

# The 2-alkyl-2*H*-indazole regioisomers of synthetic cannabinoids AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA are possible manufacturing impurities with cannabimimetic activities

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**Abstract** Indazole-derived synthetic cannabinoids (SCs) featuring an alkyl substituent at the 1-position and *L*-valinamide at the 3-carboxamide position (e.g., AB-CHMINACA) have been identified by forensic chemists around the world, and are associated with serious adverse health effects. Regioisomerism is possible for indazole SCs, with the 2-alkyl-2*H*-indazole regioisomer of AB-CHMINACA recently identified in SC products in Japan. It is unknown whether this regioisomer represents a manufacturing impurity arising as a synthetic byproduct, or was intentionally synthesized as a cannabimimetic agent. This study reports the synthesis, analytical characterization, and pharmacological evaluation of commonly encountered indazole SCs AB-CHMINACA, AB-FUBINACA, AB-

PINACA, 5F-AB-PINACA and their corresponding 2-alkyl-2*H*-indazole regioisomers. Both regioisomers of each SC were prepared from a common precursor, and the physical properties, <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance spectroscopy, gas chromatography–mass spectrometry, and ultraviolet–visible spectroscopy of all SC compounds are described. Additionally, AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA were found to act as high potency agonists at CB<sub>1</sub> (EC<sub>50</sub> = 2.1–11.6 nM) and CB<sub>2</sub> (EC<sub>50</sub> = 5.6–21.1 nM) receptors in fluorometric assays, while the corresponding 2-alkyl-2*H*-indazole regioisomers demonstrated low potency (micromolar) agonist activities at both receptors. Taken together, these data suggest that 2-alkyl-2*H*-indazole regioisomers of AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA are likely to be encountered by forensic chemists and toxicologists as the result of improper purification during the clandestine synthesis of 1-alkyl-1*H*-indazole regioisomers, and can be distinguished by differences in gas chromatography–mass spectrometry fragmentation pattern.

M. Longworth and S. D. Banister contributed equally to this work.

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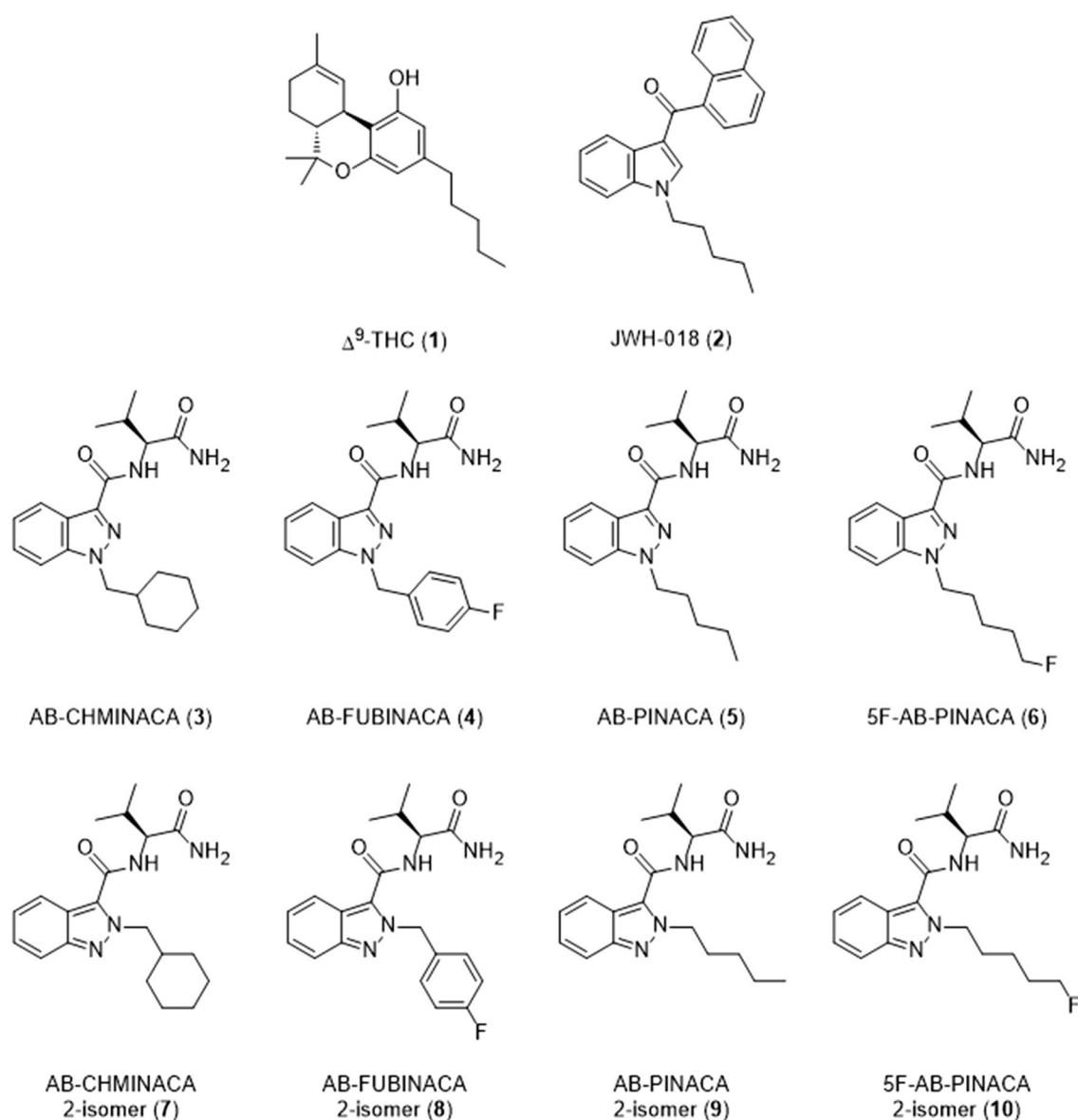
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## Introduction

Synthetic cannabinoids (SCs) are the most rapidly growing class of novel psychoactive substances (NPSs) [1]. SC “designer drugs” are intended to mimic the psychoactive effects of Δ<sup>9</sup>-tetrahydrocannabinol (Δ<sup>9</sup>-THC, **1**, Fig. 1), the principal bioactive component of cannabis. Unlike Δ<sup>9</sup>-THC—a partial agonist at both cannabinoid type 1 (CB<sub>1</sub>)



**Fig. 1** Selected natural and synthetic cannabinoids (SCs)

and type 2 ( $CB_2$ ) receptors—most SCs possess high efficacy agonist activities at both  $CB$  receptor subtypes. Since the discovery of  $CB_1/CB_2$  agonist JWH-018 (2) in consumer products in Germany, Austria, and Japan in 2008 [2, 3], more than 130 SCs have been reported in Europe, with 30 identified in 2014 alone [4].

The identification and subsequent prohibition of individual SCs have motivated clandestine chemists to produce analogues of increasing structural diversity, intended to evade legal restriction [5, 6]. Many newer SCs have no precedent in the scientific literature and little, if anything, is known of their activity or toxicity [7–14]. One particularly prevalent class of SCs, presumably inspired by recent Pfizer patents [15, 16], is comprised of an indazole core

decorated at the 1-position with various aliphatic, alicyclic, or aromatic groups, and at the 3-position with valine- or *tert*-leucine-derived carboxamides. Unlike cannabis itself, these newer SCs are associated with exposures resulting in hospitalization or death in Europe, the USA, Japan, and Russia [17–25].

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide (AB-CHMINACA, 3) was formally notified to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in 2014 following identification in Latvia [4], and was also detected in Japan [26] and Germany [27]. An analogue featuring a 4-fluorobenzene group in place of the cyclohexane ring, (*S*)-*N*-(1-amino-3-methyl-

1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (AB-FUBINACA, **4**), was found in consumer products by Japanese researchers at the National Institute of Health Sciences in 2013 [28], and was also identified in Belgium [29] and Germany [27].

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(pentyl)-1*H*-indazole-3-carboxamide (AB-PINACA, **5**), featuring the *n*-pentyl substituent of JWH-018, was also found alongside AB-FUBINACA in Japan in 2013 [28]. Consistent with the trend for incorporation of fluorine into newer SCs [30], the terminally fluorinated analogue of AB-PINACA, (*S*)-*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide (5F-AB-PINACA, **6**), was discovered in products originating from Sweden in 2013 [29], Japan in 2014 [26], and Germany in 2015 [27].

Recently, AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA were shown to act as potent and efficacious agonists at human CB<sub>1</sub> and CB<sub>2</sub> receptors in vitro [31, 32]. Moreover, AB-CHMINACA, AB-FUBINACA, and AB-PINACA exert potent cannabimimetic effects on locomotion, body temperature, heart rate, and nociception in mice and rats, as well as substituting for Δ<sup>9</sup>-THC in drug discrimination assays [31–33]. Taken together, these data indicate that a range of alkyl substituents are tolerated at the 1-indazole position in this class of SCs.

These SCs appear more toxic than earlier examples, and multiple overdoses and fatalities in the USA have been attributed to AB-CHMINACA, AB-FUBINACA, and AB-PINACA [34–36]. Due to the abuse potential and toxicity of these newer SCs, the Federal Government of the USA has used emergency scheduling laws to temporarily place AB-FUBINACA, AB-PINACA, and AB-CHMINACA into Schedule I, the most restrictive category of the Controlled Substances Act. Similar legislation has been enacted in Germany, Singapore, and elsewhere [37, 38].

Forensic chemists and toxicologists have developed methods for the detection of AB-CHMINACA [39, 40], AB-FUBINACA [41–44], AB-PINACA [41, 45, 46], 5F-AB-PINACA [45, 46], and their metabolites in various biological matrices [47]. However, the changing legality of these substances, and the development of methods for their detection, increases the likelihood that newer analogues of this class will begin to appear on the drug market.

Isomerism may be used to generate new SC analogues [48–50], and the 2*H*-indazole regioisomer of AB-CHMINACA (AB-CHMINACA 2-isomer, **7**) was recently detected in SC products in Japan [51]. It is currently unclear if this analogue is an impurity occurring as an unintended byproduct of AB-CHMINACA synthesis, or if the 2-isomer was willfully prepared as an intended cannabimimetic agent. Incidentally, the 2*H*-indazole analogue

of APINACA was reported in China recently [52]. One study of bulk powders used as the active ingredients for SC products found purity to range from 78 to 96 %, consistent with the potential presence of manufacturing impurities in the raw materials [53].

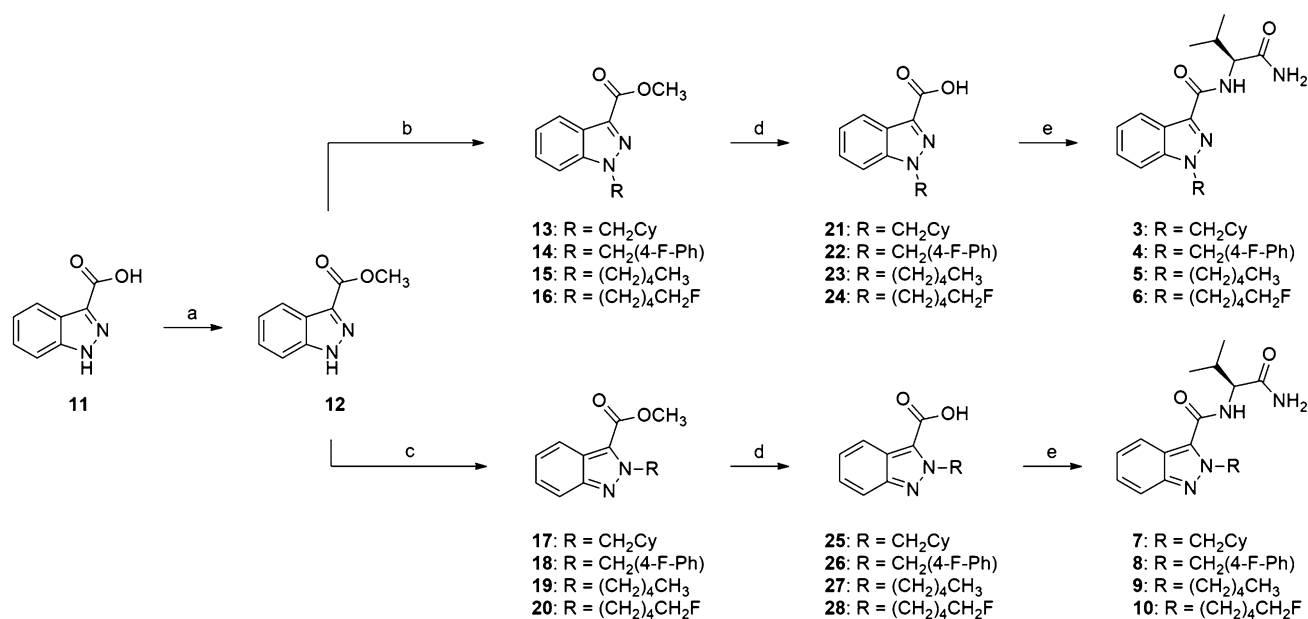
We recently reported the synthesis, structural characterization, in vitro cannabinoid activity, and in vivo biotelemetry of several indazole synthetic cannabinoid designer drugs [31]. During the preparation of 5F-AB-PINACA, it was noted that alkylation of methyl indazole-3-carboxylate predominantly produced the desired 1-alkylated intermediate, with the 2-alkyl regioisomer formed as a minor product. Failure to remove the unwanted 2-alkyl intermediate before the remaining synthetic steps would be expected to form 5F-AB-PINACA 2-isomer as an impurity. However, the cannabinoid activity of the 2*H*-indazole regioisomers of this class of SCs has never been reported, and AB-CHMINACA 2-isomer was detected in consumer products at a concentration similar to those reported for other SC products.

