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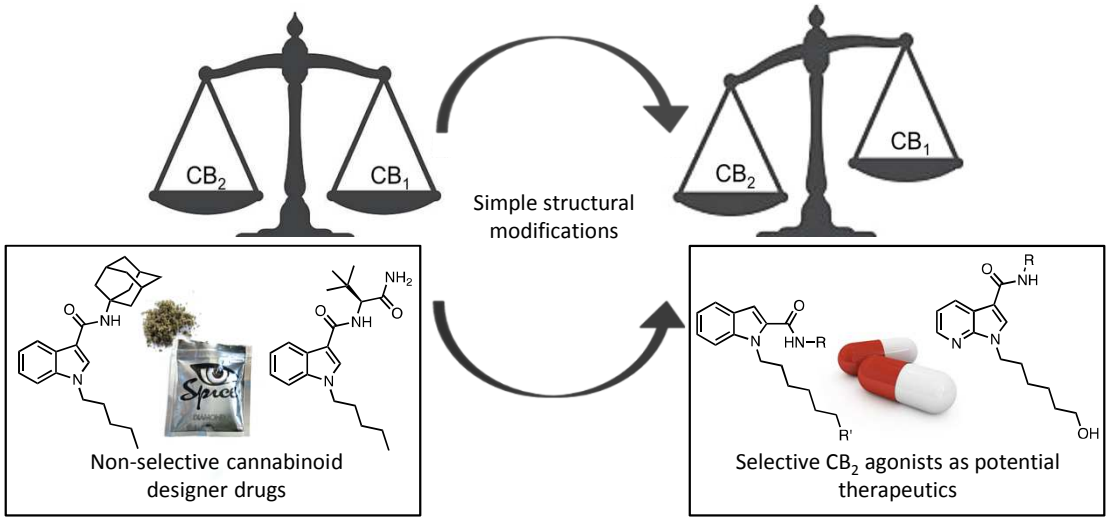
CB2
CB1
Simple structural
modifications
CB2
CB1
Non-selective cannabinoid
designer drugs
Selective CB2 agonists as potential
therapeutics

Strategies to Develop Selective CB_2 Receptor Agonists from Indole Carboxamide Synthetic Cannabinoids

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Abstract

Activation of the CB_2 receptor is an attractive therapeutic strategy for the treatment of a wide range of inflammatory diseases. However, receptor subtype selectivity is necessary in order to circumvent the psychoactive effects associated with activation of the CB_1 receptor. We aimed to use potent, non-selective synthetic cannabinoids designer drugs to develop selective CB_2 receptor agonists. Simple structural modifications such as moving the amide substituent of 3-amidoalkylindole synthetic cannabinoids to the 2-position and bioisosteric replacement of the indole core to the 7-azaindole scaffold are shown to be effective and general strategies to impart receptor subtype selectivity. 2-Amidoalkylindole **16** (EC_{50} CB_1 > 10 μM, EC_{50} CB_2 = 189 nM) and 3-amidoalkyl-7-azaindole **21** (EC_{50} CB_1 > 10 μM, EC_{50} = 49 nM) were found to be potent and selective agonists with favourable physicochemical properties. Docking studies were used to elucidate the molecular basis for the observed receptor subtype selectivity for these compounds.

Keywords: Cannabinoid; Cannabinoid type 2 receptor; Synthetic cannabinoid; Indole; Azaindole; Docking.

1. Introduction

In recent decades the endocannabinoid system has emerged as an important modulatory system that is involved in a whole range of physiological processes, including appetite, pain-sensation, mood and memory.[1] The system comprises of endogenous cannabinoids (endocannabinoids), the enzymes responsible for their synthesis and degradation and two G protein coupled receptors which are their primary targets (although other receptors may also be involved).[2] The cannabinoid type 1 (CB_1) receptor is found in high levels in the central nervous system, but also in a number of peripheral tissues.[3] Conversely, the cannabinoid type 2 (CB_2) receptor is expressed mainly on cells of the immune system, which in the CNS includes astrocytes and microglia.[3]

The discovery of the CB_2 receptor in 1993 provided an explanation for the established immunomodulatory properties of cannabinoids.[4] The broad distribution of the

endocannabinoid system throughout the body means that its manipulation can have implications in a wide range of diseases and disorders.[5] It is believed that in response to injury or damage, activation of CB_2 receptors triggers protective mechanisms for the resolution of inflammation and its associated symptoms.[6] Demonstration that CB_2 receptor activation produces desirable effects in a range of preclinical models has motivated the design of agonists for the treatment of a wide range of diseases and pathological conditions involving an inflammatory component.[7] A requirement for the development of CB_2-based therapeutics is the avoidance of unwanted psychotropic effects associated with activation of the CB_1 receptor. In light of this, much effort has been devoted to the development of selective CB_2 receptor agonists, in order to circumvent psychoactive side effects. A number of compounds developed by academic labs and pharmaceutical companies have been shown to selectively activate the CB_2 receptor (**1**-**6**, **Figure 1**).[8-14] Despite this, CB_2 agonists have fared poorly in the clinic. Potential reasons for the lack of translation from preclinical to clinical efficacy include, species differences in receptors and signalling pathways, a lack of receptor subtype selectivity in older generation agonists, issues pertaining to biased agonism or functional selectivity and concerns surrounding drug tolerance.[15] Therefore, there is a need to develop more potent, effective and safe CB_2 agonists.

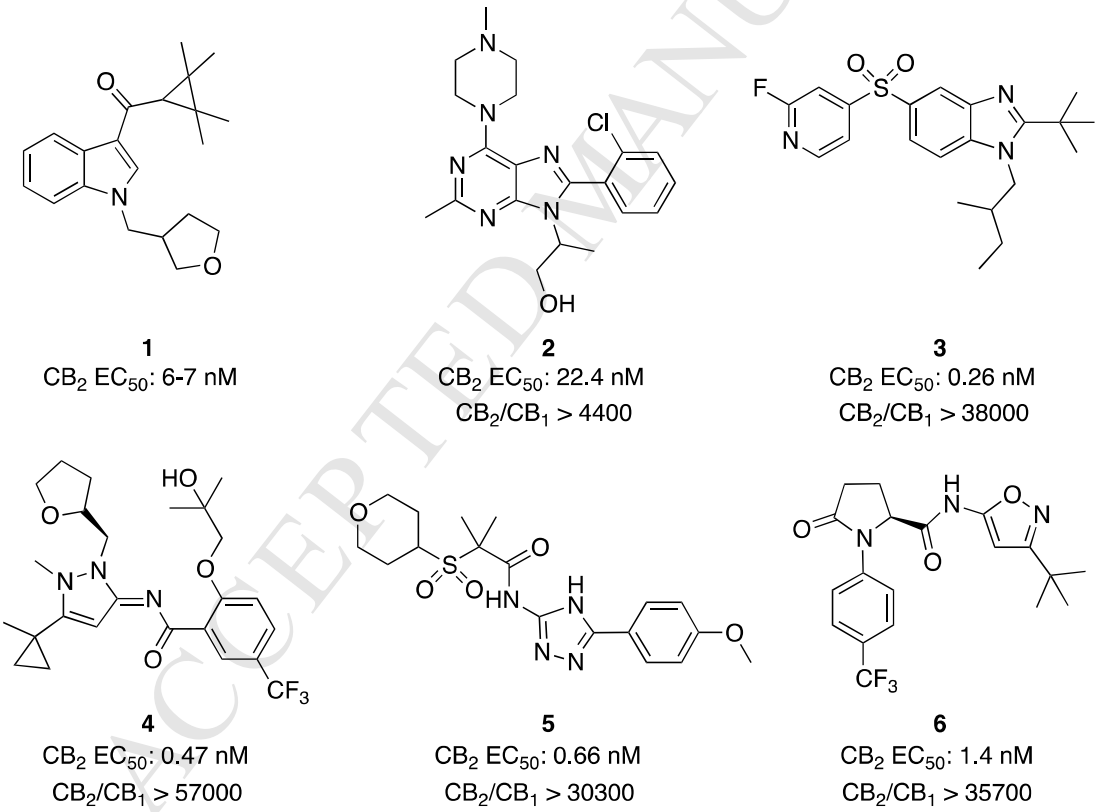


Figure 1: Representative examples of CB_2 selective agonists reported in the literature (**1**-**6**).

In the early 2000's, smokable herbal products, adulterated with synthetic cannabinoids reported in the scientific literature, appeared as legal alternatives to marijuana. In recent years we have synthesized and characterized the pharmacological activity of many such high concern synthetic cannabinoids, particularly 3-amidoalkyl indoles and indazoles (**Figure 2**).[16-20]

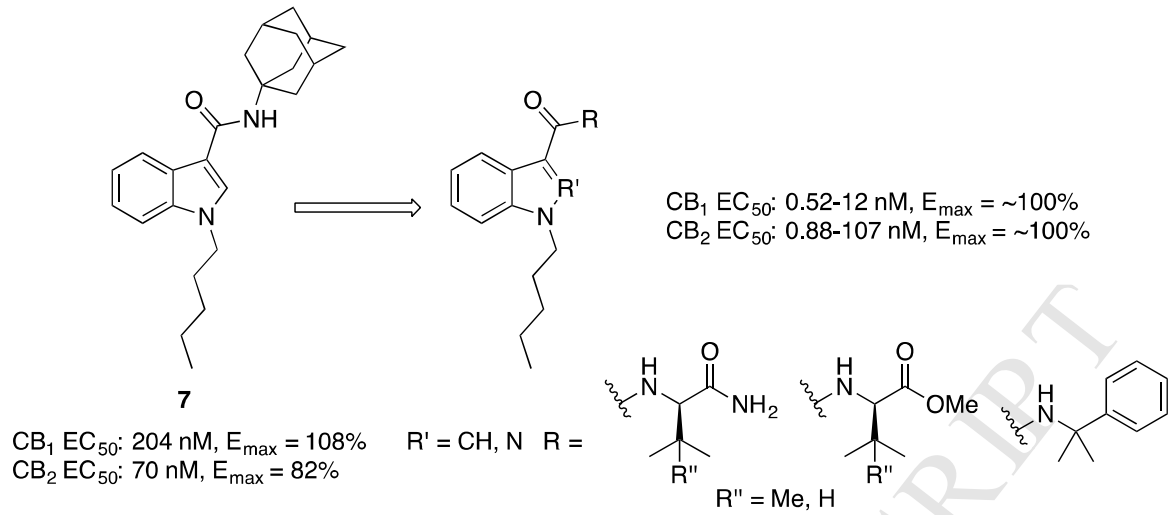


Figure 2: Evolution of synthetic cannabinoids from APICA **7**. 3-Amidoalkylindole and indazole synthetic cannabinoids (general structure shown) are generally potent, full agonists at both the CB_1 and CB_2 receptors.

The research has established simple and reliable methods to access such synthetic cannabinoids and their analogues for pharmacological evaluation. These compounds are generally highly potent, high efficacy agonists at both the CB_1 and CB_2 receptors, and have been shown to exhibit potent *in vitro* activity, cannabimimetic phenotypes *in vivo*, and anecdotal cannabimimetic activity in humans.[21]

Recently it was demonstrated that CB_2 selectivity could be realized for non-selective 3-adamantylamidoalkylindoles by simply moving the amide from the 3-position to the 2-position of the indole.[22] The 2-substituted adamantyl amide compounds (for example **8**) discovered exhibited low to moderate potencies, however are generally quite lipophilic, owing to the non-polar adamantyl group. Previous research also found that 3-substituted bulky amino acid derived amides (from L-valinamide, methyl-L-valinate, *tert*-leucinamide and *tert*-leucinate) as well as cumyl amides are amongst the most potent synthetic cannabinoids known.[16-17, 19] We have applied this strategy to such amides in an effort to obtain more potent and selective compounds (**9**, **11**, **14**, **16** and **18**) with favorable physicochemical properties, namely reduced lipophilicity (**Figure 3**). In addition, it is also reported that incorporation of a polar group, particularly a hydroxyl group, at the terminal position of the *N*-pentyl chain can improve CB_2 selectivity.[20] With this in mind we prepared the 5-hydroxyl analogues (**10**, **12**, **15**, **17** and **19**).

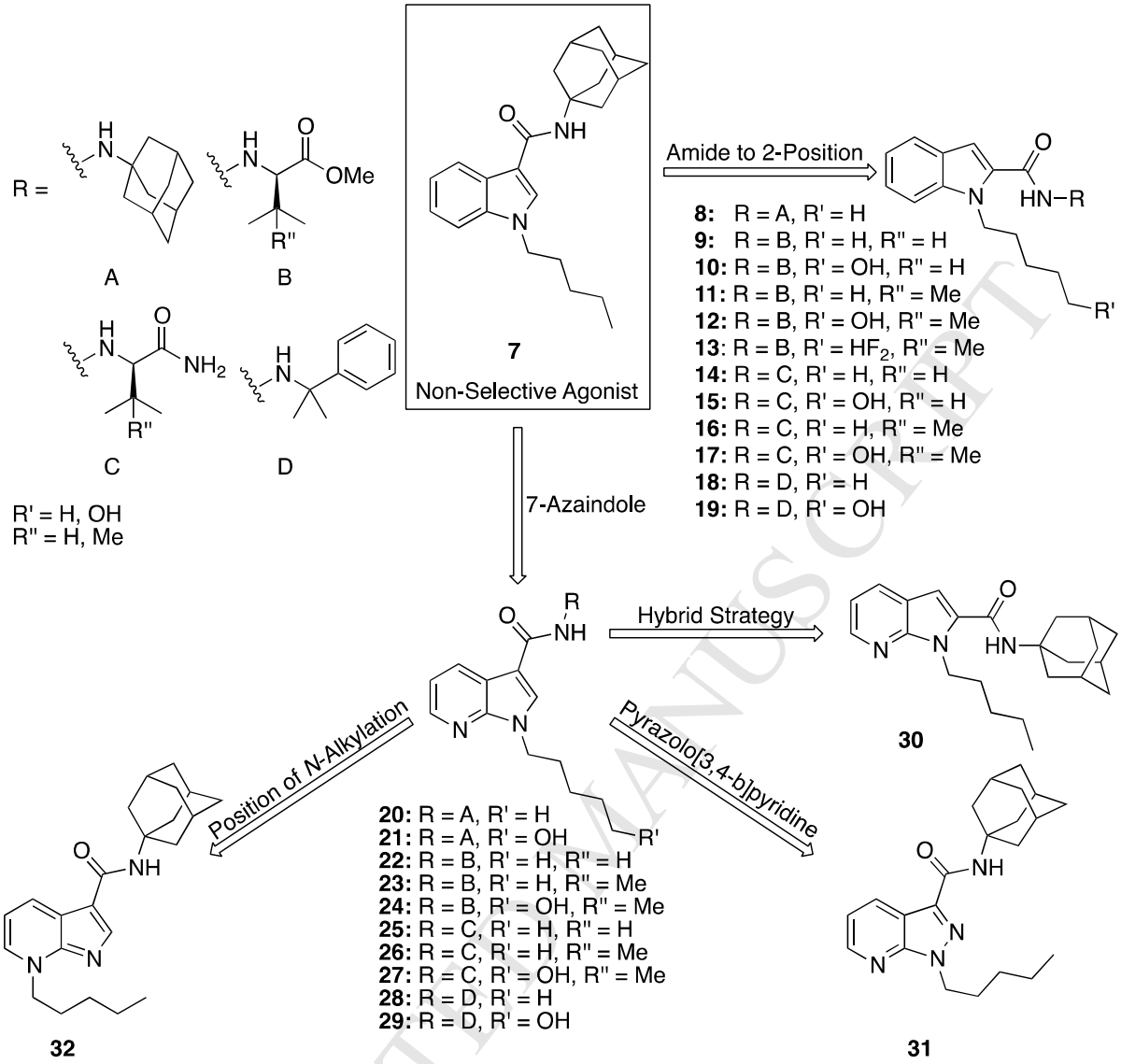


Figure 3: Strategies to develop CB_2 selective agonists from non-selective synthetic cannabinoids such as APICA **7**.

Our recent study involving the pharmacological evaluation of azaindole derived synthetic cannabinoids revealed that the 7-azaindole scaffold may impart some CB_2 selectivity.[23] Again, we sought to determine whether this is a general strategy to improve the CB_2 selectivity of potent synthetic cannabinoids (**20**-**29**, **Figure 3**). In addition to improving receptor subtype selectivity, bioisosteric replacement of the indole with a 7-azaindole moiety may improve physicochemical properties.

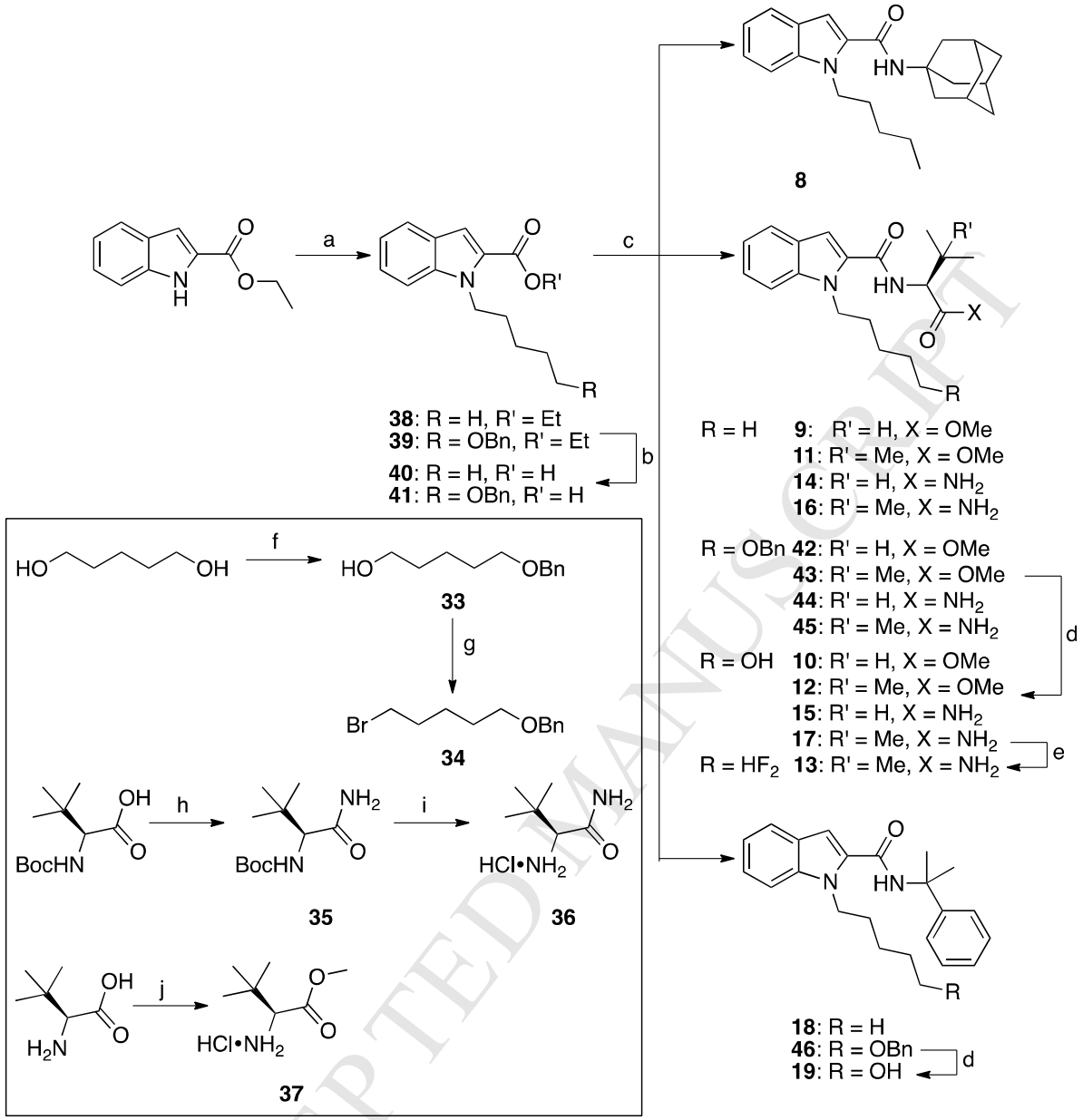
A hybrid strategy was also explored by moving the amide group from the 3-position to the 2-position of the 1-alkyl-7-azaindole moiety (**30**). Furthermore, azaindole analogues were investigated including 3-amidoalkylpyrazolo[3,4-*b*]pyridine **31** and the influence of the position of nitrogen alkylation by the preparation of pseudo-azulene compound **32**.

