme 1. Synthesis of L-*tert*-leucinamide.[a]



21 **22** **23** **20**

[a]Reagents and conditions: (a) NaOH, BnOC(O)Cl, 0 °C–rt, 2 h, 99%; (b) NH_4Cl, Et_3N, HOBt, EDCI, DMF, rt, 16 h, 84%; (c) 10% Pd/C, THF, 48%.

Scheme 2. Synthesis of indazole SCs **7**–**12**.[a]



24 **25**

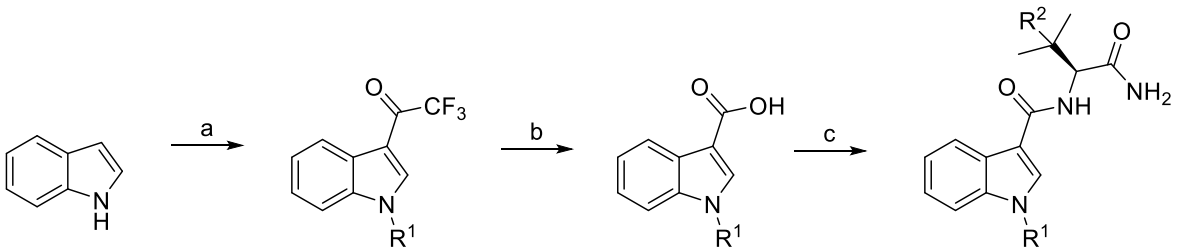
26: R^1 = 4-FBn
27: R^1 = C_5H_{11}
28: R^1 = $C_5H_{10}F$

29: R^1 = 4-FBn
30: R^1 = C_5H_{11}
31: R^1 = $C_5H_{10}F$

7: R^1 = 4-FBn; R^2 = H
8: R^1 = 4-FBn; R^2 = CH_3
9: R^1 = C_5H_{11}; R^2 = H
10: R^1 = C_5H_{11}; R^2 = CH_3
11: R^1 = $C_5H_{10}F$; R^2 = H
12: R^1 = $C_5H_{10}F$; R^2 = CH_3

[a]Reagents and conditions: (a) conc. H_2SO_4, MeOH, reflux, 4 h, 76%; (b) BrR^1, *t*-BuOK, THF, 0 °C–rt, 48 h, 67–77%; (c) NaOH, MeOH, rt, 24 h, 76–96%; (d) EDC·HCl, HOBt, DIPEA, **19** or **20**, DMF, rt, 24 h, 31–63%.

Scheme 3. Synthesis of indole SCs **13**–**18**.[a]



33

34: R^1 = 4-FBn
35: R^1 = C_5H_{11}
36: R^1 = $C_5H_{10}F$

37: R^1 = 4-FBn
38: R^1 = C_5H_{11}
39: R^1 = $C_5H_{10}F$

13: R^1 = 4-FBn; R^2 = H
14: R^1 = 4-FBn; R^2 = CH_3
15: R^1 = C_5H_{11}; R^2 = H
16: R^1 = C_5H_{11}; R^2 = CH_3
17: R^1 = $C_5H_{10}F$; R^2 = H
18: R^1 = $C_5H_{10}F$; R^2 = CH_3

[a]Reagents and conditions: (a)(i) NaH, BrR^1, DMF, 0 °C–rt, 1 h; (ii) $(CF_3CO)_2O$, DMF, 0 °C–rt, 1 h; (b) KOH, MeOH, PhMe, reflux, 2 h 54–68% (over 2 steps); (d) EDC·HCl, HOBt, DIPEA, **19** or **20**, DMF, rt, 24 h, 65–86%.

Table 1. Functional activity of Δ^9-THC, CP 55,940, JWH-018, and novel SCs **7**–**18** at CB_1 and CB_2 receptors.