In this work, we synthesized AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA (**3–6**) and the corresponding 2*H*-indazole regioisomers **7–10** from a common precursor. The characterization of these regioisomers was achieved using melting point range determinations, nuclear magnetic resonance (NMR) spectroscopy, ultraviolet (UV)-visible (Vis) spectroscopy, infrared (IR) spectroscopy, and gas chromatography–mass spectrometry (GC–MS). Additionally, the in vitro cannabinoid activities of **3–10** at human CB<sub>1</sub> and CB<sub>2</sub> receptors were evaluated using fluorometric imaging plate reader (FLIPR) assays.

## Materials and methods

### General chemical synthesis details

The synthesis of **3–10** is shown in Fig. 2. All reactions were performed under an atmosphere of nitrogen or argon unless otherwise specified. Anhydrous tetrahydrofuran (THF), methanol, acetonitrile, and dimethyl sulfoxide (DMSO) (Sigma Aldrich, St. Louis, MO, USA) were used as purchased. Commercially available chemicals (Sigma-Aldrich) were used as purchased. Analytical thin-layer chromatography was performed using Merck aluminum-backed silica gel 60 F254 (0.2 mm) plates (Merck, Darmstadt, Germany), which were visualized using short-wave (254 nm) UV fluorescence. Flash chromatography was performed using Merck Kieselgel 60 (230–400 mesh) silica gel. Melting point (m.p.) ranges were measured in open capillaries using a Stuart SMP10 m.p. apparatus (Bibby Scientific, Staffordshire, UK) and were



**Fig. 2** Synthesis of indazole SC regioisomers 3–10. Reagents and conditions: *a* conc. sulfuric acid, methanol, reflux, 24 h, 95 %; *b* bromoalkane, potassium *tert*-butoxide, tetrahydrofuran, 0 °C–rt, 48 h, 65–84 %; *c* bromoalkane, potassium carbonate, potassium

iodide, acetonitrile, reflux, 24 h, 20–32 %; *d* sodium hydroxide, methanol, rt, 24 h, 76–91 %; *e* L-valinamide, (benzotriazol-1-yl-oxo)tripyrrolidinophosphonium hexafluorophosphate, *N,N*-diisopropylethylamine, dimethyl sulfoxide, rt, 24 h, 55–87 %

uncorrected. NMR spectra were recorded at 300 K using either a Bruker AVANCE DRX400 (400.1 MHz) or AVANCE III 500 Ascend (500.1 MHz) spectrometer (Bruker, Bremen, Germany). The data are reported as chemical shift ( $\delta$  ppm) relative to the residual protonated solvent resonance, relative integral, multiplicity (s, singlet; br s, broad singlet; d, doublet; t, triplet; quin., quintet; sept., septet; dt, doublet of triplets; m, multiplet), coupling constants ( $J$  Hz) and assignment (for <sup>13</sup>C, quat., quaternary). Assignment of signals was assisted by correlation spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple-bond correlation (HMBC) experiments where necessary. Low-resolution mass spectra (LRMS) were recorded using electrospray ionization (ESI) recorded on a Finnigan LCQ ion trap mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA). High-resolution mass spectra (HRMS) were run on a Bruker 7T Apex Qe Fourier Transform Ion Cyclotron resonance mass spectrometer equipped with an Apollo II ESI/APCI/MALDI Dual source by the Mass Spectrometry Facility of the School of Chemistry at The University of Sydney. IR absorption spectra were recorded on a Bruker ALPHA FT-IR spectrometer as solid or thin film from ethanol, and the data are reported as vibrational frequencies (cm<sup>-1</sup>). Please see the supplementary material for <sup>1</sup>H and <sup>13</sup>C NMR spectra and Fourier-transform infrared (FTIR) spectra of all final compounds.

### Methyl 1*H*-indazole-3-carboxylate (**12**)

To a cooled (0 °C) solution of indazole-3-carboxylic acid (**11**, 3.0 g, 18.5 mmol) in methanol (45 mL), sulfuric acid (3 mL) was slowly added and the solution heated at reflux for 24 h. The solvent was evaporated under reduced pressure and the residue partitioned between ethyl acetate (100 mL) and water (100 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined organic phases were washed with sat. aq. sodium bicarbonate (3 × 100 mL), brine (100 mL), dried (MgSO<sub>4</sub>), and the solvent evaporated under reduced pressure. The crude material was purified using flash chromatography (hexane/ethyl acetate 50:50, *R<sub>f</sub>* 0.31) to give **12** (3.1 g, 95 %) as a white solid. m.p. 165–169 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.24 (1H, d,  $J$  = 8.2 Hz), 7.69 (1H, d,  $J$  = 8.4 Hz), 7.48 (1H, t,  $J$  = 7.0 Hz), 7.35 (1H, t,  $J$  = 7.2 Hz), 4.07 (3H, s); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  163.6 (CO), 141.5 (quat.), 136.3 (quat.), 127.6 (CH), 123.5 (CH), 122.5 (quat.), 121.8 (CH), 111.5 (CH), 52.3 (CH<sub>3</sub>); LRMS (+ESI)  $m/z$  198.95 ([M+Na]<sup>+</sup>, 54 %), 374.99 ([2M + Na]<sup>+</sup>, 100 %).

### General procedure A: 1-alkylation of methyl 1*H*-indazole-3-carboxylate using potassium *tert*-butoxide

To a cooled (0 °C) solution of 1*H*-indazole-3-carboxylate (500 mg, 2.84 mmol, 1.0 equiv.) in THF (15 mL),

potassium *tert*-butoxide (350 mg, 3.12 mmol, 1.1 equiv.) was added portionwise, and the mixture stirred at ambient temperature for 1 h. The cooled (0 °C) mixture was treated dropwise with appropriate bromoalkane (2.98 mmol, 1.05 equiv.) and stirred at ambient temperature for 48 h. The mixture was poured onto H<sub>2</sub>O (100 mL), extracted with ethyl acetate (3 × 100 mL), dried (MgSO<sub>4</sub>), and the solvent evaporated under reduced pressure. Following purification by flash chromatography (hexane/ethyl acetate 80:20 unless otherwise stated), methyl 1-alkyl-1*H*-indazole-3-carboxylate was obtained as the major product, and methyl 2-alkyl-2*H*-indazole-3-carboxylate as the minor product.

*Methyl 1-(cyclohexylmethyl)-1H-indazole-3-carboxylate (13)*

Subjecting (bromomethyl)cyclohexane (415 μL, 2.98 mmol, 1.05 equiv.) to general procedure A gave, after purification by flash chromatography (hexane/ethyl acetate 85:15), **13** (505 mg, 65 %) as a pale yellow oil. *R*<sub>f</sub> 0.60 (hexane/ethyl acetate 80:20); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.22 (1H, dt, *J* = 8.1, 0.9 Hz), 7.48–7.39 (2H, m), 7.33–7.28 (1H, m), 4.28 (2H, d, *J* = 7.5 Hz), 4.03 (3H, s), 2.08 (1H, m), 1.74–1.52 (5H, m), 1.32–0.98 (5H, m); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 163.3 (CO), 141.3 (quat.), 134.6 (quat.), 126.8 (CH), 123.7 (quat.), 123.1 (CH), 122.3 (CH), 110.0 (CH), 56.1 (CH<sub>2</sub>), 52.1 (CH<sub>3</sub>), 38.9 (CH<sub>2</sub>), 31.7 (CH), 26.3 (CH<sub>2</sub>), 25.7 (CH<sub>2</sub>); LRMS (+ESI) *m/z* 295.05 ([M+Na]<sup>+</sup>, 100 %); IR (diamond cell, thin film): 2925 (s), 2851 (m), 1710 (s), 1477 (s), 1441 (m), 1224 (s), 1161 (s), 1121 (s), 751 (s).

*Methyl 1-(4-fluorobenzyl)-1H-indazole-3-carboxylate (14)*

Subjecting 4-fluorobenzyl bromide (371 μL, 2.98 mmol, 1.05 equiv.) to general procedure A gave **14** (570 mg, 71 %) as a colorless oil. *R*<sub>f</sub> 0.35 (hexane/ethyl acetate 80:20); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.25 (1H, d, *J* = 7.8 Hz), 7.42–7.29 (3H, m), 7.21 (2H, t, *J* = 6.9 Hz), 6.99 (2H, t, *J* = 8.1 Hz), 5.67 (2H, s), 4.06 (3H, s); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 163.1 (CO), 162.6 (d, <sup>1</sup>*J*<sub>C-F</sub> = 245.3 Hz, quat.), 140.6 (quat.), 135.3 (quat.), 131.6 (d, <sup>4</sup>*J*<sub>C-F</sub> = 3.8 Hz, quat.), 129.2 (d, <sup>3</sup>*J*<sub>C-F</sub> = 8.3 Hz, CH), 127.3 (CH), 124.3 (quat.), 123.5 (CH), 122.5 (CH), 116.0 (d, <sup>2</sup>*J*<sub>C-F</sub> = 21.8 Hz, CH), 53.5 (CH<sub>2</sub>), 52.2 (CH<sub>3</sub>); <sup>19</sup>F NMR (282 MHz, CDCl<sub>3</sub>): δ -113.8 (s); LRMS (+ESI): *m/z* 307.00 ([M+Na]<sup>+</sup>, 100 %). IR (diamond cell, thin film): 3071 (w), 2952 (w), 1712 (s), 1510 (s), 1479 (s), 1268 (s), 1157 (s), 749 (s).

*Methyl 1-pentyl-1H-indazole-3-carboxylate (15)*

Subjecting 1-bromopentane (370 μL, 2.98 mmol, 1.05 equiv.) to general procedure A gave **15** (585 mg, 84 %) as a colorless oil. *R*<sub>f</sub> 0.55 (hexane/ethyl acetate 80:20); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.24 (1H, d, *J* = 8.0 Hz), 7.50–7.44 (2H, m), 7.35–7.27 (1H, m), 4.47 (2H, t, *J* = 7.4 Hz), 4.04 (3H, s), 1.97 (2H, quin., *J* = 7.0 Hz), 1.32 (4H, m), 0.87 (3H, t, *J* = 6.6 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 163.3 (CO), 140.6 (quat.), 134.6 (quat.), 126.8 (CH), 123.9 (quat.), 123.1 (CH), 122.3 (CH), 109.7 (CH), 52.1 (CH<sub>2</sub>), 50.1 (CH<sub>3</sub>), 29.7 (CH<sub>2</sub>), 29.0 (CH<sub>2</sub>), 22.4 (CH<sub>2</sub>), 14.0 (CH<sub>3</sub>); LRMS (+ESI): *m/z* 269.03 ([M+Na]<sup>+</sup>, 60 %), 515.16 ([2 M + Na]<sup>+</sup>, 100 %); IR (diamond cell, thin film): 2954 (m), 2932 (m), 2860 (w), 1709 (s), 1477 (s), 1215 (s), 1159 (s), 1117 (s), 751 (s).