Molecular modelling based on X-ray crystal structures of the CB_1 and CB_2 receptors was performed to rationalise the key binding interactions that impart receptor subtype selectivity, and inform future development of cannabinoid therapeutics.

2. Results and Discussion

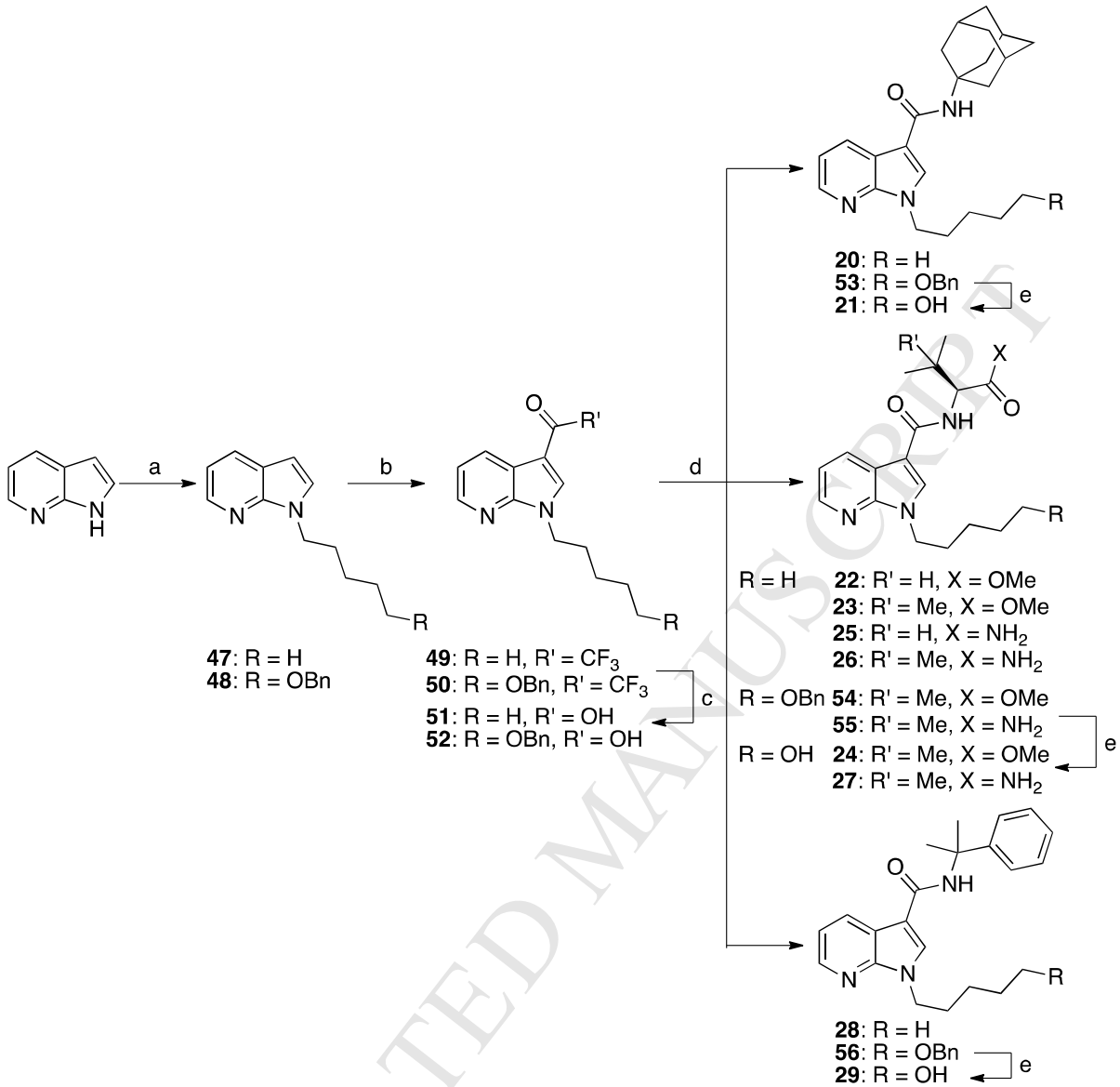
2.1 Chemistry

2-Amidoalkylindole analogues **8**-**19** were prepared from commercially available ethyl indole-2-carboxylate. Sodium hydride mediated deprotonation followed by reaction with alkyl bromides, 1-bromopentane or **34**, which was prepared from mono-benzyl protected 1,5-pentanediol **33** via an Appel reaction, gave the requisite carboxylic acid intermediates **40** and **41** after base mediated ester hydrolysis (**Scheme 1**). Operationally simple and high yielding EDCI-HOBt amide coupling using the appropriate amines gave the 11 amides **8**, **9**, **11**, **14**, **16, 18**, **42**-**46**. Amine **36** was synthesized from commercially available Boc-L-*tert*-leucine via an EDCI-HOBt mediated amide coupling and subsequent Boc group deprotection under acidic conditions. Likewise, amine **37** was prepared from commercially available L-*tert*-leucine via the corresponding acid chloride. Hydrogenolysis of the benzyl protecting groups from **42**-**46** afforded the 5-hydroxyl analogues **10**, **12**, **15**, **17** and **19**. Difluoromethyl analogue **13** was prepared in a two-step procedure from alcohol **17** via a Dess-Martin periodinane (DMP) mediated oxidation followed by treatment of the aldehyde with nucleophilic fluorinating reagent diethylaminosulfur trifluoride (DAST).



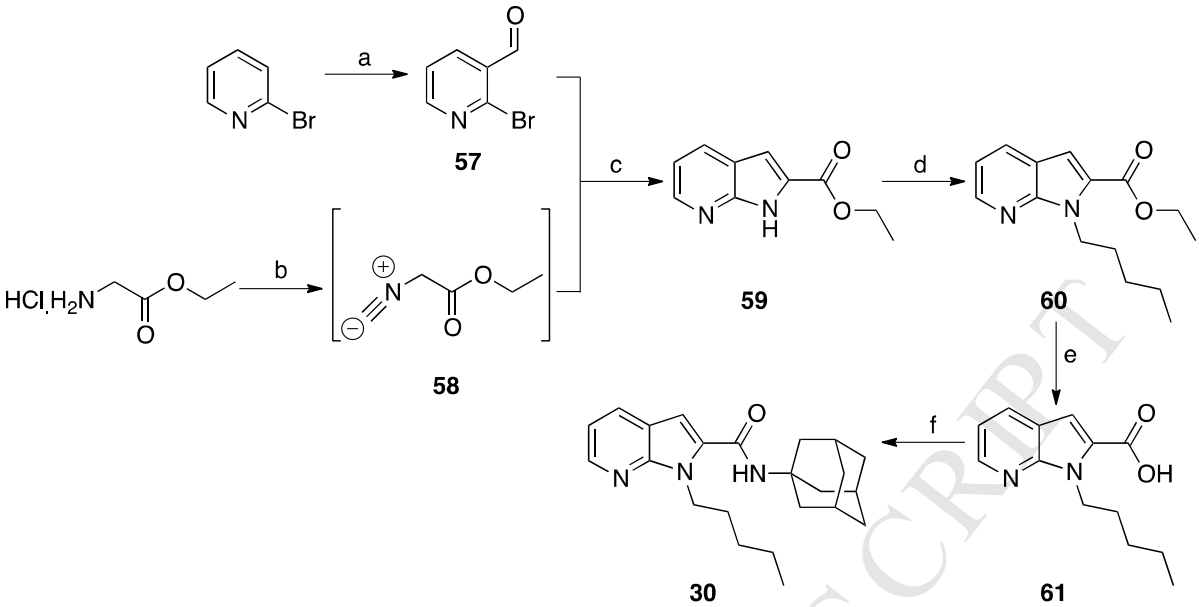
Scheme 1: Synthesis of 2-amidoalkylindoles (**8**-**19**). *Reagents and conditions*: (a) NaH, appropriate alkyl bromide (1-bromopentane or **34**), DMF, 0 °C-rt, 16 hours; (b) NaOH (aq.), EtOH, reflux, 2 hours; (c) Appropriate amine (1-adamantylamine, 2-phenylpropan-2-amine, L-valinamide hydrochloride, methyl-L-valinate hydrochloride, **36** or **37**), EDCI, HOBt, *i*Pr$_2$EtN, DMF, rt, 16 hours; (d) Pd/C, H$_2$, EtOAc, rt, 16 hours; (e) i) DMP, CH_2Cl_2, THF, rt, 16 hours, ii) DAST, CH_2Cl_2, rt, 24 hours; (f) NaH, BnBr, DMF, 0 °C-rt, 16 hours; (g) PPh_3, Br_2, pyridine, CH_2Cl_2, 0 °C-rt, 4 hours; (h) NH_4Cl, EDCI, HOBt, Et_3N, DMF, rt, 16 hours; (i) HCl, dioxane, EtOAc, rt, 16 hours; (j) $SOCl_2$, MeOH, reflux, 16 hours.

3-Amidoalkyl-7-azaindole derivatives **20**-**29** were prepared following previously reported procedures.[23] Commercially available 7-azaindole was deprotonated using sodium hydride and then reacted with alkyl bromides, 1-bromopentane or **34** to give the *N*1-alkyl intermediates **47** and **48** (**Scheme 2**). Aluminium chloride mediated Friedel Crafts acylation gave the 3-subsituted trifluoromethyl ketones **49** and **50**, which were hydrolysed under basic conditions after extended reaction times to afford carboxylic acids **51** and **52**. EDCI-HOBt mediated amide coupling gave the requisite amides **20**, **22**-**26**, **28** and **53**-**56** and hydrogenolysis to remove the benzyl protecting groups from **53**-**56** furnished the 5-hydroxyl analogues **21**, **24**, **27** and **29**.



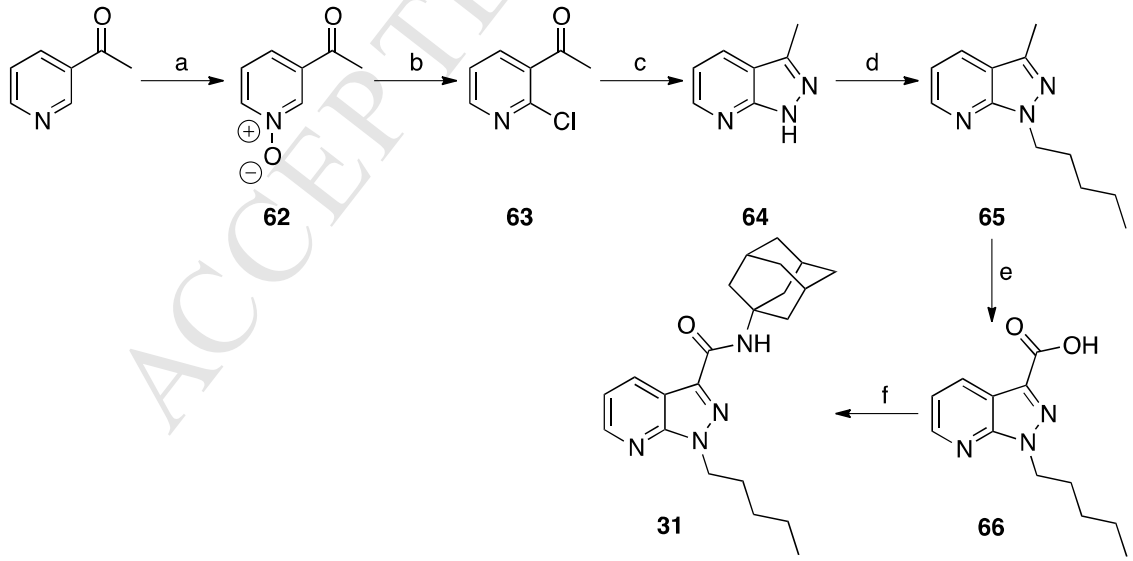
Scheme 2: Synthesis of 3-amidoalkyl-7-azaindoles (**20**-**29**). *Reagents and conditions*: (a) NaH, appropriate alkyl bromide (1-bromopentane or **34**), DMF, 0 °C-rt, 16 hours; (b) $AlCl_3$, $(CF_3CO)_2O$, DMF, 0 °C-rt, 3 hours; (c) LiOH (aq.), MeOH, reflux, 48 hours; (d) Appropriate amine (1-adamantylamine, 2-phenylpropan-2-amine, L-valinamide hydrochloride, methyl-L-valinate hydrochloride, **36** or **37**), EDCI, HOBt, iPr$_2$EtN, DMF, rt, 16 hours; (e) Pd/C, H_2, EtOAc, AcOH, rt, 16-48 hours.

2-Amidoalkyl-7-azaindole derivative **30** was prepared using a similar procedure, from ethyl 7-azaindole-2-carboxylate **59** (**Scheme 3**). The ester was synthesized using a procedure reported by Cai and co-workers via a copper mediated condensation/coupling/deformylation cascade process from 2-bromonicotinaldehyde (**57**) with ethyl isocyanoacetate (**58**).[24] *N*1-Alkylation using potassium carbonate and 1-bromopentane and base mediated ester hydrolysis gave the carboxylic acid **61**. Finally an EDCI-HOBt mediated amide coupling gave the desired amide **30**.



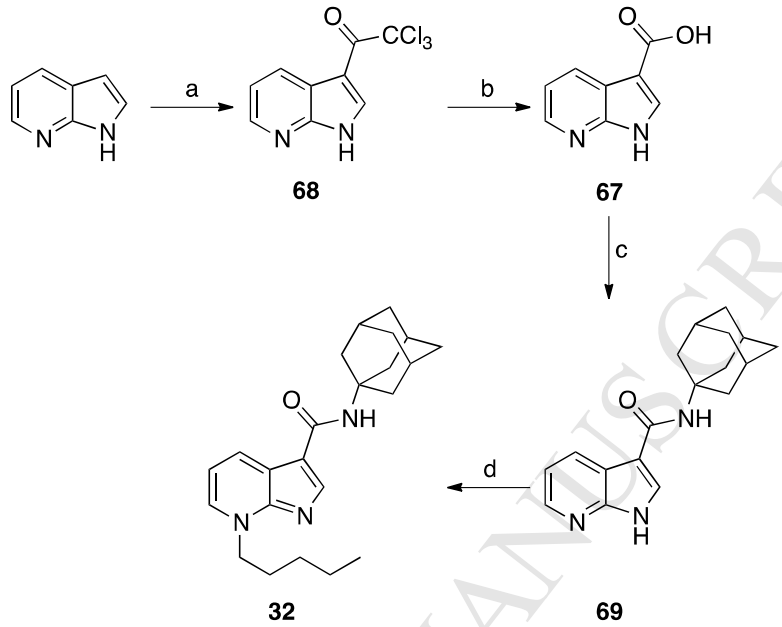
Scheme 3: Synthesis of 2-amidoalkyl-7-azaindole derivative **30.** *Reagents and conditions:* (a) LDA, DMF, THF, -78 °C-rt, 2 hours; (b) i) Ethyl formate, Et_3N, 60 °C, 16 hours; ii) $POCl_3$, Et_3N, CH_2Cl_2, 0 °C-rt, 1 hour; (c) CuI, Cs_2CO_3, DMSO, 80 °C, 16 hours; (d) K_2CO_3, 1-bromopentane, 50 °C, 16 hours; (e) NaOH (aq.), EtOH, reflux, 2 hours; (f) 1-Adamantylamine, EDCI, HOBt, *i*Pr_2EtN, DMF, rt, 16 hours.

3-Amidoalkylpyrazolo[3,4-*b*]pyridine derivative **31** was prepared from commercially available 3-acetylpyridine (**Scheme 4**). Regioselective chlorination at the 2-position of the pyridine was achieved from the *N*-oxide **62** using phosphorus oxychloride. Subsequent condensation with methyl hydrazine formed the pyrazolo[3,4-*b*]pyridine nucleus (**64**). *N*1-Alkylation with sodium hydride and 1-bromopentane followed by oxidation of the 3-methyl group using potassium permanganate gave carboxylic acid **66**. An EDCI-HOBt mediated amide coupling afforded the desired amide **31**.



Scheme 4: Synthesis of 3-amidoalkylpyrazolo[3,4-*b*]pyridine derivative **31**. *Reagents and conditions*: (a) *m*-CPBA, CH_2Cl_2, rt, 16 hours; (b) $POCl_3$, 100 °C, 2 hours; (c) Ethylene glycol, 165 °C, 16 hours; (d) NaH, 1-bromopentane, 0 °C-rt, 16 hours; (e) $KMnO_4$, NaOH (aq.), H_2O, 90 °C, 2 hours; (f) 1-Adamantylamine, EDCI, HOBt, *i*Pr_2EtN, DMF, rt, 16 hours.

N-7-Alkyl derivative **32** was formed from 3-amido-7-azaindole **69** by a regioselective alkylation under neutral conditions after basic workup (**Scheme 5**). The regiochemistry of the alkylation was confirmed by NOESY correlation between the C6 proton of the azaindole nucleus and the methylene protons adjacent to the nitrogen of the pentyl chain (see Supplementary Information).



Scheme 5: Synthesis of 3-aminoalkyl-7-azaindole derivative substituted at the 7-position **32**. *Reagents and conditions*: (a) $AlCl_3$, Cl_3COCl, CH_2Cl_2, 0 °C-rt, 4 hours; (b) NaOH (aq.), rt, 6 hours; (c) 1-Adamantylamine, EDCI, HOBt, *i*Pr_2EtN, DMF, rt, 16 hours; (d) 1-Bromopentane, TBAI, MeCN, PhMe, 150 °C, 16 hours.

2.2 Functional activity at the CB_1 and CB_2 receptors

The synthetic cannabinoid analogues **8**-**32** and lead compound **7** were assessed for their ability to activate CB_1 and CB_2 receptors. The activity of **7**-**32** was assessed in AtT-20 neuroblastoma cells stably transfected with human CB_1 or CB_2 receptors using a FLIPR membrane potential assay which measures activation of endogenously expressed G protein-gated inwardly rectifying K^+ channels (GIRKs) by CB_1 or CB_2 agonists. Compounds displaying more than 50% activation at 10 μM in the assay were evaluated further in dose–response studies. Their half maximal effective concentrations (EC_{50}) and maximal effect relative to 1 μM CP 55,940 (E_{max}) were calculated as listed in **Tables 1** and **2**. Further experimental details are provided in the Experimental Section.

In agreement with previously reported data, 3-amidoalkylindole APICA (**7**) exhibited potent functional activity at both the CB_1 and CB_2 receptors (**Table 1**).[18] The simple structural modification of moving the amide from the 3-position to the 2-position to afford 2-amidoindole **8** was found to completely supress activity at the CB_1 receptor.[22] Due to its high lipophilicity, we were unable to solubilise the compound in the assay medium at high concentrations (numerous solvent mixtures were trialled, but reliable results were not able to be obtained). It appeared from the limited data obtained that the compound is a partial agonist with low micromolar activity, similar to what has previously been reported.[22] The poor aqueous solubility of **8** highlights the need to develop more drug-like compounds using this strategy. It was envisioned that amino acid derived amides and cumyl amides **9**-**19** would retain not only the selectivity

observed for adamantyl derivative **8**, but also the high potency of their respective 3-amidoalkylindole analogues. As suspected, the receptor subtype selectivity was retained and improved sub-micromolar potency was observed, particularly for *tert*-leucinamide analogue **16** (CB_2 EC_{50} = 189 nM). Unfortunately for **11** and cumyl amide **18,** EC_{50} values could not be generated for activity at the CB_2 receptor due to poor solubility at high concentrations. The tolerance of amide substitution suggests this is a general strategy for imparting CB_2 selectivity and further optimisation of the amide and *N*-alkyl chain could further improve potency and physicochemical properties. Interestingly, 5-hydroxypentyl derivatives **10**, **12**, **15** and **17** exhibited no activity at either the CB_1 or CB_2 receptors, while cumyl amide derivative **19** retained selectivity and exhibited improved potency (CB_2 EC_{50} = 217 nM) compared to the pentyl derivative **18**. It is possible that intramolecular hydrogen bonding between the hydroxyl group and polar amino acid derived amide substituents (but not the non-polar cumyl or adamantyl amides) is unfavourable for CB_2 activation. Further exploration of other polar alkyl derivatives that would not participate in such hydrogen bonding interactions could yield more potent compounds. As fluorine is a weak hydrogen bond acceptor[25] and based on data for similar compounds, difluoromethyl analogue **13** was synthesized.[22] Introduction of the polar group reduced activity at the CB_2 receptor, but not completely, as was the case for the 5-hydroxyl analogue **12**. It is possible that interactions between the difluoromethyl group and polar amino acid derived amide still affords an unfavourable conformation for CB_2 activation. Further exploration of the *N*-alkyl moiety is required to determine the optimal functionality for potent CB_2 activity.