Compound	hCB_1	hCB_1	hCB_2	hCB_2	CB_1 sel.*
	pEC_{50} ± SEM (EC_{50}, nM)	Max ± SEM (% CP 55,940)	pEC_{50} ± SEM (EC_{50}, nM)	Max ± SEM (%CP 55,940)	
Δ^9-THC (**1**)	6.76 ± 0.09 (172)	58 ± 3	-	32 ± 1 at 30 μM	
CP 55,490 (**3**)	7.63 ± 0.09 (24)	-	7.88 ± 0.08 (13)	-	0.5
JWH-018 (**4**)	7.74 ± 0.16 (18)	116 ± 9	7.66 ± 0.16 (22)	87 ± 7	1.2
AB-FUBINACA (**7**)	8.76 ± 0.10 (1.8)	108 ± 7	8.50 ± 0.20 (3.2)	95 ± 12	1.8
ADB-FUBINACA (**8**)	8.92 ± 0.16 (1.2)	152 ± 11	8.46 ± 0.13 (3.5)	104 ± 7	2.9
AB-PINACA (**9**)	8.91 ± 0.09 (1.2)	103 ± 4	8.60 ± 0.16 (2.5)	104 ± 8	2.1
ADB-PINACA (**10**)	9.28 ± 0.08 (0.52)	117 ± 6	9.06 ± 0.31 (0.88)	107 ± 16	1.7
5F-AB-PINACA (**11**)	9.32 ± 0.10 (0.48)	94 ± 6	8.59 ± 0.25 (2.6)	110 ± 13	5.4
5F-ADB-PINACA (**12**)	9.61 ± 0.19 (0.24)	91 ± 7	8.68 ± 0.11 (2.1)	94 ± 5	8.8
AB-FUBICA (**13**)	7.67 ± 0.14 (21)	115 ± 7	7.84 ± 0.27 (15)	99 ± 10	0.7
ADB-FUBICA (**14**)	8.58 ± 0.15 (2.6)	113 ± 8	8.52 ± 0.16 (3.0)	96 ± 7	1.2
AB-PICA (**15**)	7.92 ± 0.07 (12)	99 ± 3	7.92 ± 0.21 (12)	94 ± 9	1.0
ADBICA (**16**)	9.16 ± 0.16 (0.69)	98 ± 7	8.75 ± 0.18 (1.8)	94 ± 7	2.6
5F-AB-PICA (**17**)	8.28 ± 0.21 (5.2)	123 ± 13	8.05 ± 0.53 (8.9)	121 ± 24	1.7
5F-ADBICA (**18**)	9.12 ± 0.14 (0.77)	110 ± 7	8.91 ± 14 (1.2)	92 ± 6	1.6

*CB_1 selectivity expressed as the ratio of CB_1 EC_{50} to CB_2 EC_{50}.

ASSOCIATED CONTENT

Supporting information: Table of compound names, CAS numbers, and relevant references. 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., M.M., S.M.W., M.L., and C.B. performed the synthesis, purification, and chemical characterization of compounds **7**–**18** with guidance from M.K. J.S. conducted all *in vitro* pharmacological evaluation under the supervision of M.C, and data analysis was performed by J.S., S.D.B., and M.C. K.E.W. and R.C.K carried out all behavioral pharmacology with direction from I.S.M. M.G. assisted the creation of stably transfected cells expressing hCB_2R. The manuscript was prepared by S.D.B., M.C., I.S.M., and M.K. All authors have given approval to the final version of the manuscript.

Funding Sources

Work performed at The University of Sydney and presented herein was supported in part by the European Union's Seventh Framework Programme [FP7/2007-2013] INMiND (Grant agreement No. HEALTH-F2-2011-278850). Work performed at Macquarie University and presented herein was supported by NHMRC Project Grant 1002680 awarded to M.C. and M.K., and J.S. is the recipient of an International Research Scholarship from Macquarie University

Conflict of Interest

There are no financial or other relations that could lead to a conflict of interest.

ABBREVIATIONS

CB, cannabinoid; FLIPR, fluorometric imaging plate reader; GIRK, G-protein-gated inwardly rectifying K^+ channels; i.p., intraperitoneal; NMR, nuclear magnetic resonance; p.i., post-injection; SAR, structure-activity relationship; SC, synthetic cannabinoid; Δ^9-THC, Δ^9-tetrahydrocannabinol; TLC, thin layer chromatography.

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