*Methyl 1-(5-fluoropentyl)-1H-indazole-3-carboxylate (16)*

Subjecting 1-bromo-5-fluoropentane (370 μL, 2.98 mmol, 1.05 equiv.) to general procedure A gave **16** (560 mg, 75 %) as a colorless oil. *R*<sub>f</sub> 0.30 (hexane/ethyl acetate 80:20); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.24 (1H, d, *J* = 8.1 Hz), 7.49–7.45 (2H, m), 7.32 (1H, t, *J* = 6.3 Hz), 4.52–4.47 (3H, m), 4.33 (1H, t, *J* = 5.7 Hz), 4.04 (3H, s), 2.03 (2H, quin., *J* = 7.2 Hz), 2.00–1.63 (2H, m), 1.46 (2H, quin., *J* = 12.0 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 163.2 (CO), 140.6 (quat.), 134.8 (quat.), 127.0 (CH), 123.9 (quat.), 123.2 (CH), 122.4 (CH), 109.6 (CH), 83.8 (d, <sup>1</sup>*J*<sub>C-F</sub> = 163.5 Hz, CH<sub>2</sub>F), 52.1 (CH<sub>2</sub>), 49.8 (CH<sub>3</sub>), 30.0 (d, <sup>2</sup>*J*<sub>C-F</sub> = 19.5 Hz, CH<sub>2</sub>), 29.6 (CH<sub>2</sub>), 22.8 (d, <sup>3</sup>*J*<sub>C-F</sub> = 4.5 Hz, CH<sub>2</sub>); <sup>19</sup>F NMR (282 MHz, CD<sub>3</sub>OD): δ -218.6 (m); LRMS (+ESI): *m/z* 287.03 ([M+Na]<sup>+</sup>, 100 %); IR (diamond cell, thin film): 2950 (m), 2867 (w), 1729 (s), 1710 (s), 1478 (s), 1163 (s), 1118 (s), 752 (s).

**General procedure B: 2-alkylation of methyl 1*H*-indazole-3-carboxylate using potassium carbonate**

To a stirred mixture of methyl 1*H*-indazole-3-carboxylate (500 mg, 2.84 mmol, 1.0 equiv.), potassium carbonate (785 mg, 5.68 mmol, 2.0 equiv.) and potassium iodide (24 mg, 0.14 mmol, 0.05 equiv.) in acetonitrile (5 mL), the appropriate bromoalkane (2.98 mmol, 1.05 equiv.) was added dropwise. The suspension was heated at reflux for 24 h and the cooled reaction was poured onto H<sub>2</sub>O (30 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried (MgSO<sub>4</sub>) and the solvent removed under reduced pressure. Following purification by flash chromatography (hexane/ethyl acetate 80:20 unless otherwise stated), methyl 1-alkyl-1*H*-

indazole-3-carboxylate was obtained as the major product, and methyl 2-alkyl-2*H*-indazole-3-carboxylate as the minor product.

*Methyl 2-(cyclohexylmethyl)-2H-indazole-3-carboxylate (17)*

Subjecting (bromomethyl)cyclohexane (415  $\mu$ L, 2.98 mmol, 1.05 equiv.) to general procedure B gave, after purification by flash chromatography (hexane/ethyl acetate 93:7), **17** (235 mg, 30 %) as a colorless solid. m.p. 83–85 °C;  $R_f$  0.72 (hexane/ethyl acetate 80:20);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.02 (1H, d,  $J = 8.4$  Hz), 7.79 (1H, d,  $J = 8.7$  Hz), 7.35 (1H, t,  $J = 7.2$  Hz), 7.30 (1H, t,  $J = 7.8$  Hz), 4.78 (2H, d,  $J = 7.2$  Hz), 4.03 (3H, s), 2.12–2.01 (1H, m), 1.70–1.56 (5H, m), 1.20–1.05 (5H, m);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  160.9 (CO), 147.4 (quat.), 126.3 (CH), 125.0 (CH), 123.9 (quat.), 123.6 (quat.), 121.5 (CH), 118.4 (CH), 59.3 ( $\text{CH}_2$ ), 52.0 ( $\text{CH}_3$ ), 39.6 (CH), 30.7 ( $\text{CH}_2$ ), 26.4 (CH), 25.8 ( $\text{CH}_2$ ); LRMS (+ESI):  $m/z$  295.08 ( $[\text{M}+\text{Na}]^+$ , 100 %). IR (diamond cell, thin film): 2923 (m), 2850 (m), 1711 (s), 1462 (s), 1250 (s), 1153 (s), 997 (s), 758 (s).

*Methyl 2-(4-fluorobenzyl)-2H-indazole-3-carboxylate (18)*

Subjecting 4-fluorobenzyl bromide (370  $\mu$ L, 2.98 mmol, 1.05 equiv.) to general procedure B gave, after purification by flash chromatography (hexane/ethyl acetate 85:15), **18** (225 mg, 28 %) as a white solid. m.p. 116.5–118.5 °C;  $R_f$  0.57 (hexane/ethyl acetate 80:20);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.01 (1H, d,  $J = 8.1$  Hz), 7.81 (1H, d,  $J = 8.4$  Hz), 7.35–7.28 (4H, m), 6.98 (2H, t,  $J = 7.8$  Hz), 6.07 (2H, s), 4.01 (3H, s);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.6 (d, quat.,  $^1J_{\text{C-F}} = 246.6$  Hz), 160.8 (CO), 147.8 (quat.), 132.2 (quat.), 130.0 (d,  $^3J_{\text{C-F}} = 8.3$  Hz), 126.7 (CH), 125.4 (CH), 123.8 (quat.), 123.5 (quat.), 121.6 (CH), 118.5 (CH), 115.6 (d,  $^2J_{\text{C-F}} = 21.8$  Hz, CH), 56.0 ( $\text{CH}_2$ ), 52.2 ( $\text{CH}_3$ );  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ ):  $\delta$  -114.2 (s); LRMS (+ESI):  $m/z$  307.02 ( $[\text{M}+\text{Na}]^+$ , 100 %); IR (diamond cell, thin film): 3054 (w), 2954 (w), 1710 (s), 1554 (s), 1510 (s), 1222 (s), 1210 (s), 1083 (s), 758 (s).

*Methyl 2-pentyl-2H-indazole-3-carboxylate (19)*

Subjecting 1-bromopentane (370  $\mu$ L, 2.98 mmol, 1.05 equiv.) to general procedure B gave, after purification by flash chromatography (hexane/ethyl acetate 93:7), **19** (175 mg, 25 %) as a colorless oil.  $R_f$  0.69 (hexane/ethyl acetate 80:20);  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  8.01 (1H, d,  $J = 8.5$  Hz), 7.70 (1H, d,  $J = 8.5$  Hz), 7.36 (1H, t,  $J = 7.0$  Hz), 7.28 (1H, t,  $J = 7.5$  Hz), 4.86 (2H, t,  $J = 7.0$  Hz), 4.02 (3H, s), 1.94 (2H, quin.,  $J = 7.0$  Hz),

1.39–1.31 (4H, m), 0.90 (3H, t,  $J = 6.5$  Hz);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  161.8 (CO), 148.5 (quat.), 127.7 (CH), 126.0 (CH), 124.8 (quat.), 124.6 (quat.), 122.5 (CH), 118.6 (CH), 54.4 ( $\text{CH}_2$ ), 52.5 ( $\text{CH}_3$ ), 31.6 ( $\text{CH}_2$ ), 29.8 ( $\text{CH}_2$ ), 23.2 ( $\text{CH}_2$ ), 14.2 ( $\text{CH}_3$ ); LRMS (+ESI):  $m/z$  269.03 ( $[\text{M}+\text{Na}]^+$ , 60 %), 515.16 ( $[\text{2M} + \text{Na}]^+$ , 100 %); IR (diamond cell, thin film): 2955 (m), 2930 (w), 2860 (w), 1710 (s), 1463 (s), 1278 (s), 1206 (s), 1078 (s), 758 (s).

*Methyl 2-(5-fluoropentyl)-2H-indazole-3-carboxylate (20)*

Subjecting 1-bromo-5-fluoropentane (370  $\mu$ L, 2.98 mmol, 1.05 equiv.) to general procedure B gave **20** (152 mg, 20 %) as a colorless oil.  $R_f$  0.50 (hexane/ethyl acetate 80:20);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.02 (1H, d,  $J = 8.4$  Hz), 7.78 (1H, d,  $J = 8.6$  Hz), 7.36 (1H, t,  $J = 7.9$  Hz), 7.29 (1H, t,  $J = 7.7$  Hz), 4.93 (2H, t,  $J = 7.4$  Hz), 4.43 (2H, dt,  $J = 47.5$  Hz,  $J = 6.2$  Hz), 4.04 (3H, s), 2.03 (2H, quin.,  $J = 7.5$  Hz), 1.76 (2H, m), 1.50 (2H, quin.,  $J = 7.6$  Hz);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  160.8 (CO), 147.5 (quat.), 126.4 (CH), 125.1 (CH), 123.6 (quat.), 123.5 (quat.), 121.5 (CH), 118.3 (CH), 83.9 (d,  $^1J_{\text{C-F}} = 163.5$  Hz,  $\text{CH}_2\text{F}$ ), 53.5 ( $\text{CH}_3$ ), 52.1 ( $\text{CH}_2$ ), 30.6 ( $\text{CH}_2$ ), 30.1 (d,  $^2J_{\text{C-F}} = 20.3$  Hz,  $\text{CH}_2$ ), 22.6 (d,  $^3J_{\text{C-F}} = 5.3$  Hz,  $\text{CH}_2$ );  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ ):  $\delta$  -218.7 (m); LRMS (+ESI):  $m/z$  287.05 ( $[\text{M}+\text{Na}]^+$ , 100 %); IR (diamond cell, thin film): 2954 (w), 2865 (w), 1708 (s), 1463 (s), 1278 (s), 1205 (s), 1042 (s), 758 (s).

**General procedure C: ester hydrolysis of methyl 1-alkyl-1*H*-indazole-3-carboxylates and methyl 2-alkyl-2*H*-indazole-3-carboxylates**

To a solution of the appropriately substituted indazole-3-carboxylic acid methyl ester (0.43 mmol) in methanol (5 mL), a solution of 1 M aq. sodium hydroxide (650  $\mu$ L, 0.65 mmol, 1.5 equiv.) was added dropwise, and the solution stirred at ambient temperature for 18 h. Solvent was evaporated in vacuo, and sat. aq. sodium hydrogen carbonate (50 mL) was added to the residue. The aqueous phase was washed with diethyl ether (10 mL), adjusted to pH  $\sim$ 2 using 1 M aq. hydrochloric acid solution, and extracted with diethyl ether (3  $\times$  50 mL). The combined organic phases were washed with brine (50 mL), dried ( $\text{MgSO}_4$ ), and the solvent evaporated under reduced pressure.

*1-(Cyclohexylmethyl)-1H-indazole-3-carboxylic acid (21)*

Subjecting **13** (475 mg, 1.74 mmol) to general procedure C gave **21** (388 mg, 86 %) as a white solid. m.p. 124–126 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  8.16 (1H, d,  $J = 8.1$  Hz), 7.66 (1H, d,  $J = 8.4$  Hz), 7.47 (1H, t,  $J = 7.2$  Hz), 7.31

(1H, t,  $J = 7.8$  Hz), 4.34 (2H, d,  $J = 7.2$  Hz), 2.05 (1H, m), 1.72–1.66 (3H, m), 1.56 (2H, d,  $J = 12.9$  Hz), 1.26–1.02 (5H, m);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  165.5 (CO), 142.6 (quat.), 135.9 (quat.), 128.0 (CH), 124.5 (quat.), 124.1 (CH), 123.0 (CH), 111.4 (CH), 56.5 ( $\text{CH}_2$ ), 40.0 (CH), 31.7 ( $\text{CH}_2$ ), 27.7 (CH), 26.7 ( $\text{CH}_2$ ); LRMS (–ESI):  $m/z$  257.16 ( $[\text{M} - \text{H}]^-$ , 100 %); IR (diamond cell, thin film): 3060 (bs), 2926 (s), 2851 (m), 1707 (s), 1479 (s), 1230 (s), 1174 (s), 752 (s).

#### *1-(4-Fluorobenzyl)-1H-indazole-3-carboxylic acid (22)*

Subjecting **14** (560 mg, 1.97 mmol) to general procedure C gave **22** (480 mg, 91 %) as a white solid. m.p. 198.5–199.5 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  8.17 (1H, d,  $J = 8.1$  Hz), 7.62 (1H, d,  $J = 8.7$  Hz), 7.44 (1H, t,  $J = 7.5$  Hz), 7.31 (3H, m), 7.04 (2H, t,  $J = 8.4$  Hz), 5.71 (2H, s,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  165.5 (CO), 163.9 (d,  $^1J_{\text{C-F}} = 244.5$  Hz, quat.), 142.1 (quat.), 136.7 (quat.), 133.7 (d,  $^4J_{\text{C-F}} = 3.0$  Hz, quat.), 130.6 (d,  $^3J_{\text{C-F}} = 8.3$  Hz, quat.), 128.3 (CH), 125.0 (quat.), 124.3 (CH), 123.1 (CH), 116.5 (d,  $^2J_{\text{C-F}} = 21.8$  Hz, quat.), 111.4 (CH), 53.6 ( $\text{CH}_2$ );  $^{19}\text{F}$  NMR (285 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  –118.0 (s); LRMS (–ESI):  $m/z$  269.07 ( $[\text{M} - \text{H}]^-$ , 100 %); IR (diamond cell, thin film): 3058 (bs), 2926 (w), 1696 (s), 1510 (s), 1481 (s), 1224 (s), 1170 (s), 1157 (s), 749 (s).