Table 1: Agonist activities of 2-amidoalkylindoles in AtT-20 cells expressing human CB_1 or CB_2 receptors by a FLIPR membrane potential assay[a]

	CB_1		**CB_2**	
Compound	pEC_{50} ± SEM (EC_{50} μM)	E_{max} (%)	pEC_{50} ± SEM (EC_{50} μM)	E_{max} (%)
7	7.13 ± 0.09 (0.074)	108	7.02 ± 0.08 (0.096)	85
8	NA	ND	DNC	ND
9	NA	ND	6.15 ± 0.11 (0.713)	74
10	NA	ND	NA	ND
11	NA	ND	DNC	ND
12	NA	ND	NA	ND
13	NA	ND	5.26 ± 0.73 (5.46)	128
14	NA	ND	6.13 ± 0.16 (0.741)	74
15	NA	ND	NA	ND
16	NA	ND	6.72 ± 0.12 (0.189)	73
17	NA	ND	NA	ND
18	NA	ND	DNC	ND
19	NA	ND	6.66 ± 0.09 (0.217)	88
CP 55,940	7.66 ± 0.04 (0.022)	101	7.20 ± 0.04 (0.063)	106

[a]See experimental section for more details. Data represent mean values ± SEM from at least three independent experiments each performed in duplicate, with CP 55,940 used as a positive control. [b]NA: Not active, defined as <50% activation at 10 μM in the assay. [c]ND: Not determined; for compounds defined as not active, their maximal effects

were not determined. [d]DNC: Did not converge; data does not fit a reliable curve to generate EC_{50} values (due to solubility issues at high concentration).

Our recent report suggests 3-amidoalkyl-7-azaindole **20** is a potent, CB_2 selective agonist.[23] In this study receptor subtype selectivity (roughly 3.5-fold) was also observed (**Table 2**). This selectivity however was not observed for amino acid derived amides **22**, **23**, **25** and **26** and cumyl amide **28,** which all exhibited potent activity at both receptor subtypes. 3-Amidoalkylpyrazolo[3,4-*b*]pyridine derivative **31** exhibited improved receptor subtype selectivity with a roughly 7-fold preference for CB_2 activation. Hybrid 2-amidoalkyl-7-azaindole **30** exhibited no activity at either receptor and pseudo-azulene **32** was also inactive. Fortuitously, 5-hydroxypentyl derivative **21** exhibited complete receptor subtype selectivity. With this positive result in hand we investigated whether other amide substitutions would exhibit similar selectivity. We therefore synthesized the 5-hydroxy derivatives of the two most potent 3-amidoalkyl-7-azaindole compounds **23** and **25** and also cumyl amide derivative **28** with the expectation that their potency at the CB_2 receptor would be retained with improved receptor subtype selectivity. The 5-hydroxypentyl analogues **24**, **27** and **29** were potent agonists at the CB_2 receptor with vastly improved subtype selectivity however, exhibited some activity at CB_1. Adopting this approach for the corresponding 3-amidoalkylpyrazolo[3,4-*b*]pyridine derivatives may yield even more selective compounds.

Table 2: Agonist activities of azaindole derivatives in AtT-20 cells expressing human CB_1 or CB_2 receptors by a FLIPR membrane potential assay[a]

	CB_1		**CB_2**	
Compound	pEC_{50} ± SEM (EC_{50} μM)	E_{max} (%)	pEC_{50} ± SEM (EC_{50} μM)	E_{max} (%)
20	6.82 ± 0.14 (0.152)	75	7.37 ± 0.08 (0.043)	101
21	NA	ND	7.31 ± 0.10 (0.049)	93
22	7.25 ± 0.10 (0.056)	97	7.84 ± 0.07 (0.015)	98
23	8.77 ± 0.06 (0.0017)	103	8.37 ± 0.09 (0.0043)	102
24	6.63 ± 0.12 (0.234)	99	7.72 ± 0.12 (0.019)	96
25	6.92 ± 0.14 (0.121)	117	7.23 ± 0.12 (0.058)	103
26	8.40 ± 0.06 (0.004)	110	8.29 ± 0.08 (0.005)	94
27	6.31 ± 0.08 (0.491)	98	7.30 ± 0.11 (0.050)	96
28	7.45 ± 0.09 (0.035)	106	6.98 ± 0.13 (0.104)	96
29	6.33 ± 0.12 (0.466)	110	6.82 ± 0.18 (0.152)	95
30	NA	ND	NA	ND
31	6.08 ± 0.10 (0.824)	97	6.92 ± 0.10 (0.122)	104
32	NA	ND	NA	ND
CP 55,940	7.66 ± 0.04 (0.022)	101	7.20 ± 0.04 (0.063)	106

[a]See experimental section for more details. Data represent mean values ± SEM from at least three independent experiments each performed in duplicate, with CP 55,940 used as a positive control. [b]NA: Not active, defined as

<50% activation at 10 μM in the assay. [c]ND: Not determined; for compounds defined as not active, their maximal effects were not determined.

2.3 In silico studies

Relatively small structural modifications can have profound effects on the binding affinity and functional profile of GPCR ligands. We performed docking simulations to investigate the molecular basis for the varied functional activity of the investigated compounds at the CB_1 and CB_2 receptors. A caveat to these calculations is that the inactive cannabinoid receptor structures, solved with an antagonist bound, are not ideal for predicting agonist interactions.[26-27] The manner in which agonists bind to the orthosteric site of the CB_1 receptor has been investigated by integrating docking studies, mutagenesis studies and SAR data.[28-30] Hua and co-workers have docked known CB_1 receptor agonists, including indole JWH-018, into the inactive state crystal structure and in combination with mutation studies suggest that interactions with Phe268 and Phe379 are important for functional activity.[27] Similar studies were conducted based on the active state agonist-bound crystal structure of the CB_1 receptor and found that π-π interactions with Phe177, Phe189, Phe268 and Phe379 and hydrogen bonding with Ser383 were conserved for the agonist-bound complexes.[31]

It has been extensively reported that CB_1 seems to use an extended molecular toggle switch involving a synergistic conformational change between Phe200 and Trp356 for receptor activation.[31] Agonists disrupt the π–π stacking of the side chains of Phe200 and Trp356 leading to conformational change. It is difficult however to probe the interaction of an agonist with these residues using the inactive state of the receptor.

Compounds **7**, **8**, **20** and **21** were chosen as representative compounds based on their differing receptor subtype selectivities. Docking and ΔG binding scores obtained by combining molecular mechanics (MM) terms with a generalised Born and surface area (GBSA) solvent mode (MM-GBSA)[32] are summarised in the Supplementary Information (Table S1).

APICA (**7**) is a non-selective agonist and this is reflected in the docking scores (difference of 0.09 kcal mol^{-1}) and MM-GBSA ΔG_{Bind} values (difference of 5.57 kcal mol^{-1}). At the CB_1 receptor the compound has π–π interactions with Phe379 and a hydrogen bonding interaction with Ser383, which as previously mentioned are believed to be important interactions for functional activity (See Supplementary Information, **Figure S1**). At the CB_2 receptor the agonist has π–π interactions with Phe91 and Phe183. The importance of these residues for binding have been confirmed by Feng and co-workers via site-directed mutation studies.[33]

Movement of the amide from the 3-positon to the 2-position of the indole moiety has been shown to eradicate functional activity at the CB_1 receptor. This is consistent with this docking study where a more positive MM-GBSA ΔG_{Bind} value (-55 kcal mol^{-1}) is observed for **8** compared to **7** (-73.35 kcal mol^{-1}). Higher receptor and ligand strain are also observed for this regioisomer at the CB_1 receptor. Importantly the hydrogen bonding interaction with key residue Ser383 was not observed, potentially explaining the eradication of functional activity (**Figure 4, C**). At the CB_2 receptor similar docking scores and MM-GBSA ΔG_{Bind} values (differences of 1.07 kcal mol^{-1} and 9.95 kcal mol^{-1}

respectively) were observed for the two regioisomeric compounds. Further, for **8** the indole nucleus exhibits π-π interactions with Phe94 and the carbonyl oxygen of the amide has a hydrogen bonding interaction with Ser285 (**Figure 4, D**). Li and co-workers suggest mutations of Phe94 greatly reduces the potency of CB_2 agonists,[26] while Feng and co-workers also suggest Ser285 is an important residue for ligand binding.[33]

It was found that for the azaindole scaffold, terminal hydroxylation of the *N*-1 pentyl chain precluded CB_1 functional activity. Compound **20**, a non-selective agonist, had a similar docking score and MM-GBSA ΔG_{Bind} value to **7** at both the CB_1 and CB_2 receptors, consistent with their similar functional activity data. Similar to **7** π–π interactions with Phe91 and Phe183 were observed at the CB_2 receptor (See Supplementary Information, **Figure S2**).

In comparison, **21** displayed a similar docking score and MM-GBSA ΔG_{Bind} value at the CB_2 receptor to **20**. However, the preference for CB_2 becomes quite clear when examining the MM-GBSA ΔG_{Bind} value (CB_1: -60.23 kcal mol^{-1}, CB_2 -71.63 kcal mol^{-1}) and particularly the receptor strain (30.51 kcal mol^{-1}) at the CB_1 receptor. Although this binding mode interacts with a number of important residues at CB_1 (Phe102, Phe379, Ser128 and Ser383), the high receptor strain suggests this is not a favourable pose (See Supplementary Information, **Figure S3**). For the CB_2 receptor **21** shares π–π interactions with Phe91 and Phe183 as well as hydrogen bonding interactions with His95 and Lys278.

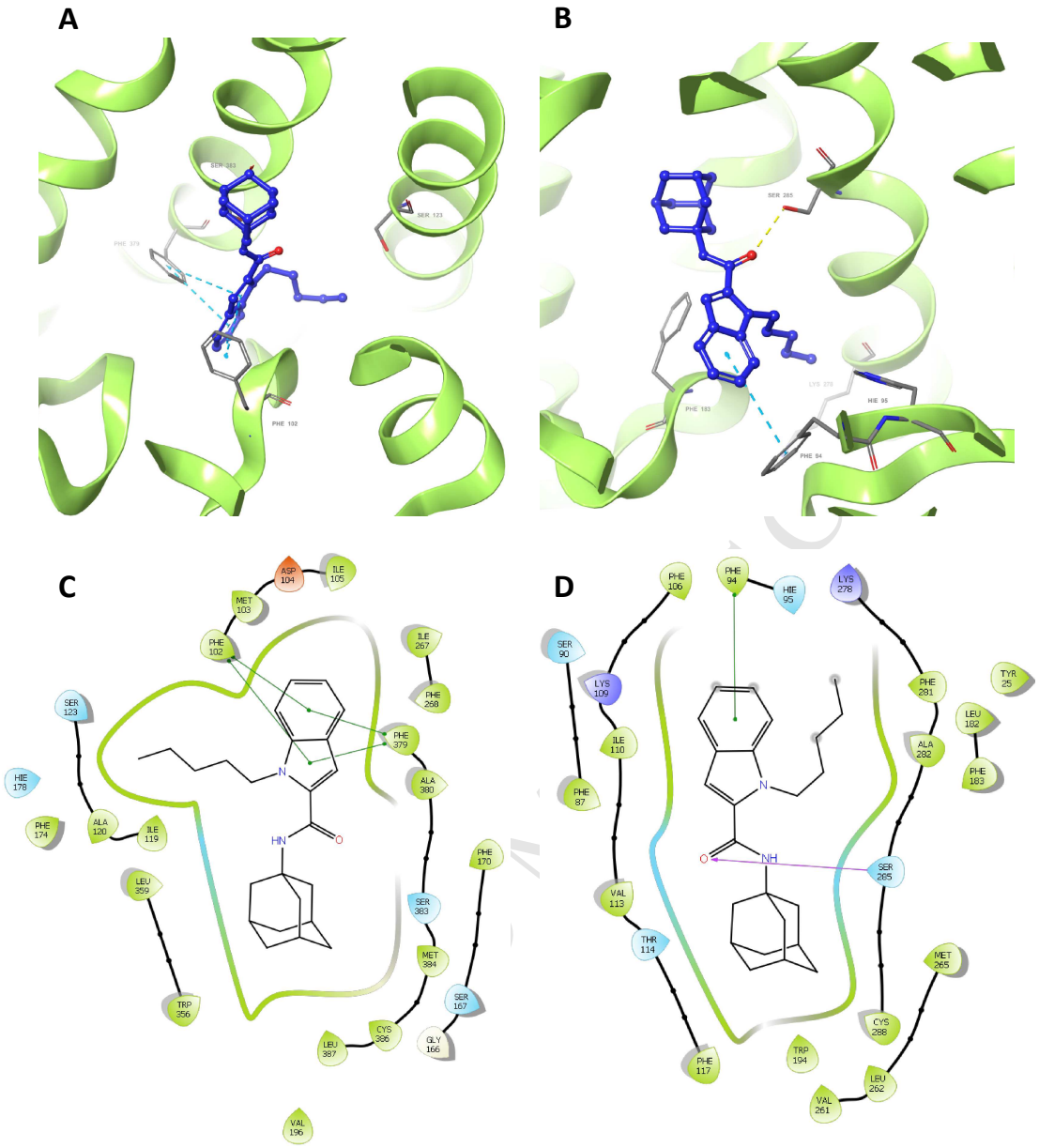


Figure 4: Docking mode of 2-amidoalkylindole **8** in the: (a) CB_1 receptor active site, and (b) CB_2 receptor active site. The π-π interactions are shown with blue dashed lines and hydrogen bonding is shown with yellow dashed lines; 2D representation of **8** in the: (c) CB_1 receptor active site, and (d) CB_2 receptor active site. The π-π interactions are shown with solid green lines and hydrogen bonding is shown with purple arrows.

With the aim of developing drug-like compounds, we performed *in silico* predictions of physiochemical and pharmacokinetic properties. The calculated properties of **16** and **21** were predominantly within the recommended values as suggested by QikProp (Schrödinger), and with similar values to 95% of known drugs (**Table 3**). Solubility issues are widely recognised in cannabinoid pharmacology because of the lipophilic nature of cannabinoid ligands.[34] Of particular note, substitution of the adamantyl group for a polar amino acid derived amide resulted in a significant drop in the calculated LogP value (6.25 for **7** and 3.66 for **16**). Further, the 7-azaindole scaffold with 5-hydroxypentyl group also resulted in a significant reduction in calculated LogP (6.25 for **7** and 4.38 for **21**).

Table 3: Calculated physicochemical and pharmacokinetic properties of lead compound **7** and analogues **16** and **21**.

Calculated properties [a]	MW (g/mol)	LogP	PSA (Å²)	Oral Absorption (%)	QPPCaco (nm/sec)	QPPMDCK (nm/sec)	QPlogBB
	Recommended Values			Range for 95% of known drugs (Schrödinger)			
	<450	<5	<70	<25% poor >80% high	<25 poor >500 great	<25 poor >500 great	-3.0 to -1.2
7	364.53	6.25	34.80	100	4948	2786	-0.13
16	343.47	3.66	80.86	100	628	568	-0.88
21	381.52	4.38	66.97	100	1325	671	-0.82

[a] Physicochemical properties (molecular weight, MW; lipophilicity, log P; polar surface area, PSA) and pharmacokinetic properties (Oral Absorption; Predicted Apparent Caco-2 Permeability, QPPCaco; Predicted apparent MDCK cell permeability, QPPMDCK; Predicted Brain/Blood Partition Coefficient, QPlogBB) were calculated with QikProp, Schrödinger v5.9 software.

3. Conclusions

We report herein the development of selective CB_2 receptor agonists from non-selective synthetic cannabinoid designer drugs. A small library of 2-amidoalkylindoles and 7-azaindole derivatives were synthesized, characterized and their activity at the cannabinoid receptors evaluated. It has been demonstrated that simple structural modifications can impart receptor subtype selectivity and here we have increased the scope of these strategies to include analogues of some of the most potent synthetic cannabinoids known. In addition, the compounds developed have improved physicochemical properties, more suitable for drug development. Substitution of the amide of 2-amidoalkylindoles has been shown to be a strategy to improve potency and optimise physicochemical properties. Bioisosteric replacement of the indole core for the 7-azaindole scaffold, in conjunction with the addition of a polar hydroxyl group at the terminal position of the pentyl chain has shown to be another strategy to significantly supress CB_1 activity. Molecular modelling results corroborated the selectivity and potency data gained from activity measurements, and this analysis recapitulated previous literature implicating significant residues at both receptors. The structure-activity relationships developed will facilitate the design of novel CB_2 agonists. Future work will focus on the optimisation of the *N*-alkyl group to further improve potency and physical properties.

4. Experimental Section

4.1 Chemistry

4.1.1 General Methods

Compound **7** was previously prepared according to the procedures reported by Banister et al.[18] Compound **20** was previously prepared according to the procedures reported by Longworth et al.[23] All reactions were performed under an atmosphere of nitrogen unless otherwise specified. Anhydrous *N,N*-dimethylformamide, dichloromethane and tetrahydrofuran were obtained from a PureSolv MD 7 solvent purification system (Innovative Technology, Inc.). All other solvents and reagents were used as received from commercial sources. Analytical thin-layer chromatography (TLC) was performed using Merck aluminium backed silica gel 60 F_{254} (0.2 mm) plates, which were visualised with shortwave (254 nm) ultraviolet light. Products were also

visualised with potassium permanganate, vanillin, cerium molybdate, bromocresol green or ninhydrin stains. Flash column chromatography was performed using Merck Kieselgel 60 (230-400 mesh) silica gel, with the eluent mixture reported as the volume:volume ratio. Melting points were measured in open capillaries using a Stanford Research System Optimelt Automated melting point apparatus. Infrared absorption spectra were recorded on a Bruker ALPHA FT-IR spectrometer as a solid or a thin film from ethanol, and the data are reported as vibrational frequencies (cm^{-1}). Nuclear magnetic resonance spectra were recorded at 300 K using either a Bruker AVANCE DRX300 (300 MHz) or AVANCE DRX400 (400 MHz) spectrometer. ^{1}H Chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 7.26), methanol (δ 3.31) and dimethyl sulfoxide (δ 2.50) as reference and are reported as chemical shift (δ); relative integral; multiplicity (s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, t= triplet, tt = triplet of triplets, q = quartet, quin = quintet, sept = septet, m = multiplet); coupling constants (*J*) reported in Hz. ^{13}C chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 77.16), methanol (δ 49.00) and dimethyl sulfoxide (δ 39.52) as reference and reported as chemical shift (δ); multiplicity; coupling constants (*J*) reported in Hz. Proton decoupled ^{19}F chemical shifts are reported as parts per million (ppm). Low-resolution mass spectrometry (LRMS) was recorded using electrospray ionisation (ESI) recorded on a Bruker AmaZon SL ion trap spectrometer. High-resolution mass spectrometry was performed on a Bruker Apex Qe 7T Fourier Transform Ion Cyclotron Resonance mass spectrometer equipped with an Apollo II ESI/MALDI dual source. Samples were run with syringe infusion at 150 µL/hr on a Cole Palmer syringe pump into the electrospray ionisation (ESI) source. Optical rotation was performed on an Optical Activity AA-65 Automatic Polarimeter. Specific rotations based on the equation $[\alpha] = (100\alpha)/(lc)$ are reported as unitless numbers and are in the form: $[\alpha]_D^T$ ±XX (c, solvent), where c is the concentration in g/l00 mL, l is the path length in decimetres, T is the temperature in °C and D refers to the sodium Fraunhofer line at 589 nm. High performance liquid chromatography (HPLC) analysis of organic purity was conducted on a Waters Alliance 2695 instrument using a SunFire™ C18 column (5 µm, 2.1 x 150 mm) and detected using a Waters 2996 photodiode array (PDA) detector set at 230 nm. Separation was achieved using water + 0.1% trifluoroacetic acid (solvent A) and acetonitrile + 0.1% trifluoroacetic acid (solvent B) at a flow rate of 0.2 mL/min and a gradient of 0% B to 100% B over 30 minutes. HPLC data is reported as percentage purity and retention time (RT) in minutes.