#### *1-Pentyl-1H-indazole-3-carboxylic acid (23)*

Subjecting **15** (600 mg, 2.58 mmol) to general procedure C gave **23** (510 mg, 85 %) as a white solid. m.p. 76.5–78.0 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  8.26 (1H, d,  $J = 8.1$  Hz), 7.52–7.44 (2H, m), 7.35 (1H, t,  $J = 7.8$  Hz), 4.48 (2H, t,  $J = 7.2$  Hz), 1.99 (2H, quin.,  $J = 7.2$  Hz), 1.34 (4H, m), 0.89 (3H, t,  $J = 6.0$  Hz);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  165.4 (CO), 142.0 (quat.), 135.9 (quat.), 127.9 (CH), 124.5 (quat.), 124.0 (CH), 122.9 (CH), 111.0 (CH), 49.7 ( $\text{CH}_2$ ), 30.4 ( $\text{CH}_2$ ), 29.8 ( $\text{CH}_2$ ), 23.1 ( $\text{CH}_2$ ), 14.1 ( $\text{CH}_3$ ); LRMS (–ESI):  $m/z$  231.12 ( $[\text{M} - \text{H}]^-$ , 100 %); IR (diamond cell, thin film): 3053 (bs), 2956 (m), 2931 (m), 2860 (w), 1687 (s), 1503 (s), 1218 (s), 1176 (s), 1121 (s), 752 (s).

#### *1-(5-Fluoropentyl)-1H-indazole-3-carboxylic acid (24)*

Subjecting **16** (750 mg, 2.84 mmol) to general procedure C gave **24** (580 mg, 81 %) as a white solid. m.p. 199–200 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  8.36 (1H, d,  $J = 7.8$  Hz), 7.52 (2H, m), 7.33 (1H, t,  $J = 7.5$  Hz), 4.53–4.49 (3H, m), 4.34 (1H, t,  $J = 5.7$  Hz), 2.03 (2H, quin.,  $J = 7.2$  Hz), 1.82–1.65 (2H, m), 1.50 (2H, quin.,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  165.5 (CO), 142.2 (quat.), 136.1 (quat.), 128.1, 124.7 (quat.), 124.2 (CH), 123.0 (CH), 111.2

(CH), 84.6 (d,  $^1J_{\text{C-F}} = 162.8$  Hz,  $\text{CH}_2$ ), 50.3 ( $\text{CH}_2$ ), 31.0 (d,  $^2J_{\text{C-F}} = 20.3$  Hz,  $\text{CH}_2$ ), 30.4 ( $\text{CH}_2$ ), 23.6 (d,  $^3J_{\text{C-F}} = 5.3$  Hz,  $\text{CH}_2$ );  $^{19}\text{F}$  NMR (282 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  –221.8 (m); LRMS (–ESI):  $m/z$  249.12 ( $[\text{M} - \text{H}]^-$ , 100 %); IR (diamond cell, thin film): 3052 (bs), 2941 (m), 2866 (w), 1685 (s), 1480 (s), 1167 (s), 1120 (s), 751 (s).

#### *2-(Cyclohexylmethyl)-2H-indazole-3-carboxylic acid (25)*

Subjecting **17** (100 mg, 0.37 mmol) to general procedure C gave **25** (72 mg, 76 %) as a white solid. m.p. 178–179.5 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  8.07 (1H, d,  $J = 8.1$  Hz), 7.69 (1H, d,  $J = 8.4$  Hz), 7.35 (1H, t,  $J = 7.2$  Hz), 7.25 (1H, t,  $J = 6.9$  Hz), 4.75 (2H, d,  $J = 7.2$  Hz), 2.03 (1H, m), 1.72–1.63 (3H, m), 1.53 (2H, m), 1.22–1.05 (5H, m);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  162.6 (CO), 148.4 (quat.), 127.6 (CH), 126.1 (quat.), 125.7 (CH), 124.9 (quat.), 122.9 (CH), 118.4 (CH), 59.7 ( $\text{CH}_2$ ), 40.8 (CH), 31.6 ( $\text{CH}_2$ ), 27.4 ( $\text{CH}_2$ ), 26.8 ( $\text{CH}_2$ ); LRMS (–ESI):  $m/z$  257.17 ( $[\text{M} - \text{H}]^-$ , 100 %); IR (diamond cell, thin film): 2923 (m), 2851 (m), 2567 (bs), 1706 (s), 1471 (s), 1271 (s), 1208 (s), 1197 (s), 1053 (s), 757 (s).

#### *2-(4-Fluorobenzyl)-2H-indazole-3-carboxylic acid (26)*

Subjecting **18** (100 mg, 0.35 mmol) to general procedure C gave **26** (75 mg, 79 %) as a white solid. m.p. 207.5–210 °C;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  8.07 (1H, d,  $J = 8.5$  Hz), 7.72 (1H, d,  $J = 8.5$  Hz), 7.37 (1H, t,  $J = 7.0$  Hz), 7.33 (2H, t,  $J = 7.0$  Hz), 7.27 (1H, t,  $J = 7.5$  Hz), 7.02 (2H, t,  $J = 8.0$  Hz), 6.09 (2H, s);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  163.8 (d,  $^1J_{\text{C-F}} = 242.5$  Hz), 162.5 (CO), 148.9 (quat.), 134.3 (quat.), 130.8 (d,  $^3J_{\text{C-F}} = 8.8$  Hz, CH), 127.9 (CH), 125.9 (CH), 125.8 (quat.), 125.0 (quat.), 122.9 (CH), 118.6 (CH), 116.3 (d,  $^2J_{\text{C-F}} = 21.3$  Hz, CH), 56.3 ( $\text{CH}_2$ );  $^{19}\text{F}$  NMR (470 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  –116.6 (s); LRMS (–ESI):  $m/z$  269.12 ( $[\text{M} - \text{H}]^-$ , 100 %); IR (diamond cell, thin film): 2922 (w), 2525 (bs), 1712 (s), 1605 (m), 1511 (s), 1214 (m), 1152 (s), 1018 (s), 755 (s).

#### *2-Pentyl-2H-indazole-3-carboxylic acid (27)*

Subjecting **19** (100 mg, 0.43 mmol) to general procedure C gave **27** (84 mg, 90 %) as a white solid. m.p. 141.5–142.5 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  8.26 (1H, d,  $J = 8.1$  Hz), 7.52–7.44 (2H, m), 7.35 (1H, t,  $J = 7.8$  Hz), 4.48 (2H, t,  $J = 7.2$  Hz), 1.99 (2H, quin.,  $J = 7.2$  Hz), 1.34 (4H, m), 0.89 (3H, t,  $J = 6.0$  Hz);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  161.0 (CO), 147.0 (CH), 134.6 (quat.), 126.1 (CH), 124.1 (CH), 123.4 (quat.), 121.3 (CH), 116.8 (CH), 52.7 ( $\text{CH}_2$ ), 30.2 ( $\text{CH}_2$ ), 28.3 ( $\text{CH}_2$ ), 21.8 ( $\text{CH}_2$ ), 12.7 ( $\text{CH}_3$ ); LRMS (–ESI):  $m/z$  231.13 ( $[\text{M} - \text{H}]^-$ ,



100 %); IR (diamond cell, thin film): 2953 (m), 2927 (m), 2869 (m), 2474 (bs), 1701 (s), 1477 (m), 1428 (m), 1274 (s), 1209 (s), 1083 (s), 753 (s).

#### 2-(5-Fluoropentyl)-2H-indazole-3-carboxylic acid (**28**)

Subjecting **20** (100 mg, 0.38 mmol) to general procedure C gave **28** (78 mg, 82 %) as a white solid. m.p. 137–138 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  8.08 (1H, d,  $J = 8.1$  Hz), 7.70 (1H, d,  $J = 8.7$  Hz), 7.37 (1H, t,  $J = 7.2$  Hz), 7.27 (1H, t,  $J = 7.2$  Hz), 4.93 (2H, t,  $J = 7.2$  Hz), 4.48 (1H, t,  $J = 6.0$  Hz), 4.32 (1H, t,  $J = 5.7$  Hz), 2.01 (2H, quin.,  $J = 7.2$  Hz), 1.80–1.66 (2H, m), 1.45 (2H, quin.,  $J = 7.5$  Hz);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  162.5 (CO), 148.6 (quat.), 127.7 (CH), 125.75 (CH), 125.74 (quat.), 124.9 (quat.), 122.8 (CH), 118.4 (CH), 84.6 (d,  $^1J_{\text{C-F}} = 162.8$  Hz,  $\text{CH}_2\text{F}$ ), 54.0 ( $\text{CH}_2$ ), 31.6 ( $\text{CH}_2$ ), 31.0 (d,  $^2J_{\text{C-F}} = 19.5$  Hz,  $\text{CH}_2$ ), 23.4 (d,  $^3J_{\text{C-F}} = 5.3$  Hz,  $\text{CH}_2$ );  $^{19}\text{F}$  NMR (282 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  -222.0 (m); LRMS (-ESI):  $m/z$  249.14 ( $[\text{M}-\text{H}]^-$ , 100 %); IR (diamond cell, thin film): 2927 (w), 2863 (w), 2548 (bs), 1692 (s), 1428 (s), 1390 (m), 1202 (s), 1079 (s).

#### General procedure D: preparation of indazole-3-carboxamides

A cooled (0 °C) solution of the appropriate 1-alkyl-1H- or 2-alkyl-2H-indazole-3-carboxylic acid (0.19 mmol, 1.0 equiv.), L-valinamide (31 mg, 0.20 mmol, 1.05 equiv.), and (benzotriazol-1-yl-oxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP<sup>®</sup>) (106 mg, 0.20 mmol, 1.05 equiv.) in DMSO (3 mL) was treated dropwise with *N,N*-diisopropylethylamine (DIPEA, 68  $\mu\text{L}$ , 0.39 mmol, 2.0 equiv.) and stirred at ambient temperature for 2 h. The reaction was poured onto sat. aq. sodium hydrogen carbonate and extracted with diethyl ether (3  $\times$  50 mL). The combined organic layers were dried ( $\text{MgSO}_4$ ) and the solvent evaporated under reduced pressure. The crude products were purified using flash chromatography.

#### (*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide (AB-CHMINACA, **3**)

Subjecting **21** (150 mg, 0.58 mmol) to general procedure D gave, following purification by flash chromatography (hexane/ethyl acetate 60:40), **3** (135 mg, 65 %) as a white solid.  $R_f$  0.25 (hexane/ethyl acetate 50:50); m.p. 88.5–92.5 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  8.16 (1H, dt,  $J = 8.0, 0.8$  Hz), 7.77 (1H, d,  $J = 8.4$  Hz), 7.68 (1H, d,  $J = 8.4$  Hz), 7.67 (1H, br s), 7.45 (1H, ddd,  $J = 8.4, 6.8, 1.2$  Hz), 7.27 (1H, ddd,  $J = 8.2, 7.2, 0.8$  Hz), 7.22 (1H, br s), 4.42 (1H, dd,  $J = 9.0, 6.4$  Hz), 4.34 (2H, d,

$J = 7.2$  Hz), 2.14–2.05 (1H, m), 2.00–1.88 (1H, m), 1.68–1.55 (3H, m), 1.54–1.43 (2H, m), 1.22–0.99 (5H, m), 0.94 (3H, d,  $J = 6.8$  Hz), 0.90 (3H, d,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ):  $\delta$ ; 172.6 (CO), 161.4 (CO), 141.2 (quat.), 136.4 (quat.), 126.6 (CH), 122.4 (CH), 121.8 (quat.), 121.6 (CH), 110.7 (CH), 56.8 (CH), 54.6 ( $\text{CH}_2$ ), 38.4 (CH), 31.3 (CH), 30.02 ( $\text{CH}_2$ ), 29.99 ( $\text{CH}_2$ ), 25.8 ( $\text{CH}_2$ ), 25.11 ( $\text{CH}_2$ ), 25.08 ( $\text{CH}_2$ ), 19.4 ( $\text{CH}_3$ ), 18.0 ( $\text{CH}_3$ ); LRMS (+ESI):  $m/z$  379.16 ( $[\text{M}+\text{Na}]^+$ , 100 %); HRMS (+ESI):  $m/z$   $[\text{M}+\text{H}]^+$  calculated 357.2291, found 357.2286; IR (diamond cell, thin film): 3388 (bs), 3190 (bs), 2926 (m), 2851 (w), 1650 (s), 1528 (s), 1491 (m), 1176 (m), 778 (w), 749 (m).