4.1.2 General Procedures

General Procedure A: N-1 Alkylation of indole and 7-azaindole derivatives

To a solution of the appropriate *NH*-aromatic heterocycle (1.0 mmol) in anhydrous *N,N*-dimethylformamide (3 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 60 mg, 1.5 eq., 1.5 mmol) and the mixture stirred for 5 minutes before the appropriate alkyl halide (1.2 eq.) in *N,N*-dimethylformamide (1-3 mL) was added dropwise. The mixture was stirred until the starting material was consumed, as determined by thin-layer chromatography. The reaction mixture was quenched with saturated aqueous ammonium chloride (20 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with water (3 x 20 mL) and aqueous

lithium chloride (1 M, 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

General Procedure B: Hydrolysis of Ethyl Esters

To a solution of the ester (1.0 mmol) in a mixture of tetrahydrofuran and methanol (1:1, 3 mL) was added aqueous sodium hydroxide (3.3 mL, 3 M, 10 eq., 10 mmol) and the mixture was stirred at room temperature or reflux until the starting material was consumed, as determined by thin-layer chromatography. The volatiles were removed under a stream of nitrogen and the aqueous residue washed with dichloromethane (10 mL) and acidified with aqueous hydrochloric acid (6 M). The aqueous suspension was extracted with dichloromethane (3 x 10 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

General Procedure C: EDCI-HOBt mediated amide coupling

The appropriate carboxylic acid (1.0 mmol), appropriate amine (1.2 eq.), hydroxybenzotriazole (203 mg, 1.5 eq., 1.5 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (288 mg, 1.5 eq., 1.5 mmol) and *N*,*N*-diisopropylethylamine (871 µL, 5 eq., 5.0 mmol) were dissolved in *N*,*N*-dimethylformamide (5 mL) and the mixture stirred for 24 hours. The reaction mixture was diluted with saturated aqueous sodium hydrogen carbonate (30 mL), extracted with ethyl acetate (3 x 20 mL), washed with saturated aqueous sodium hydrogen carbonate (30 mL), saturated aqueous ammonium chloride (30 mL) and aqueous lithium chloride (1 M, 30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

General Procedure D: Hydrogenolysis of benzyl ether protecting groups

To a solution of the benzyl ether (1.0 mmol) in ethyl acetate (5 mL) and acetic acid (1-10 drops) was added palladium on carbon (10% w/w, 106 mg, 0.1 eq., 100 µmol) under an atmosphere of nitrogen. The reaction vessel was evacuated and flushed with hydrogen three times and the mixture stirred for 16-48 hours. The suspension was filtered through a pad of Celite® and the filtrate concentrated under reduced pressure.

4.1.3 Synthetic Methods

Ethyl 1-pentyl-1H-indole-2-carboxylate **38**
General procedure A was followed using ethyl indole-2-carboxylate (3.0 g, 15.9 mmol) and 1-bromopentane (2.4 mL, 19.1 mmol) to obtain the title compound **38** (4.2 g, 100%) as a light yellow oil, $\boldsymbol{R_f}$: 0.74 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 2928, 1707, 1518, 1464, 1245, 1222, 1188, 1135, 1092, 745 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 0.75-0.88 (3H, m), 1.19-1.42 (4H, m), 1.33 (3H, t, *J* = 7.1 Hz), 1.64-1.82 (2H, m), 4.30 (2H, q, *J* = 7.1 Hz), 4.42-4.56 (2H, m), 7.06 (1H, ddd, *J* = 8.0, 6.7, 1.2 Hz), 7.17-7.41 (3H, m), 7.56-7.65 (1H, m) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): 14.2, 14.5, 22.6, 29.3, 30.5, 44.9, 60.6, 110.5, 110.6, 120.5, 122.7, 124.9, 126.1, 127.6, 139.2, 162.1 ppm; **LRMS** (+ESI) *m/z*: 282.2 ($[M+Na]^+$ 100%).

Ethyl 1-(5-(benzyloxy)pentyl)-1H-indole-2-carboxylate **39**

General procedure A was followed using ethyl indole-2-carboxylate (3.0 g, 15.9 mmol) and alkyl bromide **34** (4.1 g, 15.9 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent to obtain the title compound **39** (4.6 g, 76%) as a light yellow oil, R_f: 0.55 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 2928, 1707, 1454, 1246, 1178, 1092, 745 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 1.31 (3H, t, *J* = 7.1 Hz), 1.30-1.43 (2H, m), 1.50-1.63 (2H, m), 1.67-1.80 (2H, m), 3.35 (2H, t, *J* = 6.4 Hz), 4.27 (2H, q, *J* = 7.1 Hz), 4.38 (2H, s), 4.43-4.52 (2H, m), 7.04 (1H, ddd, *J* = 8.0, 6.6, 1.3 Hz), 7.14-7.33 (8H, m), 7.53-7.62 (1H, m) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): 14.5, 23.8, 29.6, 30.6, 44.8, 60.6, 70.3, 73.0, 110.5, 110.6, 120.5, 122.7, 124.9, 124.9, 126.1, 127.6, 127.6, 127.7, 128.4, 138.7, 139.1, 162.1 ppm; **LRMS** (+ESI) *m/z*: 388.2 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{23}H_{27}NO_3$ $[M+Na]^+$: 388.18886, found 388.18814.

1-Pentyl-1H-indole-2-carboxylic acid **40**

General procedure C was followed using ethyl ester **38** (4.0 g, 15.4 mmol) to obtain the title compound **40** (3.4 g, 94%) as a white solid, **mp**: 108.2-111.4 °C; **IR** (ν_{max} (neat)): 3032, 2952, 2859, 2601, 1677, 1519, 1426, 1265, 1225, 1133, 910, 740 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 0.70-0.90 (3H, m), 1.16-1.37 (4H, m), 1.65-1.83 (2H, m), 4.50 (2H, t, *J* = 7.4 Hz), 7.00-7.12 (1H, m), 7.22-7.45 (3H, m), 7.54-7.66 (1H, m) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): 14.1, 22.6, 29.2, 30.5, 45.0, 110.5, 112.9, 120.8, 123.1, 125.7, 126.1, 126.3, 139.8, 167.2 ppm; **LRMS** (-ESI) *m/z*: 230.0 ($[M-H]^-$ 100%).

1-(5-(Benzyloxy)pentyl)-1H-indole-2-carboxylic acid **41**

General procedure B was followed using ethyl ester **39** (4.0 g, 10.9 mmol) at reflux for 3 hours. The crude product was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound **41** (3.7 g, 100%) as a viscous yellow oil, R_f: 0.30 (1:49 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 2921, 2859, 2588, 1677, 1519, 1237, 1093, 903, 728 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 1.39-1.61 (2H, m), 2.84 (2H, quin, *J* = 6.7 Hz), 1.89 (2H, quin, *J* = 7.7 Hz), 3.51 (2H, t, *J* = 6.3 Hz), 4.54 (2H, s), 4.58-4.68 (2H, m), 7.20 (1H, ddd, *J* = 7.9, 6.3, 1.5 Hz), 7.26-7.54 (8H, m), 7.75 (1H, d, *J* = 8.1 Hz) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): 23.7, 29.6, 30.5, 44.8, 70.2, 73.0, 110.8, 112.9, 120.8, 123.1, 125.7, 126.0, 126.3, 127.6, 127.8, 128.5, 138.6, 139.8, 167.0 ppm; **LRMS** (+ESI) *m/z*: 360.2 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{21}H_{23}NO_3$ $[M+Na]^+$: 360.15756, found 360.15681.

N-(-Adamantan-1-yl)-1-pentyl-1H-indole-2-carboxamide **8**

General procedure C was followed using carboxylic acid **40** (120 mg, 520 μmol) and 1-adamantylamine (94 mg, 620 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:19) as an eluent and recrystallization from dichloromethane, hexane to obtain the title compound **8** (190 mg, 77%) as a white solid, the spectroscopic data of which corresponded to previously described.[22] **mp**: 163.3-163.7 °C; R_f: 0.41 (1:19 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3290, 2906, 2853, 1632, 1535, 1454, 1343, 1280, 1220, 808, 747, 730 cm^{-1}; **LRMS** (+ESI) *m/z*: 387.3 ($[M+Na]^+$ 100%), 751.4 ($[2M+Na]^+$ 24%); **HRMS** $(ESI)^+$ Cald for $C_{24}H_{32}N_2O$ $[M+Na]^+$: 387.24123, found 387.24047; **HPLC**: 99.8%, RT: 33.2 mins.

(S)-Methyl 3-methyl-2-(1-pentyl-1H-indole-2-carboxamido)butanoate **9**

General procedure C was followed using carboxylic acid **40** (200 mg, 870 μmol) and methyl-L-valinate hydrochloride (170 mg, 1.0 mmol). The crude product was purified by

flash column chromatography using ethyl acetate, hexane (1:9) as an eluent and recrystallization from hexane to obtain the title compound **9** (190 mg, 64%) as a colourless solid, **mp**: 72.9-73.8 °C; R_f: 0.69 (2:3 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3365, 3282, 3195, 2957, 1650, 1630, 1530, 1460, 1303, 748 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.83-0.91 (3H, m), 1.00 (3H, d, *J* = 6.9 Hz), 1.04 (3H, d, *J* = 6.9 Hz), 1.25-1.37 (4H, m), 1.74-1.86 (2H, m), 2.24-2.36 (1H, m), 3.79 (3H, s), 4.48-4.56 (2H, m), 4.76 (1H, dd, *J* = 8.9, 5.0 Hz), 6.66 (1H, d, *J* = 8.9 Hz), 6.95 (1H, s), 7.14 (1H, ddd, *J* = 7.9, 6.9, 1.0 Hz), 7.31 (1H, ddd, *J* = 8.4, 6.9, 1.2 Hz), 7.37-7.42 (1H, m), 7.65 (1H, dt, *J* = 8.0, 1.0 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.1, 18.1, 19.2, 21.6, 29.3, 30.5 , 31.8, 44.7, 52.4, 57.2, 104.5, 110.6, 120.6, 122.1, 124.2, 126.2, 131.4, 138.6, 162.5, 172.6 ppm; **LRMS** (+ESI) *m/z*: 352.2 ($[M+Na]^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{20}H_{28}N_2O_3$ $[M+Na]^+$: 367.19976, found 367.19903; **$[\alpha]_D^{27}$**: +35.2 (0.62, $CHCl_3$); **HPLC**: 95.8%, RT: 31.1 mins.

(S)-Methyl 3,3-dimethyl-2-(1-pentyl-1H-indole-2-carboxamido)butanoate **11**
General procedure C was followed using carboxylic acid **40** (250 mg, 1.1 mmol) and amine hydrochloride salt **37** (230 mg, 1.3 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:19) as an eluent to obtain the title compound **11** (210 mg, 54%) as a colourless oil, R_f: 0.42 (1:19 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3256, 2952, 1737, 1633, 1622, 1536, 1251, 1014, 741 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.83-0.89 (3H, m), 1.07 (9H, s), 1.26-1.37 (4H, m), 1.79 (2H, quin, *J* = 7.7 Hz), 3.78 (3H, s), 4.49-4.55 (2H, m), 4.67 (1H, d, *J* = 9.6 Hz), 6.70 (1H, d, *J* = 9.5 Hz), 6.93 (1H, d, *J* = 0.8 Hz), 7.14 (1H, ddd, *J* = 7.9, 6.9, 1.0 Hz), 7.31 (1H, ddd, *J* = 8.4, 6.9, 1.2 Hz), 7.37-7.42 (1H, m), 7.65 (1H, dt, *J* = 8.0, 1.0 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.1, 22.6, 26.8, 29.3, 30.5, 35.4, 44.8, 52.1, 60.0, 104.4, 110.6, 120.6, 122.0, 124.2, 126.2, 131.5, 138.6, 162.3, 172.2 ppm; **LRMS** (+ESI) *m/z*: 381.2 ($[M+Na]^+$ 100%), 739.3 ($[2M+Na]^+$ 43%); **HRMS** (ESI)$^+$ Cald for $C_{21}H_{30}N_2O_3$ $[M+Na]^+$: 381.21541, found 381.21466; **$[\alpha]_D^{27}$**: +21.1 (1.42, $CHCl_3$); **HPLC**: 99.3%, RT: 31.7 mins.

(S)-N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indole-2-carboxamide **14**
General procedure C was followed using carboxylic acid **40** (120 mg, 520 μmol) and L-valinamide hydrochloride (95 mg, 620 μmol). The crude product was purified by recrystallization from ethyl acetate to obtain the title compound **14** (170 mg, 100%) as a white solid, **mp**: 246.1-248.9 °C; R_f: 0.55 (9:1 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3365, 3282, 3195, 2957, 1650, 1630, 1530, 1460, 1303, 748 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 0.80 (3H, t, *J* = 7.1 Hz), 0.93 (3H, d, *J* = 6.8 Hz), 0.95 (3H, d, *J* = 6.8 Hz), 1.11-1.29 (4H, m), 1.65 (2H, quin, *J* = 7.4 Hz), 2.11 (1H, sept, *J* = 6.9 Hz), 4.26 (1H, dd, *J* = 8.9, 7.5 Hz), 4.51 (2H, t, *J* = 7.3 Hz), 7.05-7.13 (2H, m), 7.16 (1H, s), 7.25 (1H, ddd, *J* = 8.3, 6.9, 1.2 Hz), 7.44 (1H, bs), 7.53 (1H, dd, *J* = 8.4, 0.9 Hz), 7.64 (1H, d, *J* = 7.9 Hz), 8.14 (1H, d, *J* = 8.9 Hz) ppm; **^{13}C NMR** (100 MHz, DMSO-d_6): δ 13.8, 18.5, 19.5, 21.9, 28.4, 29.9, 30.1, 43.5, 58.3, 105.0, 110.5, 120.0, 121.6, 123.4, 125.7, 131.7, 137.7, 161.8, 172.9 ppm; **LRMS** (+ESI) *m/z*: 352.2 ($[M+Na]^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{19}H_{27}N_3O_2$ $[M+Na]^+$: 352.20010, found 352.19950; **$[\alpha]_D^{27}$**: +70.2 (0.57, DMSO); **HPLC**: 98.8%, RT: 26.9 mins.

(S)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1H-indole-2-carboxamide **16**
General procedure C was followed using carboxylic acid **40** (200 mg, 870 μmol), and amine hydrochloride salt **36** (170 mg, 1.0 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 2:3) as an eluent to obtain the title compound **16** (190 mg, 64%) as a colourless solid, **mp**: 92.9-96.6 °C; R_f: 0.31 (1:1 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3370, 3196, 2955, 1638, 1523, 1399,

1228, 741 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.86 (3H, t, *J* = 6.8 Hz), 1.12 (9H, s), 1.25-1.37 (4H, m), 1.72-1.82 (2H, m), 4.41-4.56 (2H, m), 4.61 (1H, d, *J* = 9.4 Hz), 5.99 (1H, bs), 6.42 (1H, bs), 6.96 (1H, s), 7.00 (1H, d, *J* = 9.5 Hz), 7.13 (1H, ddd, *J* = 7.9, 6.9, 1.1 Hz), 7.30 (1H, ddd, *J* = 8.4, 6.9, 1.2 Hz), 7.35-7.40 (1H, m), 7.61 (1H, dt, *J* = 8.0, 1.0 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.1, 22.6, 26.8, 29.3, 30.5, 35.0, 44.7, 60.0, 104.9, 110.5, 120.7, 122.1, 124.2, 126.3, 131.4, 138.5, 162.6, 172.8 ppm; **LRMS** (+ESI) *m/z*: 366.2 ($[M+Na]^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{20}H_{29}N_3O_2$ $[M+Na]^+$: 366.21575, found 366.21501; **$[\alpha]_D^{27}$**: +48.2 (0.52, DMSO); **HPLC**: 99.6%, RT: 28.1 mins.

1-Pentyl-N-(2-phenylpropan-2-yl)-1H-indole-2-carboxamide **18**
General procedure C was followed using carboxylic acid **40** (150 mg, 650 μmol) and 1-methyl-1-phenylethylamine (110 μL, 780 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent and recrystallization from dichloromethane, hexane to obtain the title compound **40** (130 mg, 58%) as a white solid, **mp**: 122.6-124.1 °C; ***R*$_f$**: 0.55 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3412, 3253, 2928, 1635, 1538, 1457, 729 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.86 (3H, t, *J* = 6.8 Hz), 1.12 (9H, s), 1.20-1.36 (4H, m), 1.74 (2H, quin, *J* = 7.5 Hz), 1.83 (6H, s), 4.47-4.54 (2H, m), 6.50 (1H, bs), 6.87 (1H, d, *J* = 0.8 Hz), 7.14 (1H, ddd, *J* = 8.0, 6.9, 1.0 Hz), 7.23-7.33 (2H, m), 7.33-7.40 (3H, m), 7.46-7.51 (2H, m), 7.64 (1H, dt, *J* = 8.1, 1.0 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.1, 22.6, 26.8, 29.3, 30.5, 35.0, 44.7, 60.0, 104.9, 110.5, 120.7, 122.1, 124.2, 126.3, 131.4, 138.5, 162.6, 172.8 ppm; **LRMS** (+ESI) *m/z*: 371.3 ($[M+Na]^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{23}H_{28}N_2O$ $[M+Na]^+$: 371.20993, found 371.20918; **HPLC**: 99.8%, RT: 31.8 mins.

(S)-Methyl 2-(1-(5-(benzyloxy)pentyl)-1H-indole-2-carboxamido)-3-methylbutanoate **42**
General procedure C was followed using carboxylic acid **41** (300 mg, 930 μmol) and methyl-L-valinate hydrochloride (190 mg, 1.1 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound **42** (260 mg, 62%) as a light yellow oil, ***R*$_f$**: 0.15 (1:9 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3278, 2932, 1738, 1656, 1523, 1455, 1204, 1090, 743 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 1.02 (3H, d, *J* = 6.9 Hz), 1.06 (3H, d, *J* = 6.9 Hz), 1.38-1.53 (2H, m), 1.60-1.74 (2H, m), 1.85 (2H, quin, *J* = 7.7 Hz), 2.20-2.42 (1H, m), 3.46 (2H, t, *J* = 6.5 Hz), 3.81 (3H, s), 4.49 (2H, s), 4.52-4.62 (2H, m), 4.77 (1H, dd, *J* = 8.9, 4.9 Hz), 6.68 (1H, d, *J* = 8.9 Hz), 6.97 (1H, s), 7.17 (1H, ddd, *J* = 7.9, 6.8, 1.1 Hz), 7.25-7.48 (7H, m), 7.68 (1H, dt, *J* = 8.0, 1.0 Hz) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ 18.1, 19.2, 23.8, 29.6, 30.6, 31.8, 44.7, 52.4, 57.2, 70.3, 73.0, 104.6, 110.5, 120.6, 122.1, 123.2, 126.2, 127.6, 127.7, 128.5, 131.3, 138.6, 138.8, 162.4, 172.6 ppm; **LRMS** (+ESI) *m/z*: 473.3 ($[M+Na]^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{27}H_{34}N_2O_4$ $[M+Na]^+$: 473.24163, found 473.24102.

(S)-Methyl 2-(1-(5-(benzyloxy)pentyl)-1H-indole-2-carboxamido)-3,3-dimethylbutanoate **43**
General procedure C was followed using carboxylic acid **41** (300 mg, 930 μmol) and amine hydrochloride salt **37** (200 mg, 1.1 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent to obtain the title compound **43** (170 mg, 40%) as a colourless oil, ***R*$_f$**: 0.25 (1:9 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3260, 2939, 1736, 1639, 1534, 1453, 1085, 742 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 0.99 (9H, s), 1.27-1.43 (2H, m), 1.56 (2H, quin, *J* = 6.7 Hz), 1.75 (2H, quin, *J* = 7.7 Hz), 3.36 (2H, t, *J* = 6.5 Hz), 3.69 (3H, s), 4.39 (2H, s), 4.41-4.50 (2H, m), 4.58 (1H, d, *J* = 9.5 Hz), 6.62 (1H, d, *J* = 9.6 Hz), 6.85 (1H, s), 7.07 (1H, ddd, *J* =

8.0, 6.8, 1.1 Hz), 7.14-7.35 (7H, m), 7.57 (1H, d, *J* = 7.9 Hz) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ 23.8, 26.8, 29.7, 30.6, 35.3, 44.7, 52.1, 60.0, 70.3, 73.0, 104.5, 110.6, 120.6, 122.1, 124.3, 126.2, 127.6, 127.7, 128.5, 131.4, 138.6, 138.8, 162.2, 172.1 ppm; **LRMS** (+ESI) *m/z*: 487.3 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{28}H_{36}N_2O_4$ $[M+Na]^+$: 487.25728, found 487.25661.