#### (*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide (AB-FUBINACA, **4**)

Subjecting **22** (75 mg, 0.28 mmol) to general procedure D gave, following purification by flash chromatography (hexane/ethyl acetate 50:50), **4** (84 mg, 81 %) as a white solid.  $R_f$  0.15 (hexane/ethyl acetate 50:50); m.p. 163.0–165.5 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  8.18 (1H, d,  $J = 8.4$  Hz), 7.77 (1H, t,  $J = 9.4$  Hz), 7.66 (1H, br s), 7.46 (1H, ddd,  $J = 8.4, 6.8, 0.8$  Hz), 7.34–7.27 (3H, m), 7.22 (1H, br s), 7.18–7.15 (2H, m), 5.78 (2H, s), 4.42 (1H, dd,  $J = 9.2, 6.4$  Hz), 2.15–2.06 (1H, m), 0.95 (3H, d,  $J = 6.8$  Hz), 0.90 (3H, d,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  172.6 (CO), 161.6 (d, CF,  $J = 243.8$  Hz), 161.3 (CO), 140.6 (quat.), 137.1 (quat.), 133.0 (d, quat.,  $J = 3.0$  Hz), 129.5 (d, CH,  $J = 8.4$  Hz), 127.0 (CH), 122.7 (CH), 122.3 (quat.), 121.8 (CH), 115.5 (d, CH,  $J = 21.6$  Hz), 110.6 (quat.), 56.9 (CH), 51.6 ( $\text{CH}_2$ ), 31.2 (CH), 19.4 ( $\text{CH}_3$ ), 18.0 ( $\text{CH}_3$ ); LRMS (+ESI):  $m/z$  391.10 ( $[\text{M}+\text{Na}]^+$ , 100 %); HRMS (+ESI):  $m/z$  calculated  $[\text{M}+\text{Na}]^+$  391.1546, found 391.1541; IR (diamond cell, thin film): 3337 (bs), 3231 (bs), 2966 (w), 1683 (m), 1651 (s), 1510 (s), 1491 (s), 1470 (m), 1225 (m), 748 (m).

#### (*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide (AB-PINACA, **5**)

Subjecting **23** (50 mg, 0.22 mmol) to general procedure D gave, following purification by flash chromatography (hexane/ethyl acetate 70:30), **5** (63 mg, 87 %) as a white solid.  $R_f$  0.55 (hexane/ethyl acetate 40:60); m.p. 100–103 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  8.16 (1H, d,  $J = 8.4$  Hz), 7.77 (1H, d,  $J = 8.4$  Hz), 7.69 (1H, d,  $J = 9.2$  Hz), 7.66 (1H, br s), 7.45 (1H, ddd,  $J = 8.4, 7.2, 1.2$  Hz), 7.27 (1H, t,  $J = 7.4$  Hz), 7.22 (1H, br s), 4.49 (2H, t,  $J = 7.0$  Hz), 4.39 (1H, dd,  $J = 9.2, 6.0$  Hz), 2.14–2.06 (1H, m), 1.86 (2H, quin.,  $J = 7.2$  Hz), 1.35–1.19 (4H, m), 0.95 (3H, d,  $J = 6.8$  Hz), 0.90 (3H, d,



$J = 6.8$  Hz), 0.82 (3H, t,  $J = 7.2$  Hz);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  172.6 (CO), 161.3 (CO), 140.6 (quat.), 136.4 (quat.), 126.6 (CH), 122.4 (CH), 122.0 (quat.), 121.6 (CH), 110.4 (CH), 56.7 (CH), 48.7 (CH<sub>2</sub>), 31.3 (CH), 29.1 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 21.6 (CH<sub>2</sub>), 19.4 (CH<sub>3</sub>), 17.9 (CH<sub>3</sub>), 13.8 (CH<sub>3</sub>); LRMS (+ESI):  $m/z$  353.13 ([M+Na]<sup>+</sup>, 100 %); HRMS (+ESI):  $m/z$  calculated [M+Na]<sup>+</sup> 353.1954, found 353.1948; IR (diamond cell, thin film): 3390 (bs), 3198 (m), 2960 (m), 2931 (m), 1651 (s), 1530 (s), 1468 (m), 1182 (m), 750 (m).

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide (5*F*-AB-PINACA, **6**)

Subjecting **24** (75 mg, 0.30 mmol) to general procedure D gave, following purification by flash chromatography (hexane/ethyl acetate 60:40), **6** (70 mg, 67 %) as a white solid.  $R_f$  0.45 (hexane/ethyl acetate 40:60); m.p. 121–124 °C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  8.16 (1H, d,  $J = 8.4$  Hz), 7.79 (1H, dt,  $J = 8.4, 0.9$  Hz), 7.69 (1H, d,  $J = 9.2$  Hz), 7.66 (1H, br s), 7.46 (1H, ddd,  $J = 8.4, 6.8, 1.2$  Hz), 7.28 (1H, ddd,  $J = 8.2, 7.0, 0.8$  Hz), 7.22 (1H, br s), 4.52 (2H, t,  $J = 7.0$  Hz), 4.42 (1H, dd,  $J = 9.2, 6.0$  Hz), 4.39 (1H, dt,  $J = 47.6, 6.0$  Hz), 2.14–2.06 (1H, m), 1.91 (2H, quin.,  $J = 7.6$  Hz), 1.74–1.60 (2H, m), 1.39–1.31 (2H, m), 0.95 (3H, d,  $J = 6.8$  Hz), 0.90 (3H, d,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  172.7 (CO), 161.4 (CO), 140.6 (quat.), 136.4 (quat.), 126.7 (CH), 122.5 (CH), 122.0 (quat.), 121.7 (CH), 110.5 (quat.), 83.6 (d, CH<sub>2</sub>F,  $J = 161.8$  Hz), 56.8 (CH), 48.6 (CH<sub>2</sub>), 31.3 (CH), 29.3 (d, CH<sub>2</sub>,  $J = 19.3$  Hz), 29.0 (CH<sub>2</sub>), 22.0 (d, CH<sub>2</sub>,  $J = 5.3$  Hz), 19.4 (CH<sub>3</sub>), 17.9 (CH<sub>3</sub>);  $^{19}\text{F}$  NMR (470 MHz, DMSO- $d_6$ ):  $\delta$  -217.0 (1F, s, CH<sub>2</sub>F); LRMS (+ESI):  $m/z$  371.12 ([M+Na]<sup>+</sup>, 100 %); HRMS (+ESI):  $m/z$  calculated [M+Na]<sup>+</sup> 371.1859, found 371.1855; IR (diamond cell, thin film): 3393 (bs), 3195 (bs), 2963 (m), 2873 (w), 1650 (s), 1529 (s), 1491 (m), 1172 (m), 751 (s).

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-2-(cyclohexylmethyl)-2*H*-indazole-3-carboxamide (AB-CHMINACA 2-regioisomer, **7**)

Subjecting **25** (50 mg, 0.19 mmol) to general procedure D gave, following purification by flash chromatography (hexane/ethyl acetate 70:30), **7** (50 mg, 74 %) as a white solid.  $R_f$  0.60 (hexane/ethyl acetate 40:60); m.p. 203–204 °C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  8.36 (1H, d,  $J = 8.8$  Hz), 7.81 (1H, d,  $J = 8.8$  Hz), 7.69 (1H, d,  $J = 8.8$  Hz), 7.51 (1H, br s), 7.34–7.30 (1H, m), 7.23–7.20 (1H, m), 7.18 (1H, br s), 4.59 (1H, dd,  $J = 12.8, 7.2$  Hz), 4.49 (1H, dd,  $J = 12.8, 7.2$  Hz), 4.39 (1H, dd,  $J = 8.8, 6.8$  Hz), 2.19–2.10 (1H, m), 1.96–1.85 (1H, m), 1.66–1.54

(3H, m), 1.49–1.45 (2H, m), 1.18–1.07 (3H, m), 1.03–0.92 (2H, overlapping), 0.99 (3H, d,  $J = 6.8$  Hz), 0.97 (3H, d,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  172.6 (CO), 159.8 (CO), 146.4 (quat.), 129.1 (quat.), 125.8 (CH), 122.8 (CH), 120.4 (quat.), 120.2 (CH), 117.4 (CH), 58.4 (CH), 57.3 (CH<sub>2</sub>), 30.2 (CH), 30.01 (CH), 29.99 (CH<sub>2</sub>), 25.8 (CH<sub>2</sub>), 25.2 (CH<sub>2</sub>), 19.5 (CH<sub>3</sub>), 18.3 (CH<sub>3</sub>); LRMS (+ESI):  $m/z$  379.16 ([M+Na]<sup>+</sup>, 100 %); HRMS (+ESI):  $m/z$  calculated [M+Na]<sup>+</sup> 379.2110, found 379.2105; IR (diamond cell, thin film): 3345 (bs), 3275 (bs), 3159 (bs), 2956 (w), 2925 (m), 2854 (w), 1679 (s), 1649 (s), 1624 (s), 532 (m).

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-2-(4-fluorobenzyl)-2*H*-indazole-3-carboxamide (AB-FUBINACA 2-regioisomer, **8**)

Subjecting **26** (50 mg, 0.19 mmol) to general procedure D gave, following purification by flash chromatography (hexane/ethyl acetate 70:30), **8** (55 mg, 79 %) as a white solid.  $R_f$  0.55 (hexane/ethyl acetate 40:60); m.p. 264–266 °C;  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.38 (1H, d,  $J = 8.5$  Hz), 7.81 (1H, d,  $J = 8.5$  Hz), 7.70 (1H, d,  $J = 8.5$  Hz), 7.56 (1H, br s), 7.35–7.32 (3H, m), 7.23 (1H, ddd,  $J = 8.3, 6.8, 0.5$  Hz), 7.19 (1H, br s), 7.14–7.09 (2H, m), 5.90–5.82 (2H, m), 4.37 (1H, dd,  $J = 9.0, 7.0$  Hz), 2.15–2.08 (1H, m), 0.95 (3H, d,  $J = 6.8$  Hz), 0.91 (3H, d,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  172.9 (CO), 161.9 (d, CF,  $J = 244.0$  Hz, quat.), 159.8 (CO), 147.0 (quat.), 133.2 (d,  $J = 3.0$  Hz, quat.), 130.3 (d, CH,  $J = 8.4$  Hz, CH), 128.8 (quat.), 126.4 (CH), 123.4 (CH), 120.7 (quat.), 120.5 (CH), 117.7 (CH), 115.4 (d, CH,  $J = 21.5$  Hz, CH), 58.7 (CH), 54.4 (CH<sub>2</sub>), 30.3 (CH), 19.6 (CH<sub>3</sub>), 18.4 (CH<sub>3</sub>);  $^{19}\text{F}$  NMR (470 MHz, DMSO- $d_6$ ):  $\delta$  -114.5 (s); LRMS (+ESI):  $m/z$  391.09 ([M+Na]<sup>+</sup>, 100 %); HRMS (+ESI):  $m/z$  calculated [M+Na]<sup>+</sup> 391.1546, found 391.1541; IR (diamond cell, thin film): 3350 (bs), 3257 (bs), 2960 (w), 2926 (w), 2854 (w), 1673 (s), 1636 (s), 1618 (s), 1536 (m), 1110 (w), 755 (w).