(S)-N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-(benzyloxy)pentyl)-1H-indole-2-carboxamide **44**

General procedure C was followed using carboxylic acid **41** (350 mg, 1.1 mmol) and L-valinamide hydrochloride (200 mg, 1.3 mmol). The crude product was purified by recrystallization from ethyl acetate, hexane to obtain the title compound **44** (320 mg, 68%) as a white solid, **mp**: 179.1-182.5 °C; R_f: 0.46 (4:1 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3404, 3278, 2856, 1673, 1623, 1532, 1455, 1255, 1088 cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6): δ 0.94 (6H, d, *J* = 6.8 Hz), 1.19-1.38 (2H, m), 1.51 (2H, quin, *J* = 6.7 Hz), 1.67 (2H, quin, *J* = 7.1 Hz), 2.12 (1H, sept, *J* = 6.8 Hz), 3.35 (2H, t, *J* = 6.7 Hz), 4.28 (1H, t, *J* = 8.1 Hz), 4.49 (2H, s), 4.51 (2H, t, *J* = 7.2 Hz), 7.03-7.37 (9H, m), 7.45 (1H, bs, NH), 7.53 (1H, d, *J* = 8.4 Hz), 7.64 (1H, d, *J* = 7.9 Hz), 8.13 (1H, d, *J* = 8.9 Hz) ppm; **^{13}C NMR** (75 MHz, DMSO-d_6): δ 18.5, 19.4, 23.0, 28.9, 30.1, 43.6, 58.3, 69.4, 71.7, 115.0, 110.6, 120.0, 121.6, 123.4, 125.7, 127.2, 127.3, 128.2, 131.6, 137.7, 138.7, 161.8, 172.9 ppm; **LRMS** (+ESI) *m/z*: 458.3 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{26}H_{33}N_3O_3$ $[M+Na]^+$: 458.24196, found 458.24111.

(S)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-(benzyloxy)pentyl)-1H-indole-2-carboxamide **45**

General procedure C was followed using carboxylic acid **41** (300 mg, 930 μmol) and amine hydrochloride salt **36** (190 mg, 1.1 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (2:3 to 1:1) as an eluent to obtain the title compound **45** (290 mg, 70%) as a white foam, R_f: 0.35 (1:1 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3251, 2939, 1638, 1536, 1453, 1084, 742 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 1.13 (9H, s), 1.35-1.50 (2H, m), 1.65 (2H, quin, *J* = 6.7 Hz), 1.83 (2H, quin, *J* = 7.6 Hz), 3.45 (2H, t, *J* = 6.4 Hz), 4.46-4.57 (4H, m), 4.60 (1H, d, *J* = 9.4 Hz), 6.00 (1H, bs), 6.40 (1H, bs), 6.95-7.04 (2H, m), 7.16 (1H, ddd, *J* = 7.9, 6.7, 1.2 Hz), 7.25-7.45 (7H, m), 7.66 (1H, d, *J* = 8.0 Hz) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ 23.8, 26.8, 29.6, 30.6, 35.0, 44.6, 60.0, 70.2, 73.0, 104.9, 110.5, 120.6, 122.1, 124.3, 126.2, 127.6, 127.7, 128.5, 131.3, 138.6, 138.7, 162.6, 172.8 ppm; **LRMS** (+ESI) *m/z*: 472.3 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{27}H_{35}N_3O_3$ $[M+Na]^+$: 472.25761, found 472.25680.

1-(5-(Benzyloxy)pentyl)-N-(2-phenylpropan-2-yl)-1H-indole-2-carboxamide **46**

General procedure C was followed using carboxylic acid **41** (220 mg, 695 μmol) and 1-methyl-1-phenylethylamine (120 μL, 830 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound **46** (260 mg, 62%) as a white solid, **mp**: 77.1-80.2 °C; R_f: 0.52 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3259, 2937, 1638, 1536, 1452, 1270, 747 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 1.32-1.48 (2H, m), 1.55-1.72 (2H, m), 1.74-1.86 (2H, m), 1.84 (6H, s), 3.44 (2H, t, *J* = 6.5 Hz), 4.48 (2H, s), 4.53 (2H, t, *J* = 7.4 Hz), 6.50 (1H, bs), 6.89 (1H, s), 7.13-7.21 (1H, m), 7.23-7.54 (12H, m), 7.66 (1H, d, *J* = 7.9 Hz) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ 23.7, 29.5, 29.6, 30.6, 44.3, 56.4, 70.3, 73.0, 103.7, 110.5, 120.5, 121.8, 123.9, 124.8, 126.2, 126.9, 127.6, 127.7, 128.5, 128.7, 132.7, 138.4, 138.8, 146.9,

161.9 ppm; **LRMS** (+ESI) *m/z*: 477.3 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{30}H_{34}N_2O_2$ [M+Na]$^+$: 477.25180, found 477.25092.

(S)-Methyl 2-(1-(5-hydroxypentyl)-1H-indole-2-carboxamido)-3-methylbutanoate **10**
General procedure D was followed using benzyl ether **42** (190 mg, 420 µmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 2:3) as an eluent to obtain the title compound **10** (100 mg, 67%) as a white solid, **mp**: 87.1-90.8 °C; R_f: 0.10 (3:7 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3395, 3290, 2945, 1745, 1637, 1529, 1459, 1203, 1147, 1007, 748 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.01 (3H, d, *J* = 6.9 Hz), 1.04 (3H, d, *J* = 6.8 Hz), 1.33-1.43 (2H, m), 1.50-1.60 (2H, m), 1.70 (1H, bs), 1.83 (2H, quin, *J* = 7.4 Hz), 2.22-2.35 (1H, m), 3.59 (2H, t, *J* = 6.4 Hz), 3.79 (3H, s), 4.45-4.65 (2H, m), 4.72 (1H, dd, *J* = 8.9, 5.0 Hz), 6.66 (1H, d, *J* = 8.9 Hz), 6.95 (1H, d, *J* = 0.8 Hz), 7.14 (1H, ddd, *J* = 8.0, 6.9, 1.0 Hz), 7.31 (1H, ddd, *J* = 8.4, 6.9, 1.2 Hz), 7.36-7.41 (1H, m), 7.65 (1H, dt, *J* = 8.0, 1.0 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 18.1, 19.2, 23.3, 30.4, 31.6, 32.5, 44.5, 52.5, 57.3, 62.8, 104.7, 110.5, 120.7, 122.1, 124.3, 126.3, 131.4, 138.5, 162.6, 172.8 ppm; **LRMS** (+ESI) *m/z*: 383.2 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{20}H_{28}N_2O_4$ [M+Na]$^+$: 383.19467, found 383.19401; **$[\alpha]_D^{27}$**: +6.8 (0.75, $CHCl_3$); **HPLC**: 98.6%, RT: 25.1 mins.

(S)-Methyl 2-(1-(5-hydroxypentyl)-1H-indole-2-carboxamido)-3,3-dimethylbutanoate **12**
General procedure D was followed using benzyl ether **43** (120 mg, 260 µmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 3:7) as an eluent to obtain the title compound **12** (84 mg, 87%) as a colourless oil, R_f: 0.09 (3:7 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3357, 2935, 1732, 1647, 1523, 1458, 1320, 1215, 1163, 741 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.07 (9H, s), 1.33-1.43 (2H, m), 1.51-1.65 (3H, m), 1.83 (1H, quin, *J* = 7.4 Hz), 3.60 (2H, t, *J* = 6.4 Hz), 3.78 (3H, s), 4.47-4.62 (2H, m), 4.63 (1H, d, *J* = 9.5 Hz), 6.70 (1H, d, *J* = 9.5 Hz), 6.93 (1H, d, *J* = 0.8 Hz), 7.15 (1H, ddd, *J* = 7.9, 6.9, 1.0 Hz), 7.31 (1H, ddd, *J* = 8.3, 6.9, 1.2 Hz), 7.36-7.41 (1H, m), 7.65 (1H, dt, *J* = 8.0, 1.0 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 23.3, 26.8, 30.4, 32.5, 32.5, 44.6, 52.1, 60.1, 62.8, 104.6, 110.5, 120.7, 122.1, 124.3, 126.3, 131.5, 138.5, 162.4, 172.3 ppm; **LRMS** (+ESI) *m/z*: 397.2 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{21}H_{30}N_2O_4$ [M+Na]$^+$: 397.21033, found 397.20957; **$[\alpha]_D^{27}$**: +13.3 (0.58, $CHCl_3$); **HPLC**: 97.8%, RT: 26.4 mins.

(S)-N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-hydroxypentyl)-1H-indole-2-carboxamide **15**
A modified version of general procedure D was followed using benzyl ether **44** (250 mg, 570 µmol) in a mixture of methanol (5 mL) and tetrahydrofuran (5 mL). The crude residue was purified by recrystallization from isopropanol, diethyl ether to obtain the title compound **15** (170 mg, 83%) as a white solid, **mp**: 220.8-221.2 °C; R_f: 0.10 (4:1 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3382, 3263, 2934, 1622, 1536, 1265, 1253, 741 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 0.88-0.99 (6H, m), 1.17-1.30 (2H, m), 1.39 (2H, quin, *J* = 6.9 Hz), 1.65 (2H, quin, *J* = 7.4 Hz), 2.12 (1H, sept, *J* = 6.8 Hz), 3.28-3.40 (2H, m), 4.23-4.35 (2H, m), 4.50 (2H, t, *J* = 7.3 Hz), 7.05-7.14 (2H, m), 7.17 (1H, s), 7.25 (1H, t, *J* = 7.7 Hz), 7.46 (1H, bs), 7.53 (1H, d, *J* = 8.4 Hz), 7.64 (1H, d, *J* = 7.9 Hz), 8.15 (1H, d, *J* = 8.9 Hz) ppm; **^{13}C NMR** (100 MHz, DMSO-d_6): δ 18.5, 19.5, 22.9, 30.1, 30.2, 32.3, 43.7, 58.3, 60.6, 105.0, 110.6, 120.0, 121.6, 123.5, 125.7, 131.6, 137.7, 161.8, 172.9 ppm; **LRMS** (+ESI) *m/z*: 368.3 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{19}H_{27}N_3O_3$ [M+Na]$^+$: 368.19501, found 368.19431; **$[\alpha]_D^{27}$**: +33.3 (0.75, DMSO); **HPLC**: 95.2%, RT: 21.5 mins.

(S)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-hydroxypentyl)-1H-indole-2-carboxamide **17**

A modified version of general procedure D was followed using benzyl ether **45** (240 mg, 540 μmol) in a mixture of methanol (5 mL) and tetrahydrofuran (5 mL). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:2 to 7:3) as an eluent and recrystallization from dichloromethane, hexane to obtain the title compound **17** (160 mg, 84%) as a white solid, **mp**: 140.8-144.7 °C; R_f: 0.08 (3:2 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3415, 3331, 3189, 2933, 1632, 1527, 1457, 1403, 1354, 1314 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.02 (9H, s), 1.18-1.29 (2H, m), 1.39 (2H, quin, *J* = 6.9 Hz), 1.65 (1H, quin, *J* = 7.5 Hz), 3.29-3.37 (2H, m), 4.31 (1H, t, *J* = 5.1 Hz), 4.41 (1H, d, *J* = 9.6 Hz), 4.46-4.53 (2H, m), 7.09 (1H, ddd, *J* = 7.9, 6.9, 0.9 Hz), 7.13-7.20 (2H, m), 7.26 (1H, ddd, *J* = 8.3, 7.0, 1.2 Hz), 7.50-7.56 (2H, m), 7.64 (1H, dt, *J* = 8.0, 0.9 Hz), 7.80 (1H, d, *J* = 9.6 Hz) ppm; **^{13}C NMR** (100 MHz, DMSO-d_6): δ 23.3, 27.4, 30.7, 32.7, 34.6, 44.3, 60.6, 61.0, 105.4, 111.0, 120.5, 122.1, 124.0, 126.2, 132.1, 138.2, 162.1, 172.3 ppm; **LRMS** (+ESI) *m/z*: 382.2 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{20}H_{29}N_3O_3$ [M+Na]$^+$: 382.21066, found 382.20991; **$[\alpha]_D^{27}$**: +48.4 (0.62, DMSO); **HPLC**: 98.2%, RT: 22.7 mins.

1-(5-Hydroxypentyl)-N-(2-phenylpropan-2-yl)-1H-indole-2-carboxamide **19**

General procedure D was followed using benzyl ether **46** (140 mg, 310 μmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 2:3) as an eluent to obtain the title compound **19** (90 mg, 80%) as a white solid, **mp**: 103.1-106.2 °C; R_f: 0.12 (3:7 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3290, 2935, 1632, 1529, 1447, 1354, 1271, 981, 759 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.29-1.39 (2H, m), 1.49 (2H, m), 1.60 (1H, bs), 1.72-1.83 (2H, m), 1.82 (6H, s), 3.56 (2H, t, *J* = 6.5 Hz), 4.45-4.53 (2H, m), 6.53 (1H, bs), 6.88 (1H, d, *J* = 0.8 Hz), 7.14 (1H, ddd, *J* = 8.0, 6.9, 1.0 Hz), 7.23-7.32 (2H, m), 7.33-7.39 (3H, m), 7.45-7.50 (2H, m), 7.64 (1H, dt, *J* = 8.1, 1.0 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 23.2, 29.5, 30.4, 32.4, 44.3, 56.4, 62.7, 103.8, 110.5, 120.5, 121.9, 124.0, 124.8, 126.2, 126.9, 128.7, 132.6, 138.3, 146.9, 161.9 ppm; **LRMS** (+ESI) *m/z*: 387.3 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{23}H_{28}N_2O_2$ [M+Na]$^+$: 387.20485, found 387.20411; **HPLC**: 98.1%, RT: 26.8 mins.

(S)-N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5,5-difluoropentyl)-1H-indole-2-carboxamide **13**

To a solution of alcohol **17** (70 mg, 196 μmol) in a mixture of dichloromethane (1 mL) and tetrahydrofuran (1 mL) at 0 °C was added Dess-Martin periodinane (100 mg, 236 μmol) and the mixture was warmed to room temperature and stirred for 16 hours. The solids were removed by filtration through a pad of silica and the filtrate concentrated under reduced pressure to obtain the crude aldehyde intermediate, which was used immediately without further purification or characterization.

The crude aldehyde (50 mg, 140 μmol) was taken up in dichloromethane (2 mL) and cooled to 0 °C. Diethylaminosulfur trifluoride (43 μL, 322 μmol) was added slowly and the mixture was warmed to room temperature and stirred for 24 hours. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (10 mL) and the mixture extracted with dichloromethane (3 x 10 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the crude product purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:1) as an eluent to obtain the title compound **13** (30 mg, 40%)

as a colourless foam, R_f: 0.70 (7:3 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3323, 3196, 2961, 2928, 1641, 1525, 1455, 1403, 1123, 1026, 735 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.11 (9H, s), 1.42-1.57 (2H, m), 1.78-1.94 (4H, m), 4.45 (1H, d, *J* = 9.3 Hz), 4.51-4.60 (2H, m), 5.56 (1H, bs), 5.73 (1H, bs), 5.77 (1H, tt, *J* = 56.8, 4.5 Hz), 6.91 (1H, d, *J* = 9.1 Hz), 6.97 (1H, s), 7.15 (1H, ddd, *J* = 8.0, 6.7, 1.0 Hz), 7.29-7.40 (2H, m), 7.65 (1H, d, *J* = 8.0 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 19.8 (t, *J* = 5.7 Hz), 26.8, 30.0, 33.9 (t, *J* = 20.9 Hz), 35.0, 44.3, 60.2, 105.0, 110.3, 117.3 (t, *J* = 238.9 Hz), 120.8, 122.3, 124.5, 126.3, 131.2, 138.5, 162.5, 172.4 ppm; **^{19}F NMR** (376 MHz, $CDCl_3$): δ -115.9 ppm; **LRMS** (+ESI) *m/z*: 402.2 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{20}H_{27}F_2N_3O_2$ $[M+Na]^+$: 402.19690, found 402.19702; **$[\alpha]_D^{27}$**: +10.3 (0.66, $CHCl_3$); **HPLC**: 96.8%, RT: 22.4 mins.

1-Pentyl-1H-pyrrolo[2,3-b]pyridine **47**

General procedure A was followed using 7-azaindole (2.0 g, 16.9 mmol) and 1-bromopentane (2.5 mL, 20.5 mmol) to obtain the title compound **47** (3.2 g, 100%) as a yellow oil, the spectroscopic data of which corresponded to previously described.[35] R_f: 0.58 (2:3 ethyl acetate, hexane).

1-(5-(Benzyloxy)pentyl)-1H-pyrrolo[2,3-b]pyridine **48**

General procedure A was followed using 7-azaindole (1.5 g, 12.7 mmol) and alkyl bromide **34** (3.9 g, 15.2 mmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound **48** (3.1 g, 85%) as a light yellow oil, R_f: 0.39 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 2935, 2859, 1509, 1425, 1306, 1094, 796, 773 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.41-1.51 (2H, m), 1.63-1.73 (2H, m), 1.93 (2H, quin, *J* = 7.4 Hz), 3.47 (2H, t, *J* = 6.5 Hz), 4.32 (2H, t, *J* = 7.2 Hz), 4.50 (2H, s), 6.47 (1H, d, *J* = 3.5 Hz), 7.07 (1H, dd, *J* = 7.8, 4.7 Hz), 7.23 (1H, d, *J* = 3.5 Hz), 7.27-7.40 (5H, m), 7.93 (1H, dd, *J* = 7.8, 1.6 Hz), 8.35 (1H, dd, *J* = 4.7, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 23.7, 29.5, 30.3, 44.6, 70.2, 73.0, 99.4, 115.6, 120.7, 127.6, 127.7, 128.1, 128.4, 128.8, 138.7, 142.7, 147.5 ppm; **LRMS** (+ESI) *m/z*: 295.2 ($[M+H]^+$ 62%), 317.2 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{19}H_{22}N_2O$ $[M+Na]^+$: 317.16298, found 317.16232.

2,2,2-Trifluoro-1-(1-pentyl-1H-pyrrolo[2,3-b]pyridin-3-yl)ethanone **49**

To a solution of **47** (3.0 g, 15.9 mmol) in *N,N*-dimethylformamide (100 mL) at 0 °C was added aluminium chloride (10.6 g, 79.7 mmol) portionwise and the mixture stirred for 1 hour. Trifluoroacetic anhydride (3.4 mL, 23.9 mmol) was added dropwise and the mixture stirred at room temperature for 3 hours. The reaction mixture was quenched with ice, diluted with water (150 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were washed with water (3 x 100 mL) and aqueous lithium chloride (1 M, 100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound **49** (3.5 g, 78%) as a yellow oil, the spectroscopic data of which corresponded to previously described.[23] R_f: 0.40 (1:4 ethyl acetate, hexane).

1-(1-(5-(Benzyloxy)pentyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)-2,2,2-trifluoroethanone **50**

To a solution of **48** (2.4 g, 8.2 mmol) in anhydrous *N,N*-dimethylformamide (60 mL) at 0 °C was added aluminium chloride (5.4 g, 40.8 mmol) portionwise and the mixture stirred for 1 hour. Trifluoroacetic anhydride (1.7 mL, 12.2 mmol) was added dropwise and the mixture was warmed to room temperature and stirred for 4 hours. The reaction mixture was poured on to ice-water (100 mL) and extracted with ethyl acetate (3 x 50

mL). The combined organic extracts were washed with water (3 x 100 mL) and aqueous lithium chloride (1 M, 80 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound **50** (2.8 g, 88%) as a yellow oil, R_f: 0.28 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 2940, 2861, 1670, 1527, 1385, 1287, 1136, 882, 802, 714 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.44-1.55 (2H, m), 1.65-1.75 (2H, m), 1.94-2.04 (2H, m), 3.49 (2H, t, *J* = 6.3 Hz), 4.40 (2H, t, *J* = 7.4 Hz), 4.50 (2H, s), 7.25-7.38 (6H, m), 8.09 (1H, d, *J* = 1.7 Hz), 8.48 (1H, dd, *J* = 4.7, 1.7 Hz), 8.66 (1H, dd, *J* = 7.9, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 23.6, 29.3, 29.9, 46.0, 69.9, 73.1, 108.0, 117.0 (q, *J* = 290.6 Hz), 119.6, 119.8, 127.7, 127.7, 128.5, 131.2, 137.4 (q, *J* = 4.2 Hz), 138.5, 145.6, 148.1, 174.9 (q, *J* = 35.4 Hz) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$): δ -72.4 ppm; **LRMS** (+ESI) *m/z*: 391.2 ($[M+H]^+$ 52%), 413.2 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{21}H_{21}F_3N_2O_2$ $[M+Na]^+$: 413.14528, found 413.14448.