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-2-pentyl-2*H*-indazole-3-carboxamide (AB-PINACA 2-regioisomer, **9**)

Subjecting **27** (50 mg, 0.22 mmol) to general procedure D gave, following purification by flash chromatography (hexane/ethyl acetate 70:30), **9** (40 mg, 55 %) as a white solid.  $R_f$  0.30 (hexane/ethyl acetate 50:50); m.p. 224–226 °C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  8.30 (1H, d,  $J = 8.8$  Hz), 7.80 (1H, dt,  $J = 8.4, 1.0$  Hz), 7.69 (1H, dt,  $J = 8.4, 1.0$  Hz), 7.56 (1H, br s), 7.32 (1H, ddd,  $J = 8.4, 6.8, 1.2$  Hz), 7.22 (1H, ddd,  $J = 8.4, 6.8, 1.2$  Hz), 7.17 (1H, br s), 4.72–4.57 (2H, m), 4.39 (1H, dd,  $J = 8.8, 6.9$  Hz), 2.18–2.10 (1H, m), 1.84 (2H, quin.,  $J = 7.4$  Hz),

1.29–1.16 (4H, m), 0.98 (3H, d,  $J = 6.8$  Hz), 0.96 (3H, d,  $J = 6.8$  Hz), 0.81 (3H, t,  $J = 7.4$  Hz);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  172.9 (CO), 159.9 (CO), 146.6 (quat.), 128.8 (quat.), 126.0 (CH), 123.1 (CH), 120.5 (quat.), 120.3 (CH), 117.6 (CH), 58.6 (CH), 51.9 (CH<sub>2</sub>), 30.4 (CH), 30.1 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 21.8 (CH<sub>2</sub>), 19.6 (CH<sub>3</sub>), 18.4 (CH<sub>3</sub>), 13.9 (CH<sub>3</sub>); LRMS (+ESI):  $m/z$  353.15 ([M+Na]<sup>+</sup>, 100 %); HRMS (+ESI):  $m/z$  calculated [M+Na]<sup>+</sup> 353.1954, found 353.1948; IR (diamond cell, thin film): 3369 (bs), 3269 (bs), 3205 (bs), 2958 (w), 2932 (w), 2859 (w), 1672 (s), 1619 (s), 1528 (m), 756 (w).

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-2-(5-fluoropentyl)-2*H*-indazole-3-carboxamide (5*F*-AB-PINACA 2-regioisomer, **10**)

Subjecting **28** (50 mg, 0.20 mmol) to general procedure D gave, following purification by flash chromatography (hexane/ethyl acetate 65:35), **10** (57 mg, 82 %) as a white solid.  $R_f$  0.50 (hexane/ethyl acetate 40:60); m.p. 213–214 °C;  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.32 (1H, d,  $J = 8.8$  Hz), 7.82 (1H, d,  $J = 8.5$  Hz), 7.70 (1H, d,  $J = 8.8$  Hz), 7.55 (1H, br s), 7.34–7.31 (1H, m), 7.24–7.21 (1H, m), 7.18 (1H, br s), 4.74–4.62 (2H, m), 4.40 (1H, dd,  $J = 8.8, 6.9$  Hz), 4.39 (2H, dt,  $J = 47.5, 6.1$  Hz), 2.14 (1H, sept.,  $J = 6.8$  Hz), 1.90 (2H, quin.,  $J = 7.6$  Hz), 1.68–1.58 (2H, m), 1.33–1.27 (2H, m), 1.00 (3H, d,  $J = 6.8$  Hz), 0.97 (3H, d,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  172.6 (CO), 159.7 (CO), 146.5 (quat.), 128.7 (quat.), 125.8 (CH), 122.9 (CH), 120.4 (quat.), 120.2 (CH), 117.5 (quat.), 83.5 (d,  $J = 162.2$  Hz, CH<sub>2</sub>F), 58.4 (CH), 51.6 (CH<sub>2</sub>), 30.3 (CH), 29.9 (CH<sub>2</sub>), 29.3 (d,  $J = 18.9$  Hz, CH<sub>2</sub>), 21.8 (d,  $J = 5.0$  Hz, CH<sub>2</sub>), 19.5 (CH<sub>3</sub>), 18.3 (CH<sub>3</sub>);  $^{19}\text{F}$  NMR (470 MHz, DMSO- $d_6$ ):  $\delta$  –216.9 (s); LRMS (+ESI):  $m/z$  371.14 ([M+Na], 100 %); HRMS (+ESI):  $m/z$  calculated [M+Na]<sup>+</sup> 371.1859, found 371.1854; IR (diamond cell, thin film): 3368 (bs), 3267 (bs), 3204 (bs), 2959 (w), 2869 (w), 1672 (s), 1631 (s), 1530 (m), 758 (w).

### Ultraviolet absorption spectroscopy

UV absorption spectroscopy was performed using a Cary 60 UV–Vis Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). UV absorbances of solutions of **3–10** in methanol (0.001 % w/v) in quartz cuvettes were recorded over 200–800 nm.

### Gas chromatography–mass spectrometry

All SCs were analyzed by gas chromatography–mass spectrometry (GC–MS) using a ThermoQuest Trace gas chromatograph with Finnigan Polaris Q ion trap mass

spectrometer (ThermoQuest, Madison, CT, USA) in positive ion electron ionization (EI) mode and a Phenomenex ZB Wax column (30 m  $\times$  0.25 mm internal diameter, 0.25  $\mu\text{m}$  film thickness; Phenomenex, Torrance, CA, USA) with helium gas as carrier at 1.3 mL/min. The conditions were: electron energy, 70 eV; injector temperature, 200 °C; injection, splitless mode; injection volume, 1  $\mu\text{L}$ ; oven temperature program, 80 °C (1.2 min hold), increase at a rate of 50 °C/min to 190 °C (15 min-hold) and then increase rate of 10 °C/min to 310 °C (10 min-hold); transfer line temperature, 280 °C; scan range,  $m/z$  46–650 (3 min solvent delay).

### In vitro pharmacological assessment of **3–10**

Mouse AtT-20 neuroblastoma cells stably transfected with human CB<sub>1</sub> or human CB<sub>2</sub> have been previously described [54] and were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS), 100 U penicillin/streptomycin, and 300  $\mu\text{g}/\text{mL}$  G418. Cells were passaged at 80 % confluency as required. Cells for assays were grown in 75 cm<sup>2</sup> flasks and used at 90 % confluence. The day before, the assay cells were detached from the flask with trypsin/EDTA (Sigma-Aldrich) 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 Ca<sup>2+</sup> calcium assay). The cells were plated in volume of 90  $\mu\text{L}$  in black-walled, clear-bottomed 96-well microplates (Corning, Oneonta, NY, USA) which had been precoated with poly-L-lysine (Sigma-Aldrich). Cells were incubated overnight at 37 °C in ambient CO<sub>2</sub>.

Membrane potential was measured using a FLIPR membrane potential assay kit (blue) from Molecular Devices (Sunnyvale, CA, USA), as described previously [55]. The dye was reconstituted with assay buffer of composition (mM): NaCl 145, HEPES 22, Na<sub>2</sub>HPO<sub>4</sub> 0.338, NaHCO<sub>3</sub> 4.17, KH<sub>2</sub>PO<sub>4</sub> 0.441, MgSO<sub>4</sub> 0.407, MgCl<sub>2</sub> 0.493, CaCl<sub>2</sub> 1.26, glucose 5.56, and bovine serum albumin (0.1 mg/mL, pH 7.4, osmolarity 315  $\pm$  5). Prior to the assay, cells were loaded with 90  $\mu\text{L}/\text{well}$  of the dye solution without removal of the L-15, giving an initial assay volume of 180  $\mu\text{L}/\text{well}$ . Plates were then incubated at 37 °C at ambient CO<sub>2</sub> for 60 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  $\mu\text{L}$ . 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 always 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 (1  $\mu$ M) of CP 55,940 (Cayman Chemical, Ann Arbor, MI, USA) was added to allow for normalization between assays.

## Results and discussion

The synthesis of **3–10** started with indazole-3-carboxylic acid (**11**), which was subjected to Fischer esterification to give the corresponding methyl ester (**12**). Alkylation of **12** with the appropriate bromoalkane, in the presence of potassium *tert*-butoxide in THF at ambient temperature or potassium carbonate in acetonitrile at reflux, produced a mixture of major 1-alkyl (**13–16**) and minor 2-alkyl (**17–20**) regioisomeric intermediates. Although 1-alkylated products **13–16** always predominated, regardless of choice of base, solvent, and temperature, the overall yield of minor 2-alkylated intermediates **17–20** was greater using potassium carbonate than potassium *tert*-butoxide. Saponification of esters **13–20** gave the corresponding acids **21–28**, which were coupled to L-valinamide using PyBOP and DIPEA to furnish 1*H*-indazoles **3–6** and their 2*H*-regioisomers **7–10** (Fig. 2).

The alkylation of methyl 1*H*-indazole-3-carboxylate (**12**) proceeded regioselectively to favour the formation of 1-substituted 1*H*-indazole intermediates as major products, entirely consistent with the known reactivity of indazoles towards nucleophilic substitution [56–58]. To quantify the regioselectivity of each alkylation method, the isolated yields of the regioisomeric products obtained following alkylation of **12** under each set of conditions for two representative bromoalkanes were determined are shown in Table 1. All reactions were performed in parallel, on the same scale, using identical reagents. Alkylation of **12** with

4-fluorobenzyl bromide or 1-bromopentane using potassium *tert*-butoxide as the base produced **14** and **15**, respectively, as the major products with excellent regioselectivity for the 1-position (49:1 and 12.3:1, respectively). The corresponding 2-alkylated regioisomers (**18** and **19**, respectively) were obtained as minor products in <10 % yield. While use of potassium carbonate as a base also furnished **14** and **15** as the major products, regioselectivity was reduced and appreciable quantities of **18** and **19** were obtained. The latter set of conditions (general procedure B) required flash chromatography to separate regioisomeric intermediates of similar polarity and retention time; so it is unlikely that this method is used on an industrial scale to produce this class of SCs. In contrast, general procedure A proceeded with high regioselectivity, and it is feasible that incomplete purification of the crude product from this reaction allows 2-alkyl-2*H*-indazole intermediate to be carried through the synthesis, resulting in the production of regioisomeric SCs.

All SCs were analyzed using GC–MS, and total ion current chromatograms (TICs) and EI mass spectra for (*S*)-*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-alkyl-1*H*-indazole-3-carboxamides (**3–6**) and the corresponding 2-alkyl-2*H*-indazole regioisomers (**7–10**) are shown in Figs. 3 and 4, respectively.

All 1-alkyl regioisomers failed to show molecular ion peaks, but similar fragmentation patterns were evident in each case. For AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA, the most prominent fragment ions could be attributed to cleavage of the terminal carboxamide subunit of the pendant L-valinamide group (*m/z* 312, 324, 286, and 304 respectively), as well as C–N scission of the indazole-3-carboxamide bond (*m/z* 241, 253, 215, and 233). 5F-AB-PINACA also showed a peak at *m/z* 213, consistent with a protonated species arising from defluorination of fragment *m/z* 233.

In the cases of AB-CHMINACA, AB-PINACA, and 5F-AB-PINACA, a common fragment of 145 mass units was observed, likely due to the protonated species arising from indazole-3-carboxamide bond scission and loss of the

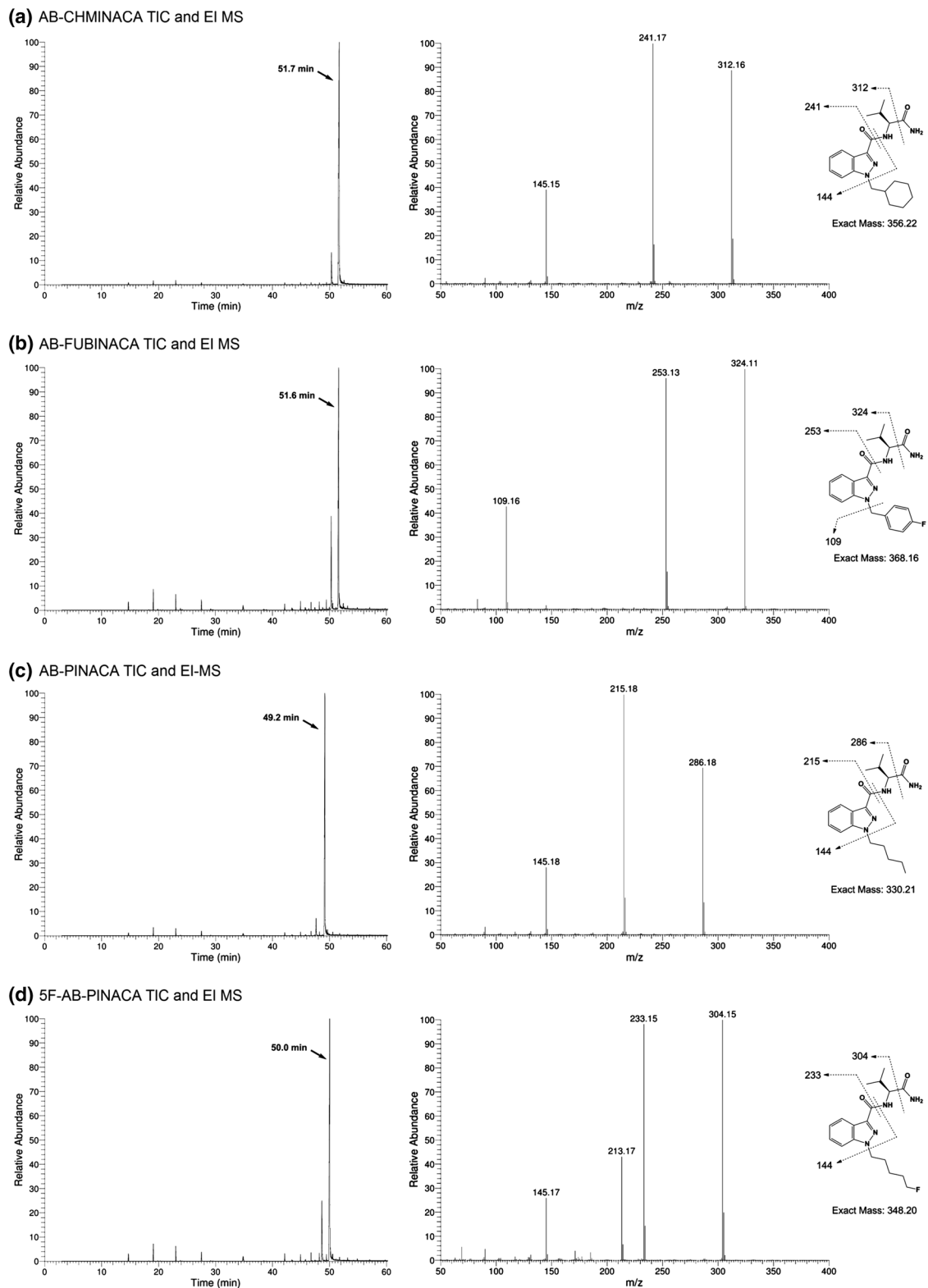
**Table 1** Regioisomeric ratios for alkylation of methyl 1*H*-indazole-3-carboxylate (**12**) in the presence of different bases

Condition <sup>a</sup>	Base	Alkylating reagent	Products (yields) <sup>b</sup>		1-Alkyl isomer selectivity <sup>c</sup>
A	Potassium <i>tert</i> -butoxide	4-Fluorobenzyl bromide	<b>14</b> (570 mg, 71 %)	<b>18</b> (12 mg, 1.5 %)	47.5
A	Potassium <i>tert</i> -butoxide	1-Bromopentane	<b>15</b> (585 mg, 84 %)	<b>19</b> (48 mg, 6.9 %)	12.2
B	K <sub>2</sub> CO <sub>3</sub>	4-Fluorobenzyl bromide	<b>14</b> (469 mg, 58 %)	<b>18</b> (225 mg, 28 %)	2.1
B	K <sub>2</sub> CO <sub>3</sub>	1-Bromopentane	<b>15</b> (488 mg, 70 %)	<b>19</b> (175 mg, 25 %)	2.8

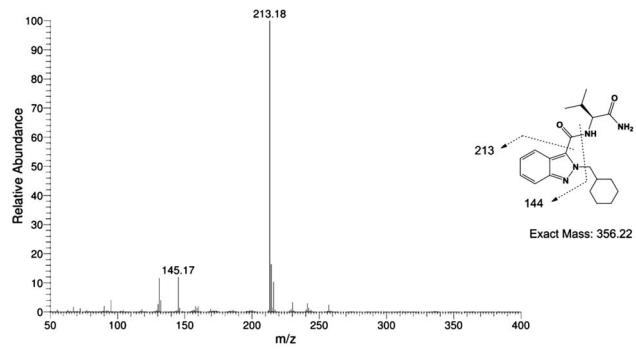
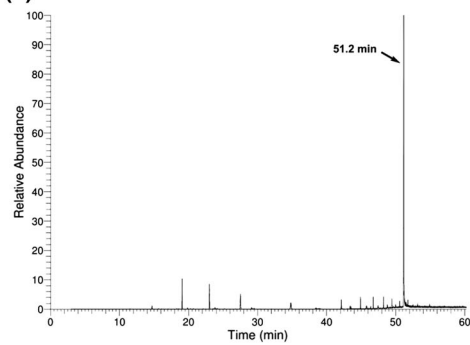
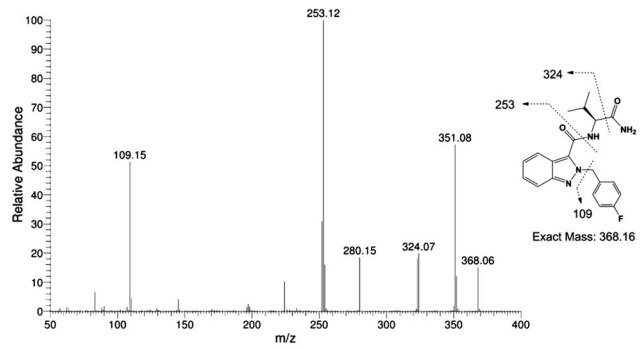
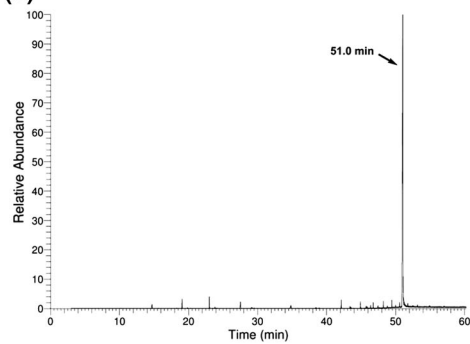
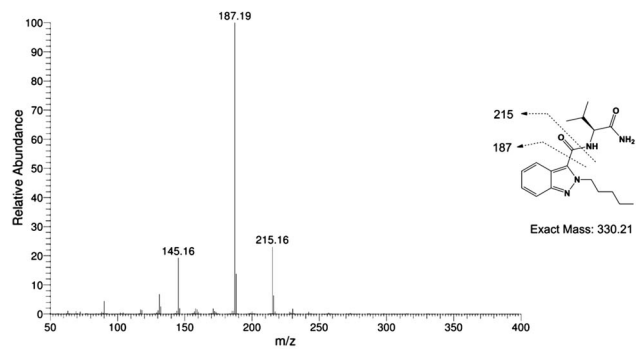
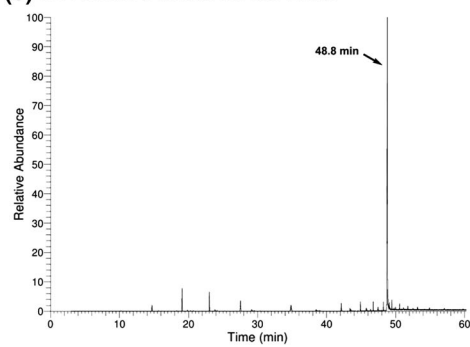
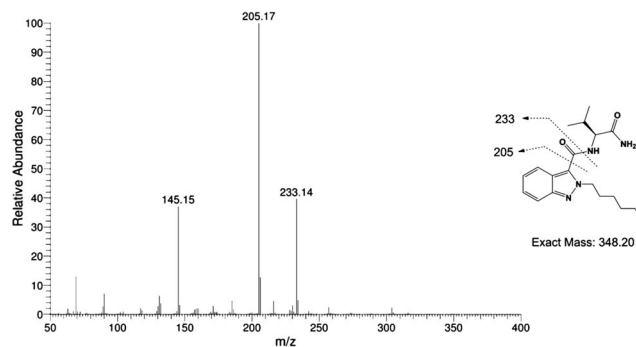
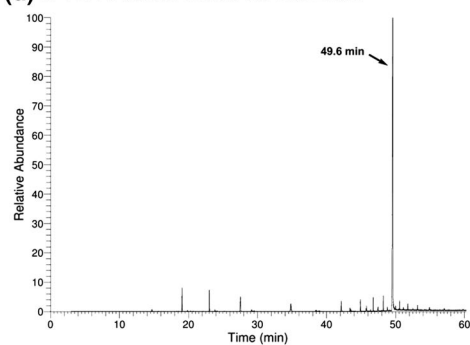
<sup>a</sup> Denotes general procedure A or B for alkylation of **12** as described in full in the experimental procedures

<sup>b</sup> Isolated yield of each regioisomer following purification by flash chromatography

<sup>c</sup> Selectivity presented as ratio of 1-alkyl to 2-alkyl products



**Fig. 3** Gas chromatography–mass spectrometry (GC–MS) analyses showing total ion current chromatograms (TICs) (*left*) and electron ionization (EI)-mass spectra (*right*) for **a** **3**, **b** **4**, **c** **5**, and **d** **6**. Proposed fragmentation ions are superimposed

**(a)** AB-CHMINACA 2-isomer TIC and EI MS**(b)** AB-FUBINACA 2-isomer TIC and EI MS**(c)** AB-PINACA 2-isomer TIC and EI MS**(d)** 5F-AB-PINACA 2-isomer TIC and EI MS

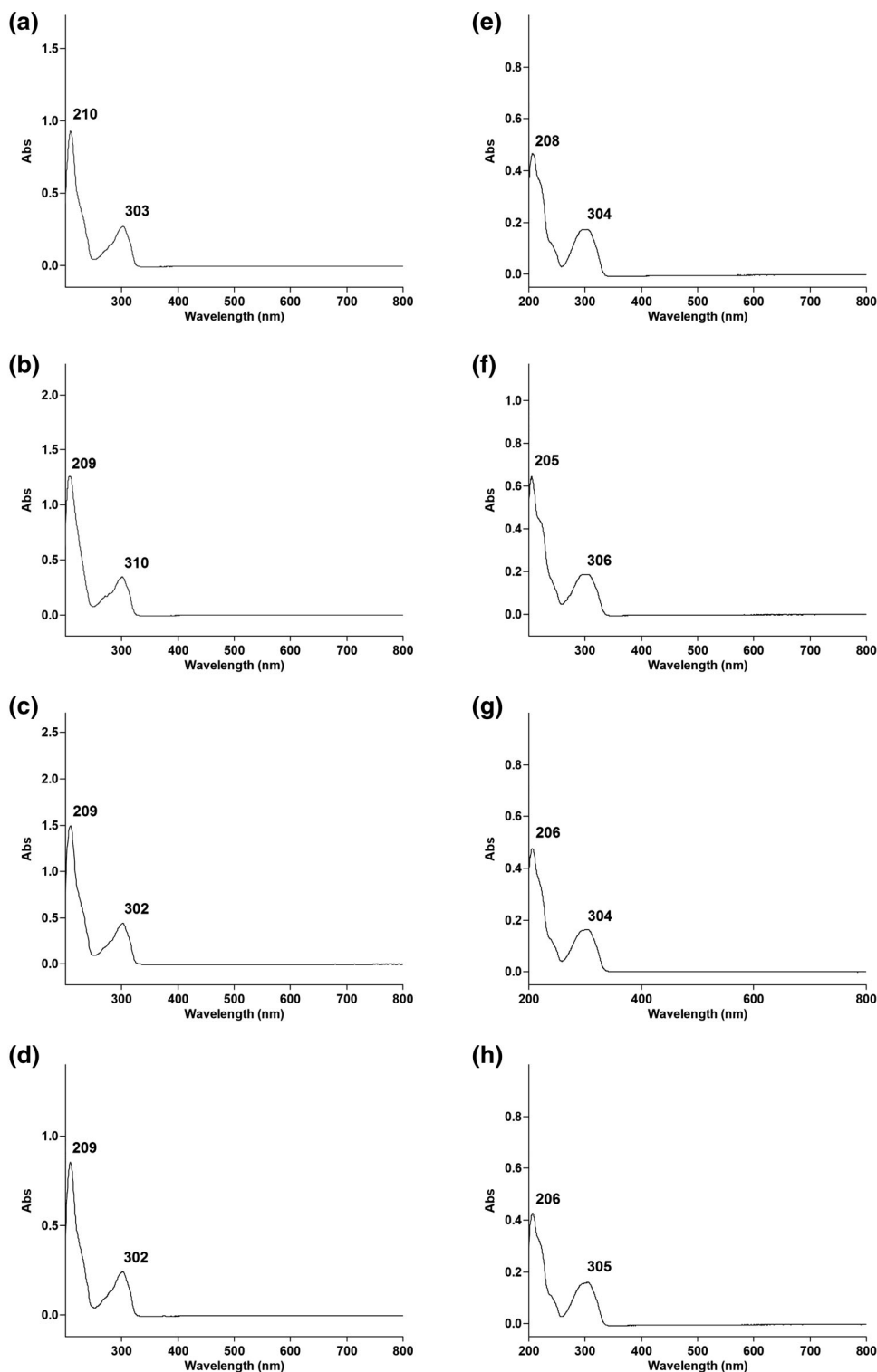
**Fig. 4** GC–MS analyses showing TICs (*left*) and EI-mass spectra (*right*) for **a** 7, **b** 8, **c** 9, and **d** 10. Proposed fragmentation ions are superimposed

1-alkyl group. AB-FUBINACA was devoid of peak at  $m/z$  145, but did feature the 4-fluorobenzyl fragmentation ion ( $m/z$  109).