1-Pentyl-1H-pyrrolo[2,3-b]pyridine-3-carboxylic acid **51**
To a solution of **49** (3.5 g, 12.3 mmol) in methanol (100 mL) was added aqueous lithium hydroxide (3 M, 10.3 mL, 30.8 mmol) and the mixture stirred at reflux for 48 hours. The volatiles were removed under a stream of nitrogen and the aqueous residue washed with dichloromethane (20 mL), acidified with aqueous hydrochloric acid (6 M) and extracted with dichloromethane (3 x 50 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound **51** (2.4 g, 84%) as an off-white solid, the spectroscopic data of which corresponded to previously described.[23] R_f: 0.07 (1:4 ethyl acetate, hexane).

1-(5-(Benzyloxy)pentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxylic acid **52**
A solution of **50** (2.5 g, 6.4 mmol) in a mixture of methanol and tetrahydrofuran (1:1, 30 mL) was added aqueous lithium hydroxide (2 M, 16 mL, 32 mmol) and the mixture was heated at reflux for 72 hours. The volatiles were removed under a stream of nitrogen and the aqueous residue washed with dichloromethane (30 mL), acidified with aqueous hydrochloric acid (6 M) and extracted with dichloromethane (3 x 50 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to afford the title compound **52** (1.6 g, 74%) as a light yellow solid, **mp**: 108.1-110.8 °C; R_f: 0.28 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3102, 2061, 2855, 2502, 1686, 1533, 1260 1095, 800, 758, 730 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.43-1.53 (2H, m), 1.69 (2H, quin, *J* = 6.6 Hz), 1.97 (2H, quin, *J* = 7.4 Hz), 3.47 (2H, t, *J* = 6.4 Hz), 4.38 (2H, t, *J* = 7.2 Hz), 4.50 (2H, s), 7.24-7.38 (6H, m), 8.06 (1H, s), 8.43 (1H, dd, *J* = 4.7, 1.7 Hz), 8.50 (1H, dd, *J* = 7.9, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 23.7, 29.4, 30.0, 45.5, 70.0, 73.1, 105.0, 118.4, 119.6, 127.7, 127.8, 128.5, 130.3, 135.4, 138.6, 144.2, 148.0, 169.3 ppm; **LRMS** (+ESI) *m/z*: 361.2 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{20}H_{22}N_2O_3$ $[M+Na]^+$: 361.15281, found 361.15205.

(S)-Methyl 3-methyl-2-(1-pentyl-1H-pyrrolo[2,3-b]pyridine-3-carboxamido)butanoate **22**
General procedure C was followed using carboxylic acid **51** (250 mg, 1.1 mmol) and methyl-L-valinate hydrochloride (220 mg, 1.3 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 2:3) as an eluent and recrystallization from dichloromethane, hexane to obtain the title compound **22** (240 mg, 65%) as a white solid, **mp**: 86.9-89.8 °C; R_f: 0.20 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3286, 2961, 2931, 1743, 1619, 1541, 1523, 1400, 1286, 1196, 1142, 776 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.87 (3H, t, *J* = 6.9 Hz), 1.01 (3H, d, *J* = 6.9 Hz), 1.04 (3H, d, *J* = 6.9 Hz), 1.26-1.41 (4H, m), 1.88 (2H, quin, *J* = 7.4 Hz), 2.22-2.37 (1H, m), 3.78 (3H, s),

4.26-4.34 (2H, m), 4.84 (1H, dd, *J* = 8.6, 4.9 Hz), 6.42 (1H, d, *J* = 8.6 Hz), 7.20 (1H, dd, *J* = 8.0, 4.7 Hz), 7.83 (1H, s), 8.32 (1H, dd, *J* = 8.0, 1.6 Hz), 8.37 (1H, dd, *J* = 4.7, 1.6 Hz), 8.48 (1H, s) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.0, 18.2, 19.2, 21.4, 29.0, 30.0, 31.8, 45.2, 52.4, 57.0, 108.9, 117.7, 118.3, 128.8, 131.0, 143.9, 147.8, 164.4, 173.3 ppm; **LRMS** (+ESI) *m/z*: 368.2 ([M+Na]$^+$ 100%), 713.3 ([2M+Na]$^+$ 43%); **HRMS** (ESI)$^+$ Cald for $C_{19}H_{27}N_3O_3$ [M+Na]$^+$: 368.19501, found 368.19429; **$[\alpha]_D^{27}$**: +34.5 (0.58, $CHCl_3$); **HPLC**: 99.5%, RT: 25.9 mins.

(S)-Methyl 3,3-dimethyl-2-(1-pentyl-1H-pyrrolo[2,3-b]pyridine-3-carboxamido)butanoate **23**

General procedure C was followed using carboxylic acid **51** (200 mg, 860 μmol) and amine hydrochloride salt **37** (190 mg, 1.0 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as an eluent to obtain the title compound **23** (140 mg, 45%) as a white solid, **mp**: 68.3-70.1 °C; ***R*$_f$**: 0.37 (2:3 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3334, 3101, 2956, 1738, 1614, 1537, 1515, 1426, 1267, 1217, 1140, 777 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 6.9 Hz), 1.08 (9H, s), 1.28-1.41 (4H, m), 1.89 (2H, quin, *J* = 7.4 Hz), 3.76 (3H, s), 4.27-4.34 (2H, m), 4.76 (1H, d, *J* = 9.4 Hz), 6.42 (1H, d, *J* = 9.3 Hz), 7.21 (1H, dd, *J* = 7.9, 4.7 Hz), 7.83 (1H, s), 8.31 (1H, dd, *J* = 8.0, 1.5 Hz), 8.38 (1H, dd, *J* = 4.7, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.1, 22.4, 26.9, 29.0, 30.1, 35.3, 45.3, 52.1, 59.8, 108.9, 117.7, 118.2, 128.7, 131.1, 143.9, 147.8, 164.3, 172.9 ppm; **LRMS** (+ESI) *m/z*: 382.3 ([M+Na]$^+$ 100%), 741.4 ([2M+Na]$^+$ 32%); **HRMS** (ESI)$^+$ Cald for $C_{20}H_{29}N_3O_3$ [M+Na]$^+$: 382.21066, found 382.20991; **$[\alpha]_D^{27}$**: +30.5 (1.15, $CHCl_3$); **HPLC**: 99.8%, RT: 27.4 mins.

(S)-N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-pyrrolo[2,3-b]pyridine-3-carboxamide **25**

General procedure C was followed using carboxylic acid **51** (230 mg, 1.0 mmol) and L-valinamide hydrochloride (180 mg, 1.2 mmol). The crude product was purified by recrystallization from ethyl acetate to obtain the title compound **25** (240 mg, 73%) as a white solid, **mp**: 210.0-213.4 °C; ***R*$_f$**: 0.19 (ethyl acetate); **IR** (ν_{max} (neat)): 3372, 3293, 3193, 2934, 1649, 1621, 1530, 1516, 1426, 1297, 1144 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 0.84 (3H, t, *J* = 7.1 Hz), 0.96 (3H, d, *J* = 6.7 Hz), 0.98 (3H, d, *J* = 6.7 Hz), 1.20-1.38 (4H, m), 1.84 (2H, quin, *J* = 7.2 Hz), 2.09 (1H, sept, *J* = 6.8 Hz), 4.19-4.38 (3H, m), 7.07 (1H, bs), 7.20 (1H, dd, *J* = 7.9, 4.7 Hz), 7.49 (1H, bs), 7.69 (1H, d, *J* = 8.8 Hz), 8.30 (1H, dd, *J* = 4.7, 1.7 Hz), 8.41 (1H, dd, *J* = 7.9, 1.7 Hz), 8.48 (1H, s) ppm; **^{13}C NMR** (100 MHz, DMSO-d_6): δ 13.8, 18.5, 19.5, 21.7, 28.3, 29.2, 30.3, 44.1, 57.5, 107.9, 117.1, 118.9, 129.4, 131.2, 143.2, 147.1, 163.5, 173.4 ppm; **LRMS** (+ESI) *m/z*: 353.3 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{18}H_{26}N_4O_2$ [M+Na]$^+$: 353.19534, found 353.19461; **$[\alpha]_D^{27}$**: +22.8 (0.88, DMSO); **HPLC**: 96.3%, RT: 21.8 mins.

(S)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1H-pyrrolo[2,3-b]pyridine-3-carboxamide **26**

General procedure C was followed using carboxylic acid **51** (250 mg, 1.1 mmol) and amine hydrochloride salt **36** (220 mg, 1.3 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (9:1 to 1:0) as an eluent to obtain the title compound **26** (310 mg, 84%) as a white solid, **mp**: 90.1-94.3 °C; ***R*$_f$**: 0.21 (ethyl acetate); **IR** (ν_{max} (neat)): 3334, 3187, 2954, 1674, 1620, 1535, 1513, 1398, 1367, 1261, 1140 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 0.84 (3H, t, *J* = 7.1 Hz), 1.01 (9H, s), 1.19-1.38 (4H, m), 1.85 (2H, quin, *J* = 7.3 Hz), 4.19-4.36 (2H, m), 4.48 (1H, d, *J* = 9.5 Hz),

7.11 (1H, bs), 7.21 (1H, dd, *J* = 7.9, 4.7 Hz), 7.40 (1H, d, *J* = 9.5 Hz), 7.55 (1H, bs), 8.31 (1H, dd, *J* = 4.7, 1.6 Hz), 8.39 (1H, dd, *J* = 7.9, 1.6 Hz), 8.55 (1H, s) ppm; **^{13}C NMR** (100 MHz, DMSO-d_6): δ 13.8, 21.7, 26.9, 28.4, 29.2, 34.1, 44.1, 59.3, 107.8, 117.1, 118.8, 129.2, 131.4, 143.2, 147.2, 163.3, 172.4 ppm; **LRMS** (+ESI) *m/z*: 367.2 ([M+Na]$^+$ 100%), 711.3 ([2M+Na]$^+$ 20%); **HRMS** (ESI)$^+$ Cald for $C_{19}H_{28}N_4O_2$ [M+Na]$^+$: 367.21100, found 367.21025; **[α]$_D^{27}$**: +25.5 (1.57, DMSO); **HPLC**: 98.4%, RT: 23.2 mins.

1-Pentyl-N-(2-phenylpropan-2-yl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide **28**

General procedure C was followed using carboxylic acid **51** (110 mg, 470 μmol) and 1-methyl-1-phenylethylamine (70 mg, 520 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as an eluent to obtain the title compound **28** (84 mg, 51%) as a white solid, **mp**: 171.8-174.3 °C; ***R*$_f$**: 0.30 (2:3 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3328, 3093, 2923, 1620, 1538, 1516, 1426, 1270, 1198 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.91 (3H, t, *J* = 6.8 Hz), 1.29-1.46 (4H, m), 1.84-1.97 (8H, m), 4.33 (2H, t, *J* = 7.3 Hz), 6.13 (1H, bs), 7.20 (1H, dd, *J* = 7.9, 4.7 Hz), 7.26-7.31 (1H, m), 7.38 (2H, t, *J* = 7.7 Hz), 7.50-7.56 (2H, m), 7.77 (1H, s), 8.29 (1H, dd, *J* = 7.9, 1.6 Hz), 8.39 (1H, dd, *J* = 4.8, 1.5 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.1, 22.4, 29.1, 29.7, 30.1, 45.2, 56.4, 110.2, 117.4, 118.3, 124.9, 126.9, 128.7, 128.8, 130.6, 143.8, 147.4, 147.8, 163.8 ppm; **LRMS** (+ESI) *m/z*: 372.3 ([M+Na]$^+$ 100%), 721.4 ([2M+Na]$^+$ 46%); **HRMS** (ESI)$^+$ Cald for $C_{22}H_{27}N_3O$ [M+Na]$^+$: 372.20518, found 372.20444; **HPLC**: 99.9%, RT: 27.4 mins.

N-(Adamantan-1-yl)-1-(5-(benzyloxy)pentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide **53**

General procedure C was followed using carboxylic acid **52** (120 mg, 35 μmol) and 1-adamantylamine (64 mg, 43 μmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 2:3) as an eluent to obtain the title compound **53** (110 mg, 65%) as a white gummy solid, ***R*$_f$**: 0.29 (2:3 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3365, 2907, 2848, 1620, 1536, 1516, 1422, 1278, 1251, 1095, 754 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.37-1.48 (2H, m), 1.59-1.80 (8H, m), 1.90 (2H, quin, *J* = 7.4 Hz), 2.10-2.21 (9H, m), 3.44 (2H, t, *J* = 6.4 Hz), 4.30 (2H, t, *J* = 7.2 Hz), 4.47 (2H, s), 5.55 (1H, bs), 7.17 (1H, dd, *J* = 7.9, 4.7 Hz), 7.23-7.36 (5H, m), 7.68 (1H, s), 8.27 (1H, dd, *J* = 8.0, 1.5 Hz), 8.35 (1H, dd, *J* = 4.7, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 23.7, 29.4, 29.7, 30.2, 36.6, 42.3, 45.1, 52.3, 70.1, 73.0, 110.7, 117.3, 118.4, 127.7, 127.8, 128.5, 128.9, 130.1, 138.6, 143.7, 147.7, 163.9 ppm; **LRMS** (+ESI) *m/z*: 494.3 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{30}H_{37}N_3O_2$ [M+H]$^+$: 494.27835, found 494.27749.

(S)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-(benzyloxy)pentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide **55**

General procedure C was followed using carboxylic acid **52** (420 mg, 1.3 mmol) and amine hydrochloride salt **36** (250 mg, 1.5 mmol). The crude product was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound **55** (510 mg, 91%) as a colourless foam, ***R*$_f$**: 0.29 (1:19 methanol, dichloromethane); **IR** (ν_{max} (neat)): 3103, 2938, 2856, 1685, 1535, 1260, 1096 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.16 (9H, s), 1.38-1.48 (2H, m), 1.66 (2H, quin, *J* = 6.7 Hz), 1.90 (2H, quin, *J* = 7.4 Hz), 3.45 (2H, t, *J* = 6.4 Hz), 4.29 (2H, td, *J* = 7.1, 1.9 Hz), 4.48 (2H, s), 4.79 (1H, d, *J* = 9.2 Hz), 5.98 (1H, bs), 6.81 (1H, d, *J* = 9.3 Hz), 6.94 (1H, bs), 7.19 (1H, dd, *J* = 8.0, 4.7 Hz), 7.24-7.37 (5H, m), 7.84 (1H, s), 8.33 (1H, dd, *J* = 8.0, 1.6 Hz), 8.37 (1H, dd, *J* = 4.7, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 23.6, 26.9,

29.4, 30.1, 34.9, 45.1, 59.9, 70.0, 73.0, 108.9, 117.7, 118.3, 127.6, 127.7, 128.5, 128.8, 130.9, 138.6, 143.9, 147.7, 164.5, 173.5 ppm; **LRMS** (+ESI) *m/z*: 473.3 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{26}H_{34}N_4O_3$ [M+Na]$^+$: 473.25286, found 473.25201.

1-(5-(Benzyloxy)pentyl)-N-(2-phenylpropan-2-yl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide **56**

General procedure C was followed using carboxylic acid **52** (250 mg, 740 µmol) and 1-methyl-1-phenylethylamine (250 mg, 890 µmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 3:2) as an eluent to obtain the title compound **56** (290 mg, 85%) as a white solid, **mp**: 137.8-138.8 °C; $\boldsymbol{R_f}$: (0.19 2:3 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3337, 2926, 2854, 1621, 1537, 1275, 1094, 760 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.38-1.48 (2H, m), 1.60 (2H, m), 1.86 (6H, s), 1.86-1.95 (2H, m), 3.44 (2H, t, *J* = 6.4 Hz), 4.30 (2H, t, *J* = 7.2 Hz), 4.46 (2H, s), 6.13 (1H, s), 7.16 (1H, dd, *J* = 8.0, 4.7 Hz), 7.22-7.38 (8H, m), 7.48-7.53 (2H, m), 7.73 (1H, s), 8.27 (1H, dd, *J* = 8.0, 1.6 Hz), 8.36 (1H, dd, *J* = 4.7, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 23.7, 29.4, 29.7, 30.2, 45.1, 56.3, 70.1, 73.2, 110.2, 117.4, 118.3, 124.9, 126.8, 127.7, 127.7, 128.5, 128.6, 128.9, 130.4, 138.7, 143.8, 147.4, 147.8, 163.7 ppm; **LRMS** (+ESI) *m/z*: 456.3 ([M+H]$^+$ 31%), 478.3 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{26}H_{34}N_4O_3$ [M+Na]$^+$: 473.25286, found 473.25201.

N-(Adamantan-1-yl)-1-(5-hydroxypentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide **21**

General procedure D was followed using benzyl ether **53** (70 mg, 148 µmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:1 to 3:2) as an eluent to obtain the title compound **21** (49 mg, 86%) as a white solid, **mp**: 110.9-113.4 °C; $\boldsymbol{R_f}$: 0.16 (3:2 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3320, 2904, 2849, 1618, 1534, 1517, 1425, 1290, 1160, 776 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.34-1.45 (2H, m), 1.53-1.63 (2H, m), 1.66-1.79 (6H, m), 1.85-1.94 (3H, m), 2.09-2.20 (9H, m), 3.61 (2H, t, *J* = 6.4 Hz), 4.29 (2H, t, *J* = 7.1 Hz,), 5.62 (1H, bs), 7.16 (1H, dd, *J* = 7.9, 4.7 Hz), 8.26 (1H, dd, *J* = 7.9, 1.5 Hz), 8.33 (1H, dd, *J* = 4.7, 1.5 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 23.1, 29.7, 30.1, 32.4, 36.6, 42.3, 45.0, 52.4, 62.5, 110.6, 117.4, 118.4, 129.0, 130.3, 143.6, 147.6, 164.0 ppm; **LRMS** (+ESI) *m/z*: 404.3 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{23}H_{31}N_3O_2$ [M+Na]$^+$: 404.23140, found 404.23067; **HPLC**: 99.0%, RT: 24.5 mins.

(S)-Methyl 2-(1-(5-hydroxypentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamido)-3,3-dimethylbutanoate **24**

General procedure C was followed using carboxylic acid **52** (36 mg, 106 µmol) and amine hydrochloride salt **37** (23 mg, 127 µmol). The crude residue contained an inseparable mixture of products and hence was taken on to the next step without further purification or characterization.

General procedure D was followed using the crude benzyl protected intermediate. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:1 to 1:4) as an eluent to obtain the title compound **24** (16 mg, 40% over 2 steps) as a colourless foam, $\boldsymbol{R_f}$: 0.08 (1:1 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3323, 2999, 2946, 2927, 1739, 1547, 1520, 1476, 1426, 1331, 1214, 1162, 1055 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.08 (9H, s), 1.40-1.51 (2H, m), 1.59-1.68 (2H, m), 1.92-2.02 (2H, m), 3.65 (2H, t, *J* = 6.2 Hz), 3.77 (3H, s), 4.34-4.50 (2H, m), 4.76 (1H, d, *J* = 9.3 Hz), 6.48 (1H, d, *J* = 9.3 Hz), 7.23-7.32 (1H, m), 7.87 (1H, s), 8.35-8.51 (2H, m) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 22.9, 26.9, 29.9, 31.9, 35.4, 52.1, 59.8, 62.5, 77.4, 109.9, 117.7, 117.8, 131.4, 131.4, 143.3, 146.9, 163.8, 172.9 ppm; **LRMS** (+ESI) *m/z*: 398.5 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$

Cald for $C_{20}H_{29}N_3O_4$ $[M+Na]^+$: 398.20557, found 398.20480; **$[\alpha]_D^{27}$**: +29.8 (1.1, $CHCl_3$); **HPLC**: 97.0%, RT: 26.6 mins.

(S)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-hydroxypentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide **27**

A modified version of General procedure D was followed using benzyl ether **55** (150 mg, 330 µmol), palladium on carbon (10% w/w, 35 mg, 33 µmol) and palladium hydroxide on carbon (20% w/w, 23 mg, 33 µmol) in methanol (10 mL). The crude residue was purified by flash column chromatography using methanol, dichloromethane (1:19) as an eluent to obtain the title compound **27** (81 mg, 68%) as a white solid, **mp**: 90.1-92.2 °C; ***R*f**: 0.10 (1:19 methanol, dichloromethane); **IR** (ν_{max} (neat)): 3331, 3192, 2868, 1673, 1573, 1534, 1426, 1398, 1261, 1133 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.12 (9H, s), 1.31-1.41 (2H, m), 1.49-1.60 (2H, m), 1.80-1.90 (2H, m), 2.71 (1H, s), 3.53-3.64 (2H, m), 4.15-4.34 (2H, m), 4.72 (1H, d, *J* = 9.3 Hz), 6.03 (1H, bs), 6.88-7.01 (2H, m), 7.14 (1H, dd, *J* = 7.7, 4.9 Hz), 7.89 (1H, s), 8.27-8.36 (2H, m) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 23.1, 27.0, 29.8, 32.0, 34.9, 45.0, 60.0, 62.4, 108.7, 117.7, 118.6, 129.1, 131.2, 143.9, 147.7, 164.7, 173.8 ppm; **LRMS** (+ESI) *m/z*: 383.2 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{19}H_{28}N_4O_3$ $[M+Na]^+$: 383.20591, found 383.20516; **$[\alpha]_D^{27}$**: +24.8 (0.91, DMSO); **HPLC**: 98.7%, RT: 23.1 mins.

1-(5-Hydroxypentyl)-N-(2-phenylpropan-2-yl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide **29**

A modified version of General procedure D was followed using benzyl ether **56** (150 mg, 410 µmol), palladium on carbon (10% w/w, 44 mg, 41 µmol) and palladium hydroxide on carbon (20% w/w, 29 mg, 41 µmol) in a mixture of methanol (10 mL) and tetrahydrofuran (10 mL). The crude residue was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound **29** (106 mg, 71%) as a white solid, **mp**: 198.8-200.4 °C; ***R*f**: 0.25 (4:1 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3334, 2925, 2855, 1621, 1539, 1428, 1277, 760 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.33 (2H, tt, *J* = 9.6, 6.0 Hz), 1.42-1.52 (2H, m), 1.69 (6H s), 1.86 (2H, quin, *J* = 7.4 Hz), 3.35-3.44 (2H, m), 4.29 (2H, t, *J* = 7.2 Hz), 4.37 (1H, bs), 7.11-7.19 (2H, m), 7.27 (2H, dd, *J* = 8.5, 7.0 Hz), 7.38-7.44 (2H, m), 7.98 (1H, s), 8.28 (1H, dd, *J* = 4.6, 1.6 Hz), 8.33 (1H, dd, *J* = 7.9, 1.7 Hz), 8.45 (1H, s) ppm; **^{13}C NMR** (100 MHz, DMSO-d_6): δ 22.8, 29.5, 29.9, 32.0, 44.1, 55.0, 60.4, 108.8, 116.9, 119.0, 124.7, 125.6, 127.8, 129.5, 130.9, 143.1, 147.1, 148.5, 163.1 ppm; **LRMS** (+ESI) *m/z*: 366.3 ($[M+H]^+$ 16%), 388.2 ($[M+Na]^+$ 100%; **HRMS** $(ESI)^+$ Cald for $C_{22}H_{27}N_3O_2$ $[M+Na]^+$: 388.20010, found 388.20103; **HPLC**: 99.7%, RT: 23.3 mins.

2-Bromonicotinaldehyde **57**

Prepared according to the methods reported by Subota and co-workers.[36] The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound **57** (2.2 g, 37%) as a colourless oil which crystallised over time to form large colourless shards, the spectroscopic data of which corresponded to previously described.[36] **mp**: 74.6-76.6 °C; ***R*f**: 0.29 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3347, 3025, 2870, 1692, 1655, 1570, 1369, 1048 cm^{-1}; **LRMS** (+ESI) *m/z*: 240.0/242.0 ($[M+MeOH+Na]^+$ 100%) (observed as hemiacetal in methanol).

Ethyl 1H-pyrrolo[2,3-b]pyridine-2-carboxylate **59**

Synthesized according to the procedures reported by Cai and co-workers.[24] The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7) as an eluent to obtain the title compound **59** (660 mg, 55% over 2 steps) as a light green solid, the spectroscopic data of which corresponded to previously described.[37] **mp**: 160.8-164.2 °C; R_f: 0.36 (2:3 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 2976, 2969, 1707, 1522, 1424, 1271, 1208, 1023, 841, 769 cm^{-1}; **LRMS** (+ESI) *m/z*: 213.1 ($[M+Na]^+$ 100%).

Ethyl 1-pentyl-1H-pyrrolo[2,3-b]pyridine-2-carboxylate **60**

To a solution of **59** (100 mg, 530 µmol) in anhydrous *N,N*-dimethylformamide (5 mL) was added potassium carbonate (220 mg, 1.5 mmol) followed by 1-bromopentane (78 µL, 630 µmol) and the mixture stirred at 50 °C for 16 hours. After cooling to room temperature, water (20 mL) was added and the mixture was extracted with ethyl acetate (3 x 15 mL), washed with water (3 x 20 mL) and aqueous lithium chloride (1 M, 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19) as an eluent to obtain the title compound **60** (86 mg, 63%) as a colourless oil, R_f: 0.30 (1:19 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 2956, 1711, 1458, 1223, 1189, 1093, 747 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.85-0.93 (3H, m), 1.31-1.41 (4H, m), 1.44 (3H, t, *J* = 7.1 Hz), 1.76-1.88 (2H, m), 4.42 (2H, q, *J* = 7.1 Hz), 4.71-4.78 (2H, m), 7.12 (1H, dd, *J* = 7.9, 4.6 Hz), 7.27 (1H, s), 8.00 (1H, dd, *J* = 7.9, 1.6 Hz), 8.50 (1H, dd, *J* = 4.6, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.2, 14.5, 22.6, 29.2, 30.7, 43.5, 60.9, 108.3, 117.0, 118.6, 128.1, 130.9, 146.6, 149.2, 161.8 ppm; **LRMS** (+ESI) *m/z*: 261.2 ($[M+H]^+$ 100%).

1-Pentyl-1H-pyrrolo[2,3-b]pyridine-2-carboxylic acid **61**

General procedure B was followed using ethyl ester **60** (75 mg, 290 µmol) in ethanol (3 mL) to obtain the title compound **61** (61 mg, 91%) as an off-white solid, **mp**: 173 °C (decomposition); **IR** (ν_{max} (neat)): 3193, 2946, 2485, 1696, 1514, 1220, 1063 cm^{-1}; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 0.79 (3H, t, *J* = 7.0 Hz), 1.13-1.31 (4H, m), 1.70 (2H, quin, *J* = 7.5 Hz), 4.72 (2H, t, *J* = 7.3 Hz), 7.06 (1H, s), 7.11 (1H, dd, *J* = 7.9, 4.6 Hz), 8.02 (1H, dd, *J* = 7.9, 1.6 Hz), 8.35 (1H, dd, *J* = 4.6, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, DMSO-d_6): δ 13.9, 21.9, 28.5, 30.1, 42.2, 105.5, 116.3, 118.3, 129.8, 129.9, 144.7, 148.3, 165.2 ppm; **LRMS** (-ESI) *m/z*: 231.2 ($[M-H]^-$ 100%).

N-(Adamantan-1-yl)-1-pentyl-1H-pyrrolo[2,3-b]pyridine-2-carboxamide **30**

General procedure C was followed using carboxylic acid **61** (72 mg, 280 µmol) and 1-adamantylamine (51 mg, 340 µmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent to obtain the title compound **30** (60 mg, 59%) as a white solid, **mp**: 175.9-178.7 °C; R_f: 0.52 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3318, 3098, 2924, 1740, 1613, 1540, 1517, 1450, 1305 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.86 (3H, t, *J* = 6.9 Hz), 1.23-1.38 (4H, m), 1.68-1.83 (8H, m), 2.10-2.19 (9H, m), 4.64-4.72 (2H, m), 5.88 (1H, bs), 6.66 (1H, s), 7.07 (1H, dd, *J* = 7.9, 4.6 Hz), 7.89 (1H, dd, *J* = 7.9, 1.6 Hz), 8.41 (1H, dd, *J* = 4.7, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.2, 22.6, 29.2, 29.7, 30.7, 36.5, 41.9, 43.0, 52.7, 101.0, 116.7, 118.8, 129.7, 134.0, 145.2, 148.8, 161.8 ppm; **LRMS** (+ESI) *m/z*: 366.3 ($[M+H]^+$ 46%), 388.3 ($[M+Na]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{23}H_{31}N_3O$ $[M+Na]^+$: 388.23648, found 388.25383; **HPLC**: 99.6%, RT: 30.9 mins.

3-Acetylpyridine 1-oxide **62**

Prepared according to the procedures described by Roudesly and co-workers.[38] The crude residue was purified by flash column chromatography using methanol, ethyl acetate (1:9) as an eluent to obtain the title compound **62** (2.0 g, 58%) as a white solid, the spectroscopic data of which corresponded to previously described.[38] R_f: 0.23 (1:9 methanol, ethyl acetate); **LRMS** (+ESI) *m/z*: 160.1 ($[M+Na]^+$ 37%), 297.1 ($[2M+Na]^+$ 100%).

1-(2-Chloropyridin-3-yl)ethanone **63**

A solution of **62** (1.9 g, 13.9 mmol) in phosphorus oxychloride (23 mL, 249 mmol) was heated at 100 °C for 2 hours. Excess phosphorus oxychloride was removed under a stream of nitrogen and to the residue was added ice-water (200 mL) and the mixture extracted with diethyl ether (3 x 100 mL). The combined organic extracts were washed with water (2 x 100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The product was isolated by flash column chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound **63** (1.1 g, 50%) as a yellow oil, R_f: 0.55 (1:1 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3020, 1683, 1574, 1559, 1395, 1276, 1116, 1063, 803 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 2.69 (3H, s), 7.34 (1H, dd, *J* = 7.6, 4.8 Hz), 7.90 (1H, dd, 7.6, 2.0 Hz), 8.49 (1H, dd, *J* = 4.8, 2.0 Hz), 8.69 (1H, s) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$): δ 26.9, 124.7, 126.2, 135.8, 139.5, 142.4, 193.8 ppm.

3-Methyl-1H-pyrazolo[3,4-b]pyridine **64**

The pyridine **63** (1.0 g, 6.3 mmol) in ethylene glycol (5 mL) was heated at 165 °C for 16 hours. After cooling to room temperature, water was added (50 mL) and the mixture extracted with ethyl acetate (5 x 40 mL), washed with water (50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound **64** (680 mg, 81%) as a light yellow solid, **mp**: 144.9-150.7 °C; R_f: 0.20 (3:7 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3134, 3097, 2893, 1590, 1394, 1285, 916, 764 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 2.62 (3H, s), 7.12 (1H, dd, *J* = 8.0, 4.6 Hz), 8.05 (1H, dd, 8.0, 1.5 Hz), 8.60 (1H, dd, *J* = 4.7, 1.5 Hz), 8.69 (1H, s), 12.89 (1H, bs) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 12.6, 115.1, 116.2, 129.9, 142.5, 148.7, 152.8 ppm; **LRMS** (+ESI) *m/z*: 156.1 ($[M+Na]^+$ 100%).

3-Methyl-1-pentyl-1H-pyrazolo[3,4-b]pyridine **65**

General procedure A was followed using **64** (300 mg, 2.3 mmol) and 1-bromopentane (340 µL, 2.7 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:4) as an eluent to obtain the title compound **65** (210 mg, 46%) as a colourless oil, R_f: 0.38 (1:4 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 2929, 2859, 1599, 1574, 1500, 1459, 1388, 1267, 771 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.86 (3H, t, *J* = 7.0 Hz), 1.26-1.40 (4H, m), 1.87-1.98 (2H, m), 2.56 (3H, s), 4.41-4.47 (2H, m), 7.04 (1H, dd, *J* = 8.0, 4.5 Hz), 7.96 (1H, dd, *J* = 8.0, 1.6 Hz), 8.49 (1H, dd, *J* = 4.5, 1.6 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 12.5, 14.1, 22.5, 29.1, 29.8, 46.9, 115.1, 115.7, 129.4, 140.3, 148.6, 150.9 ppm; **LRMS** (+ESI) *m/z*: 204.2 ($[M+H]^+$ 100%); **HRMS** $(ESI)^+$ Cald for $C_{12}H_{17}N_3$ $[M+H]^+$: 204.15007, found 204.14946.

1-Pentyl-1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid **66**

To a suspension of **65** (300 mg, 1.5 mmol) in aqueous sodium hydroxide (1 M, 10.8 mL, 10.8 mmol) at 80 °C was added a solution of potassium permanganate (850 mg, 5.4 mmol) in water (10 mL) over 2 hours. The mixture was warmed to 90 °C and stirred for

an additional 2 hours. The mixture was filtered, hot, through a pad of Celite®, washed with dichloromethane (20 mL), acidified with aqueous hydrochloric acid (6 M) and extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound **66** (120 mg, 36%) as an orange gummy mass, **IR** (ν_{max} (neat)): 2955, 2929, 2553, 1702, 1581, 1501, 1252, 1177, 1146, 1124, 815 cm^{-1}; **^{1}H NMR** (400 MHz, MeOD): δ 0.84-0.90 (3H, m), 1.21-1.41 (4H, m), 1.93-2.03 (2H, m), 4.59 (2H, t, *J* = 7.1 Hz), 7.37 (1H, dd, *J* = 8.1, 4.5 Hz), 8.52 (1H, dd, *J* = 8.2, 1.7 Hz), 8.60 (1H, dd, *J* = 4.5, 1.7 Hz) ppm; **^{13}C NMR** (100 MHz, MeOD): δ 14.2, 23.2, 29.9, 30.4, 48.8, 116.7, 120.3, 132.8, 135.4, 150.6, 161.7, 164.7 ppm; **LRMS** (+ESI) *m/z*: 278.1 ([M+2Na-H]$^+$ 100%); **HRMS** (ESI)$^+$ Cald for $C_{12}H_{15}N_3O_2$ [M+2Na-H]$^+$: 278.08814, found 278.08478.

N-(Adamantan-1-yl)-1-pentyl-1H-pyrazolo[3,4-b]pyridine-3-carboxamide **31**
General procedure C was followed using carboxylic acid **66** (50 mg, 220 μmol) and 1-adamantylamine (41 mg, 270 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:4) as an eluent to obtain the title compound **31** (42 mg, 52%) as a white solid, **mp**: 121.1-123.5 °C; $\boldsymbol{R_f}$: 0.54 (3:7 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3259, 2900, 2846, 1658, 1541, 1275, 1155 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 7.0 Hz), 1.26-1.43 (4H, m), 1.66-1.80 (6H, m), 1.96 (2H, quin, *J* = 7.6 Hz), 2.11-2.22 (9H, m), 4.47-4.54 (2H, m), 6.80 (1H, bs), 7.20 (1H, dd, *J* = 8.1, 4.5 Hz), 8.53 (1H, dd, *J* = 4.5, 1.7 Hz), 8.65 (1H, dd, *J* = 8.1, 1.7 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.1, 22.4, 29.0, 29.6, 29.7, 36.6, 42.0, 47.7, 52.1, 114.8, 118.6, 132.3, 137.3, 149.1, 151.2, 161.5 ppm; **LRMS** (+ESI) *m/z*: 389.3 ([M+Na]$^+$ 100%), 755.4 ([2M+Na]$^+$ 53%); **HRMS** (ESI)$^+$ Cald for $C_{22}H_{30}N_4O$ [M+Na]$^+$: 389.23173, found 389.23101; **HPLC**: 99.2%, RT: 33.4 mins.

2,2,2-Trichloro-1-(1H-pyrrolo[2,3-b]pyridin-3-yl)ethanone **67**
To a solution of 7-azaindole (500 mg, 4.2 mmol) in anhydrous dichloromethane (20 mL) at 0 °C was added aluminium chloride (2.8 g, 21.2 mmol) portionwise and the mixture was stirred for 15 minutes before trichloroacetyl chloride (710 μL, 6.4 mmol) was added slowly. The mixture was warmed to room temperature and stirred for 4 hours then quenched with ice-cold water (80 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic extracts were washed with aqueous hydrochloric acid (1 M, 3 x 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound **67** (1.0 g, 91%) as a white solid, **mp**: 220 °C (decomposition); $\boldsymbol{R_f}$: 0.24 (1:1 ethyl acetate, hexane); **IR** (ν_{max} (neat)): 3139, 3011, 2833, 1661, 1411, 1292, 841, 742 cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6): δ 7.37 (1H, dd, *J* = 7.9, 4.7 Hz), 8.42 (1H, dd, *J* = 4.8, 1.7 Hz), 8.51 (1H, dd, *J* = 8.0, 1.7 Hz), 8.67 (1H, s), 13.19 (1H, bs) ppm; **^{13}C NMR** (75 MHz, DMSO-d_6): δ 79.2, 103.8, 119.1, 119.5, 129.9, 137.0, 145.1, 148.4, 176.6 ppm; **LRMS** (+ESI) *m/z*: 263.0/265.0 ([M+H]$^+$ 100%).

1H-Pyrrolo[2,3-b]pyridine-3-carboxylic acid **68**
A suspension of **67** (3.0 g, 11.4 mmol) in aqueous sodium hydroxide (3 M, 65 mL, 200 mmol) was stirred at room temperature for 6 hours. The solution was acidified with aqueous hydrochloric acid (6 M) and the precipitate obtained by filtration. The solid was dried *in vacuo* to obtain the title compound **68** (1.8 g, 100%) as an off-white powder, the spectroscopic data of which corresponded to previously described.[39]

N-(Adamantan-1-yl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide **69**

General procedure C was followed using carboxylic acid **68** (300 mg, 1.9 mmol) and 1-adamantylamine (340 mg, 2.2 mmol). The crude product was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound **69** (330 mg, 61%) as a white solid, **mp**: 274.1-280.2 °C; R_f: 0.35 (1:19 methanol, dichloromethane); **IR** (ν_{max} (neat)): 3322, 2905, 1626, 1531, 1416, 1302, 799, 773 cm^{-1}; **^{1}H NMR** (300 MHz, DMSO-d_6): δ 1.57-1.76 (6H, m), 1.99-2.17 (9H, m), 7.10-7.17 (2H, m), 8.18-8.26 (2H, m), 8.41 (1H, dd, *J* = 7.9, 1.7 Hz), 11.97 (1H, bs) ppm; **^{13}C NMR** (75 MHz, DMSO-d_6): δ 29.0, 36.2, 41.4, 51.0, 110.3, 116.6, 118.7, 127.9, 129.3, 143.2, 148.3, 163.6 ppm; **LRMS** (+ESI) *m/z*: 318.2 ($[M+Na]^+$ 100%).

N-(Adamantan-1-yl)-7-pentyl-7H-pyrrolo[2,3-b]pyridine-3-carboxamide **32**
A solution of **69** (50 mg, 170 μmol), 1-bromopentane (110 μL, 850 μmol) and tetrabutylammonium iodide (55 mg, 170 μmol) in a mixture of anhydrous toluene and acetonitrile (1:1, 3 mL) was heated at 150 °C in a sealed vessel for 16 hours. The volatiles were removed under a stream of nitrogen and the residue taken up in saturated methanolic ammonia, adsorbed to Celite® and purified by flash column chromatography using saturated methanolic ammonia, dichloromethane (0:1 to 1:49) as an eluent to obtain a solid which was recrystallised from isopropyl ether, hexane to afford the title compound **32** (37 mg, 60%) as a light yellow solid, **mp**: 158.1-160.0 °C; R_f: 0.31 (1:19 methanol, dichloromethane); **IR** (ν_{max} (neat)): 3269, 2904, 2849, 1618, 1558, 1456, 1358, 1307, 1178, 1145, 1096, 746 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.83-0.91 (3H, m), 1.30-1.40 (4H, m), 1.66-1.79 (6H, m), 1.98-2.08 (2H, m), 2.09-2.20 (9H, m), 4.67 (2H, t, *J* = 7.4 Hz), 5.64 (1H, bs), 7.04 (1H, dd, *J* = 7.6, 6.2 Hz), 7.67 (1H, dd, *J* = 6.2, 1.2 Hz), 8.06 (1H, s), 8.82 (1H, dd, *J* = 7.6, 1.2 Hz) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.0, 22.4, 28.9, 29.6, 29.8, 36.7, 42.4, 52.0, 53.9, 111.8, 112.2, 129.1, 130.2, 133.9, 145.0, 149.5, 165.0 ppm; **LRMS** (+ESI) *m/z*: 366.3 ($[M+H]^+$ 100%), 388.3 ($[M+Na]^+$ 43%), 731.4 ($[2M+H]^+$ 35%); **HRMS** (ESI)$^+$ Cald for $C_{23}H_{31}N_3O$ $[M+H]^+$: 366.25454, found 366.25392; **HPLC**: 96.2%, RT: 22.6 mins.