The fragmentation patterns for the corresponding 2-alkyl-2*H*-indazoles **7–10** were distinct from their 1-alkyl

regioisomers. Only in the case of AB-FUBINACA 2-isomer, the molecular ion ( $m/z$  368) and fragment corresponding to cleavage of the terminal carboxamide group ( $m/z$  324) were observed. Again, common fragment  $m/z$  145 arising from dealkylated indazole-3-acylium ion could

**Fig. 5** Ultraviolet absorption spectra of **a** **3**, **b** **4**, **c** **5**, **d** **6**, **e** **7**, **f** **8**, **g** **9**, and **h** **10**



be seen in the spectra of **7**, **9**, and **10**. A fragment corresponding to scission of the entire 3-substituent of indazoles **7**, **9**, and **10** ( $m/z$  213, 187, and 205 respectively) was present as the base peak in these spectra. Fragmentation of the neighboring amide bond proximal to the indazole core was seen for **8**, **9**, and **10** ( $m/z$  253, 215, and 233 respectively), but not for **7**.

The UV–Vis absorption spectra of **3–10** are shown in Fig. 5, and peak absorbances are similar for each 1-alkyl-1*H*-indazoles and their regioisomers, indicating that ultraviolet spectroscopy is not a suitable method for differentiation of regioisomeric indazole cannabinoids of this class.

The cannabinoid activities of indazole SCs **3–10** at CB<sub>1</sub> and CB<sub>2</sub> receptors are shown in Table 2. Murine AtT-20 neuroblastoma cells were stably transfected with human CB<sub>1</sub> or CB<sub>2</sub> receptors, and activities of CP 55,940 and **3–10** were evaluated using FLIPR membrane potential assays whereby endogenously expressed G protein-gated inwardly rectifying K<sup>+</sup> channels (GIRKs) are activated by agonists at the coexpressed CB<sub>1</sub> or CB<sub>2</sub> receptors [55, 59]. The maximum effects of **3–10** were compared to the high efficacy CB<sub>1</sub>/CB<sub>2</sub> receptor agonist CP 55,940, which produced a maximal decrease in fluorescence, corresponding to cellular hyperpolarization, of 14 ± 2 % in the AtT-20-CB<sub>1</sub> cells and 33 ± 2 % in the AtT-20-CB<sub>2</sub> cells.

AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA, and their 2-alkylated regioisomers displayed distinct profiles at CB<sub>1</sub> and CB<sub>2</sub> receptors. The

1-alkyl isomers AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA showed potent activity at CB<sub>1</sub> receptors, with nanomolar EC<sub>50</sub> values (EC<sub>50</sub> = 2.1–7.8 nM) exceeding that of CP 55,940 itself. Moreover, **3–6** were highly efficacious CB<sub>1</sub> agonists, eliciting 132–151 % of the response achieved by 1 μM CP 55,940. The **3–6** showed slightly lower potency at CB<sub>2</sub> receptors (1.5–3.2 times less), but were still potent, efficacious agonists.

In contrast, the 2-regioisomers **7–10** were not potent at stimulating CB<sub>1</sub> and CB<sub>2</sub> receptor coupling to GIRKs. EC<sub>50</sub> values could not be determined for **7** or **8** at either receptor, and **9** and **10** showed only micromolar potency for CB<sub>1</sub> (EC<sub>50</sub> = 4080 and 1930 nM, respectively) and CB<sub>2</sub> (EC<sub>50</sub> = 4120 and 2980 nM, respectively).

Of the 2-alkyl-2*H*-indazoles, **7**, **9**, and **10**, displayed a maximum effect at CB<sub>1</sub> receptors comparable to 1 μM CP 55,940 at the highest concentration tested, 30 μM. At 30 μM, **8** achieved only 61 % of the CP 55,940 hyperpolarization. At CB<sub>2</sub> receptors, 30 μM of **9** elicited a response equivalent to 1 μM CP 55,940, while **7**, **8**, and **10** at 30 μM achieved only 25–47 % of the maximum.

The dramatic difference in potency and efficacy of the 1-alkyl-1*H*-indazoles **3–6** and 2-alkyl-2*H*-indazoles **7–10** at CB<sub>1</sub> and CB<sub>2</sub> receptors is shown in Fig. 6a, b respectively. The non-linear regression fit of CP 55,940 is included as a dashed line for comparison. The 1-alkyl compounds **3–6** (white data points) were all more potent

**Table 2** Functional activities of CP 55,940, and **3–10** at human CB<sub>1</sub> and human CB<sub>2</sub> receptors

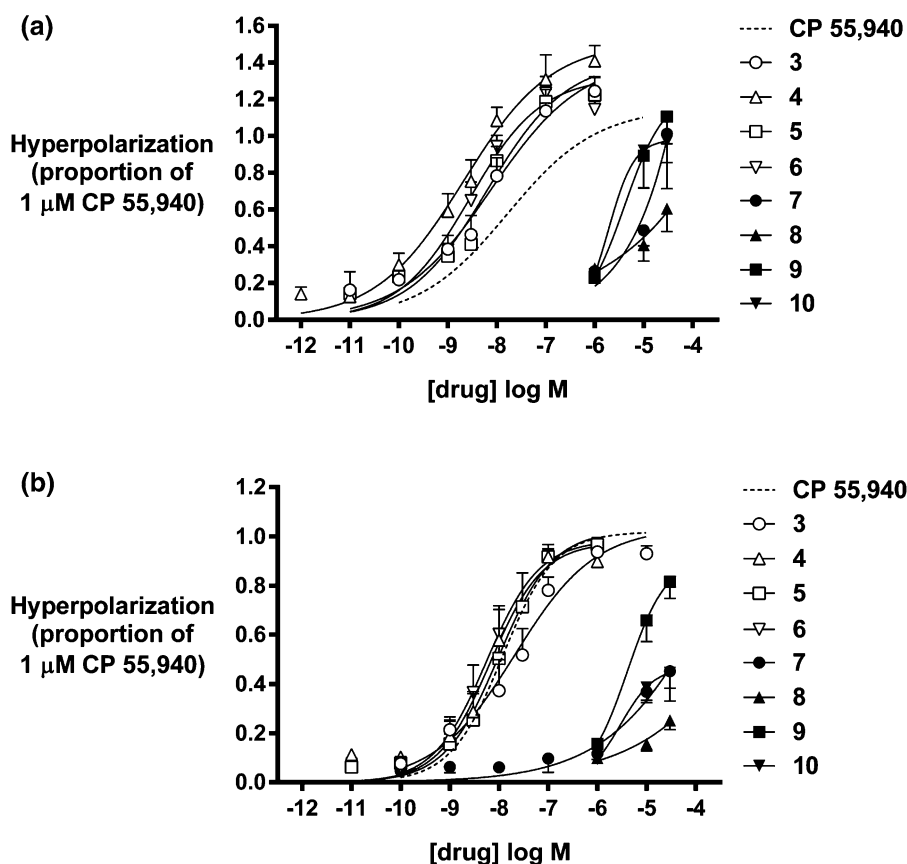
Compound	hCB <sub>1</sub>		hCB <sub>2</sub>		CB <sub>1</sub> selectivity <sup>a</sup>
	pEC <sub>50</sub> ± SEM (EC <sub>50</sub> , nM)	Max ± SEM (% CP 55,940)	pEC <sub>50</sub> ± SEM (EC <sub>50</sub> , nM)	Max ± SEM (% CP 55,940)	
CP 55,940	7.93 ± 0.08 (12)	–	7.78 ± 0.19 (17)	–	1.4
<b>3</b> (AB-CHMINACA)	8.11 ± 0.32 (7.8)	142 ± 18	7.68 ± 0.24 (21)	104 ± 11	2.7
<b>4</b> (AB-FUBINACA)	8.67 ± 0.24 (2.1)	151 ± 14	8.17 ± 0.19 (6.7)	98 ± 10	3.2
<b>5</b> (AB-PINACA)	8.19 ± 0.23 (6.5)	142 ± 15	8.01 ± 0.16 (9.9)	102 ± 9	1.5
<b>6</b> (5F-AB-PINACA)	8.55 ± 0.15 (2.8)	132 ± 9	8.25 ± 0.16 (5.6)	98 ± 8	2.0
<b>7</b> (AB-CHMINACA 2-isomer)	n.d.	101 ± 16 (at 30 μM)	n.d.	45 ± 7 (at 30 μM)	–
<b>8</b> (AB-FUBINACA 2-isomer)	n.d.	61 ± 12 (at 30 μM)	n.d.	25 ± 4 (at 30 μM)	–
<b>9</b> (AB-PINACA 2-isomer)	5.39 ± 0.42 (4080)	124 ± 38 (at 30 μM)	5.39 ± 0.27 (4120)	91 ± 18 (at 30 μM)	1.0
<b>10</b> (5F-AB-PINACA 2-isomer)	5.72 ± 0.56 (1930)	98 ± 27 (at 30 μM)	5.53 ± 0.49 (2980)	47 ± 15 (at 30 μM)	1.5

hCB<sub>1</sub> human CB<sub>1</sub> receptor, hCB<sub>2</sub> human CB<sub>2</sub> receptor, pEC<sub>50</sub> ± SEM negative logarithm of the EC<sub>50</sub> ± standard error of the mean, Max ± SEM the maximum drug effect as a percentage of that achieved by a maximally effective concentration of CP 55,940, EC<sub>50</sub> concentration giving 50 % of the maximum drug effect, n.d., not determined

<sup>a</sup> CB<sub>1</sub> selectivity expressed as the ratio of CB<sub>2</sub> EC<sub>50</sub> to CB<sub>1</sub> EC<sub>50</sub>



**Fig. 6** Hyperpolarization of **a** CB<sub>1</sub> and **b** CB<sub>2</sub> receptors induced by **3–10** as a proportion of that produced by 1  $\mu$ M CP 55,940. Membrane potential was measured using a fluorescent dye, as outlined in the text. Each point represents the mean  $\pm$  standard error of at least five independent determinations, each performed in duplicate. Data was fitted with a four parameter logistic equation in GraphPad Prism



and efficacious than CP 55,940 at CB<sub>1</sub> receptors, while 2-alkyl regioisomers **7–10** (black data points) were substantially less potent, with three reaching efficacy comparable to maximum CP 55,940 only at the highest concentration tested (Fig. 6a). At CB<sub>2</sub> receptors, **3–6** demonstrate potency and efficacy comparable to CP 55,940, but **7–10**, with the exception of **9**, show efficacy approximately 20–40 % that of a maximally efficacious concentration of CP 55,940 (Fig. 6b).

## Conclusions

This study represents the first pharmacological characterization of the 2-alkyl-2*H*-indazole regioisomers of SC designer drugs AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA. A general synthetic route to both 1-alkyl-1*H*- and 2-alkyl-2*H*-indazole-3-carboxamides was demonstrated, and may be of use to cannabinoid researchers for the preparation of related analogues. Additionally, characteristic differences in the fragmentation patterns of these regioisomers were described, enabling their differentiation by GC–MS.

AB-CHMINACA, AB-FUBINACA, AB-PINACA, 5F-AB-PINACA, and the corresponding 2-alkyl-2*H*-

indazole regioisomers **7–10** were evaluated for their activity at human CB<sub>1</sub> and CB<sub>2</sub> receptors in vitro using FLIPR membrane potential assays. AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA are potent, efficacious agonists of CB<sub>1</sub> and CB<sub>2</sub> receptors, while the corresponding 2-alkyl-2*H*-indazole regioisomers possessed only low potency as CB<sub>1</sub>/CB<sub>2</sub> agonists. Having demonstrated the weak cannabimimetic properties of the 2-alkyl-2*H*-indazole regioisomers of AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA, and the synthesis of both regioisomers from a common precursor, it is possible that 2-alkyl-2*H*-indazole-3-carboxamide SCs occur in recreational products as synthesis byproducts of 1-alkyl-1*H*-indazole-3-carboxamides rather than analogues produced deliberately as SCs in their own right.

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## Compliance and ethical standards

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

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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