4.2 Biological Methods: Functional Activity in Membrane Potential Assay

Mouse AtT-20 neuroblastoma cells stably transfected with human CB_1 or human CB_2 were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U penicillin/streptomycin, and 80 μg/mL Hygromycin B.[40] Cells were passaged at 80% confluence, 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. The cells were plated in a volume of 90 μL in black-walled, clear-bottomed 96-well microplates (Corning) that 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.[41] The dye was reconstituted with assay buffer of the following 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, and glucose 5.56 (pH 7.4, osmolarity 315 ± 5 mOsm). 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 minutes. 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 seconds for at least 2 minutes, 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 negligible for drugs dissolved in assay buffer or DMSO. The final concentration of DMSO was not more than 0.1%. Data were analysed with PRISM (GraphPad Software Inc., SanDiego, CA), using four-parameter non-linear 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.

4.3 In Silico Studies

4.3.1 Target protein preparation

The X-ray crystal structures of the CB_1 and CB_2 proteins (PDB ID: 5TGZ and 5ZTY)[26-27] were obtained from the RCSB Protein Data Bank (https://www.rcsb.org/).
The protein structures were prepared using preparation and refinement protocols, directed by the Protein Preparation Wizard[42] embedded in Maestro v11.9 (Schrödinger, LLC, New York, USA). This process includes assigning bond orders, adding hydrogen atoms, creating zero-order bonds to metals and disulphide bonds, deleting water molecules beyond 5 Å from heteroatoms and filling in missing side chains using Prime v5.5.[43] Missing side chains and atoms were added using Prime for the both systems. The hydrogen bond network within the protein was also optimised with all het groups within the receptor grid bounding box previously removed and the protein structure minimised to a root mean square deviation (RMSD) of 0.3 Å using the OPLS3e force field.[42, 44]

4.3.2 Ligand preparation

All ligands were prepared using the LigPrep v4.9[45] module to generate possible low energy conformations of the ligands as well as generating all potential ionisation states at pH 7±2.

4.3.3 Docking studies

The ligands were docked into the receptor with Induced Fit Docking[46] with default settings. Standard protocol and OPLS3e force field[44] was used for the calculations. Box centre was set with the ligand bound to the reported protein structure. Receptor van der Waals scaling and Ligand van der Waals scaling was set to 0.50. Residues within 5 Å of ligand poses were refined, with optimisation of side chains. Ligands were re-docked into the protein structure with Extra Precision (XP)[47] to refine binding energy estimates. All ligands were docked with flexible states to allow sampling of the effect of nitrogen inversion, changing ring conformations and non-planar amide functional groups were penalised.
Prime MM-GBSA calculations[48] which combines molecular mechanics (MM) terms, and a generalised Born and surface area (GBSA) solvent mode,[32] was utilised to calculate the free energy of binding for the ligands. The output poses from Induced Fit Docking were used as the basis for these calculations. The calculations were performed using the

variable-dielectric generalised Born (VSGB)[49] solvation model and OPLS3 force field.[44] Residues within 20 Å from the ligand were set flexible for the calculation.

5. Supporting information available

Procedures for the preparation of **33**-**37** and corresponding characterization data. NOESY NMR of **32**. Docking study results for **7**, **8**, **20** and **21.** ^{1}H and ^{13}C NMR spectra of representative compounds from each chemotype. These materials are available in the supplementary data.

Conflict of interest

The authors declare no competing financial interest.

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Abbreviations used

EC_{50}, the molar concentration of an drug, which produces 50% of the maximum possible response; E_{max}, the maximum response achievable from an applied or dosed agent; CB, cannabinoid; GPCR, G-protein-coupled receptor; FLIPR, fluorescence imaging plate reader; DMF, *N,N*-dimethylformamide; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, hydroxybenzotriazole; TBAI, tetrabutylammonium iodide; *m*-CPBA, *meta*-chloroperoxybenzoic acid; LDA, lithium diisopropylamide; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; Boc, *tert*-butoxycarbonyl; DMP, Dess-Martin periodinane; DAST, diethylaminosulfur trifluoride; TM, transmembrane; MM-GBSA, molecular mechanics/generalized Born surface area**,** SEM, standard error of the mean; MW, molecular weight; PSA, polar surface area; QPPCaco, predicted Apparent Caco-2 Permeability; QPPMDCK, Predicted apparent MDCK cell permeability; QPlogBB, Predicted Brain/Blood Partition Coefficient; Eq., equivalents; Cald, calculated.

Appendix A. Supplementary data

Supplementary data to this article can be found online at

References

1. Lu, H.-C.; Mackie, K., An Introduction to the Endogenous Cannabinoid System. *Biol. Psychiatry* **2016,** *79* (7), 516-525.
2. Graham, E. S.; Ashton, J. C.; Glass, M., Cannabinoid Receptors: A brief history and what not. *Front. Biosci. (Landmark Ed)* **2009,** *14*, 944-57.
3. Mackie, K., Cannabinoid Receptors: Where They are and What They do. *J. Neuroendocrinol.* **2008,** *20* (s1), 10-14.
4. Munro, S.; Thomas, K. L.; Abu-Shaar, M., Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **1993,** *365* (6441), 61-65.

5. Tabrizi, M. A.; Baraldi, P. G.; Borea, P. A.; Varani, K., Medicinal Chemistry, Pharmacology, and Potential Therapeutic
Benefits of Cannabinoid CB2 Receptor Agonists. *Chem. Rev.* **2016,** *116* (2), 519-560.
6. Pacher, P.; Mechoulam, R., Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog. Lipid Res.* **2011,** *50* (2), 193-211.
7. Han, S.; Thatte, J.; Buzard, D. J.; Jones, R. M., Therapeutic Utility of Cannabinoid Receptor Type 2 (CB2) Selective Agonists. *J. Med. Chem.* **2013,** *56* (21), 8224-8256.
8. Frost, J. M.; Dart, M. J.; Tietje, K. R.; Garrison, T. R.; Grayson, G. K.; Daza, A. V.; El-Kouhen, O. F.; Yao, B. B.; Hsieh, G. C.; Pai, M.; Zhu, C. Z.; Chandran, P.; Meyer, M. D., Indol-3-ylcycloalkyl Ketones: Effects of N1 Substituted Indole Side Chain Variations on CB2 Cannabinoid Receptor Activity. *J. Med. Chem.* **2010,** *53* (1), 295-315.
9. Astles, P. C.; Guidetti, R.; Tidwell, M. W.; Hollinshead, S. P. Preparation of piperazinyl purine compounds for the treatment of pain. US20100160288A1, 2010.
10. Pasquini, S.; De Rosa, M.; Pedani, V.; Mugnaini, C.; Guida, F.; Luongo, L.; De Chiaro, M.; Maione, S.; Dragoni, S.; Frosini, M.; Ligresti, A.; Di Marzo, V.; Corelli, F., Investigations on the 4-Quinolone-3-carboxylic Acid Motif. 4. Identification of New Potent and Selective Ligands for the Cannabinoid Type 2 Receptor with Diverse Substitution Patterns and Antihyperalgesic Effects in Mice. *J. Med. Chem.* **2011,** *54* (15), 5444-5453.
11. Gijsen, H. J. M.; De Cleyn, M. A. J.; Surkyn, M. Benzimidazole derivatives as cannabinoid receptor agonists and their preparation, pharmaceutical compositions and use in the treatment of diseases. WO2008119694A1, 2008.
12. Carroll, W. A.; Dart, M. J.; Frost, J. M.; Latshaw, S. P.; Kolasa, T.; Li, T.; Peddi, S.; Liu, B.; Perez-Medrano, A.; Patel, M.; Wang, X.; Nelson, D. W. Heteroarylidene-benzamide derivatives as cannabinoid receptor ligands and their preparation, pharmaceutical compositions and use in the treatment of diseases. US20100069348A1, 2010.
13. Berry, A.; Riether, D.; Zindell, R.; Blumire, N. J. Preparation of 2-sulfamoyl-2-methylpropionamides as CB2 receptor modulators for treating diseases. WO2009061652A1, 2009.
14. Hickey, E. R.; Zindell, R.; Cirillo, P. F.; Wu, L.; Ermann, M.; Berry, A. K.; Thomson, D. S.; Albrecht, C.; Gemkow, M. J.; Riether, D., Selective CB2 receptor agonists. Part 1: The identification of novel ligands through computer-aided drug design (CADD) approaches. *Biorg. Med. Chem. Lett.* **2015,** *25* (3), 575-580.
15. Dhopeshwarkar, A.; Mackie, K., CB2 Cannabinoid receptors as a therapeutic target-what does the future hold? *Mol. Pharmacol.* **2014,** *86* (4), 430-437.
16. Banister, S. D.; Longworth, M.; Kevin, R.; Sachdev, S.; Santiago, M.; Stuart, J.; Mack, J. B. C.; Glass, M.; McGregor, I. S.; Connor, M.; Kassiou, M., Pharmacology of Valinate and tert-Leucinate Synthetic Cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and Their Analogues. *ACS Chem. Neurosci.* **2016,** *7* (9), 1241-1254.
17. Banister, S. D.; Moir, M.; Stuart, J.; Kevin, R. C.; Wood, K. E.; Longworth, M.; Wilkinson, S. M.; Beinat, C.; Buchanan, A. S.; Glass, M.; Connor, M.; McGregor, I. S.; Kassiou, M., 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. *ACS Chem. Neurosci.* **2015,** *6* (9), 1546-1559.
18. Banister, S. D.; Stuart, J.; Kevin, R. C.; Edington, A.; Longworth, M.; Wilkinson, S. M.; Beinat, C.; Buchanan, A. S.; Hibbs, D. E.; Glass, M.; Connor, M.; McGregor, I. S.; Kassiou, M., Effects of Bioisosteric Fluorine in Synthetic Cannabinoid Designer Drugs JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135. *ACS Chem. Neurosci.* **2015,** *6* (8), 1445-1458.

19. Longworth, M.; Banister, S. D.; Boyd, R.; Kevin, R. C.; Connor, M.; McGregor, I. S.; Kassiou, M., Pharmacology of Cumyl-Carboxamide Synthetic Cannabinoid New Psychoactive Substances (NPS) CUMYL-BICA, CUMYL-PICA, CUMYL-5F-PICA, CUMYL-5F-PINACA, and Their Analogues. *ACS Chem. Neurosci.* **2017,** *8* (10), 2159-2167.
20. Longworth, M.; Connor, M.; Banister, S. D.; Kassiou, M., Synthesis and Pharmacological Profiling of the Metabolites of Synthetic Cannabinoid Drugs APICA, STS-135, ADB-PINACA, and 5F-ADB-PINACA. *ACS Chem. Neurosci.* **2017,** *8* (8), 1673-1680.
21. Banister, S. D.; Connor, M., The Chemistry and Pharmacology of Synthetic Cannabinoid Receptor Agonist New Psychoactive Substances: Evolution. In *New Psychoactive Substances : Pharmacology, Clinical, Forensic and Analytical Toxicology*, Maurer, H. H.; Brandt, S. D., Eds. Springer International Publishing: Cham, 2018; pp 191-226.
22. Shi, Y.; Duan, Y.-H.; Ji, Y.-Y.; Wang, Z.-L.; Wu, Y.-R.; Gunosewoyo, H.; Xie, X.-Y.; Chen, J.-Z.; Yang, F.; Li, J.; Tang, J.; Xie, X.; Yu, L.-F., Amidoalkylindoles as Potent and Selective Cannabinoid Type 2 Receptor Agonists with in Vivo Efficacy in a Mouse Model of Multiple Sclerosis. *J. Med. Chem.* **2017,** *60* (16), 7067-7083.
23. Longworth, M.; Reekie, T. A.; Blakey, K.; Boyd, R.; Connor, M.; Kassiou, M., New-generation azaindole-adamantyl-derived synthetic cannabinoids. *Forensic Toxicol.* **2019**.
24. Cai, Q.; Li, Z.; Wei, J.; Ha, C.; Pei, D.; Ding, K., Assembly of indole-2-carboxylic acid esters through a ligand-free copper-catalysed cascade process. *Chem. Commun.* **2009,** (48), 7581-7583.
25. Champagne, P. A.; Desroches, J.; Paquin, J.-F., Organic Fluorine as a Hydrogen-Bond Acceptor: Recent Examples and Applications. *Synthesis* **2015,** *47* (03), 306-322.
26. Li, X.; Hua, T.; Vemuri, K.; Ho, J.-H.; Wu, Y.; Wu, L.; Popov, P.; Benchama, O.; Zvonok, N.; Locke, K. a.; Qu, L.; Han, G. W.; Iyer, M. R.; Cinar, R.; Coffey, N. J.; Wang, J.; Wu, M.; Katritch, V.; Zhao, S.; Kunos, G.; Bohn, L. M.; Makriyannis, A.; Stevens, R. C.; Liu, Z.-J., Crystal Structure of the Human Cannabinoid Receptor CB2. *Cell* **2019,** *176* (3), 459-467.
27. Hua, T.; Vemuri, K.; Pu, M.; Qu, L.; Han, G. W.; Wu, Y.; Zhao, S.; Shui, W.; Li, S.; Korde, A.; Laprairie, R. B.; Stahl, E. L.; Ho, J.-H.; Zvonok, N.; Zhou, H.; Kufareva, I.; Wu, B.; Zhao, Q.; Hanson, M. A.; Bohn, L. M.; Makriyannis, A.; Stevens, R. C.; Liu, Z.-J., Crystal Structure of the Human Cannabinoid Receptor CB1. *Cell* **2016,** *167* (3), 750-762.
28. Ahn, K. H.; Bertalovitz, A. C.; Mierke, D. F.; Kendall, D. A., Dual role of the second extracellular loop of the cannabinoid receptor 1: ligand binding and receptor localization. *Mol. Pharmacol.* **2009,** *76* (4), 833-42.
29. Bertalovitz, A. C.; Ahn, K. H.; Kendall, D. A., Ligand binding sensitivity of the extracellular loop two of the cannabinoid receptor 1. *Drug Dev. Res.* **2010,** *71* (7), 404-411.
30. Aung, M. M.; Griffin, G.; Huffman, J. W.; Wu, M.-J.; Keel, C.; Yang, B.; Showalter, V. M.; Abood, M. E.; Martin, B. R., Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB1 and CB2 receptor binding. *Drug Alcohol Depend.* **2000,** *60* (2), 133-140.
31. Hua, T.; Vemuri, K.; Nikas, S. P.; Laprairie, R. B.; Wu, Y.; Qu, L.; Pu, M.; Korde, A.; Jiang, S.; Ho, J.-H.; Han, G. W.; Ding, K.; Li, X.; Liu, H.; Hanson, M. A.; Zhao, S.; Bohn, L. M.; Makriyannis, A.; Stevens, R. C.; Liu, Z.-J., Crystal structures of agonist-bound human cannabinoid receptor CB1. *Nature* **2017,** *547*, 468.

32. Lyne, P. D.; Lamb, M. L.; Saeh, J. C., Accurate Prediction of the Relative Potencies of Members of a Series of Kinase Inhibitors Using Molecular Docking and MM-GBSA Scoring. *J. Med. Chem.* **2006,** *49* (16), 4805-4808.
33. Feng, Z.; Alqarni, M. H.; Yang, P.; Tong, Q.; Chowdhury, A.; Wang, L.; Xie, X. Q., Modeling, molecular dynamics simulation, and mutation validation for structure of cannabinoid receptor 2 based on known crystal structures of GPCRs. *J. Chem. Inf. Model.* **2014,** *54* (9), 2483-99.
34. Huestis, M. A., Human Cannabinoid Pharmacokinetics. *Chem. Biodivers.* **2007,** *4* (8), 1770-1804.
35. Hodgkinson, R. C.; Schulz, J.; Willis, M. C., Palladium-catalysed tandem alkenyl- and aryl-C–N bond formation: a cascade N-annulation route to 1-functionalised 7-azaindoles. *Tetrahedron* **2009,** *65* (44), 8940-8949.
36. Subota, A. I.; Artamonov, O. S.; Gorlova, A.; Volochnyuk, D. M.; Grygorenko, O. O., Approach to 5-substituted 6,7,8,9-tetrahydro-5H-pyrido[3,2-c]azepines. *Tetrahedron Lett.* **2017,** *58* (20), 1989-1991.
37. Baltus, C. B.; Jorda, R.; Marot, C.; Berka, K.; Bazgier, V.; Kryštof, V.; Prié, G.; Viaud-Massuard, M.-C., Synthesis, biological evaluation and molecular modeling of a novel series of 7-azaindole based tri-heterocyclic compounds as potent CDK2/Cyclin E inhibitors. *Eur. J. Med. Chem.* **2016,** *108*, 701-719.
38. Roudesly, F.; Veiros, L. F.; Oble, J.; Poli, G., Pd-Catalyzed Direct C–H Alkenylation and Allylation of Azine N-Oxides. *Org. Lett.* **2018,** *20* (8), 2346-2350.
39. Allegretti, M.; Anacardio, R.; Cesta, M. C.; Curti, R.; Mantovanini, M.; Nano, G.; Topai, A.; Zampella, G., A Practical Synthesis of 7-Azaindolylcarboxy-endo-tropanamide (DF 1012). *Org. Process Res. Dev.* **2003,** *7* (2), 209-213.
40. Knapman, A.; Santiago, M.; Connor, M., Buprenorphine signalling is compromised at the N40D polymorphism of the human μ opioid receptor in vitro. *Br. J. Pharmacol.* **2014,** *171* (18), 4273-4288.
41. Knapman, A.; Santiago, M.; Du, Y. P.; Bennallack, P. R.; Christie, M. J.; Connor, M., A Continuous, Fluorescence-based Assay of μ-Opioid Receptor Activation in AtT-20 Cells. *J. Biomol. Screen.* **2012,** *18* (3), 269-276.
42. Madhavi Sastry, G.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W., Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *J. Comput. Aided Mol. Des.* **2013,** *27* (3), 221-234.
43. Jacobson, M. P.; Pincus, D. L.; Rapp, C. S.; Day, T. J. F.; Honig, B.; Shaw, D. E.; Friesner, R. A., A hierarchical approach to all-atom protein loop prediction. *Proteins* **2004,** *55* (2), 351-367.
44. Harder, E.; Damm, W.; Maple, J.; Wu, C.; Reboul, M.; Xiang, J. Y.; Wang, L.; Lupyan, D.; Dahlgren, M. K.; Knight, J. L.; Kaus, J. W.; Cerutti, D. S.; Krilov, G.; Jorgensen, W. L.; Abel, R.; Friesner, R. A., OPLS3: A Force Field Providing Broad Coverage of Drug-like Small Molecules and Proteins. *J. Chem. Theory Comput.* **2016,** *12* (1), 281-296.
45. *Schrödinger Release 2019-2: LigPrep*, Schrödinger: LLC, New York, NY, 2019.
46. Sherman, W.; Day, T.; Jacobson, M. P.; Friesner, R. A.; Farid, R., Novel Procedure for Modeling Ligand/Receptor Induced Fit Effects. *J. Med. Chem.* **2006,** *49* (2), 534-553.
47. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T., Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein−Ligand Complexes. *J. Med. Chem.* **2006,** *49* (21), 6177-6196.

48. Jacobson, M. P.; Friesner, R. A.; Xiang, Z.; Honig, B., On the Role of the Crystal Environment in Determining Protein Side-chain Conformations. *J. Mol. Biol.* **2002,** *320* (3), 597-608.
49. Li, J.; Abel, R.; Zhu, K.; Cao, Y.; Zhao, S.; Friesner, R. A., The VSGB 2.0 model: a next generation energy model for high resolution protein structure modeling. *Proteins* **2011,** *79* (10), 2794-812.

Highlights:

- 2-Amidoalkylindoles are selective CB_2 agonists irrespective of amide substitution
- Amino acid derived amides improve potency and physicochemical properties
- 1-(5-Hydroxypentyl)-3-amidoalkyl-7-azaindoles are selective CB_2 agonists
- Docking studies explain the molecular basis for receptor subtype selectivity