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Synthesis and *in Vitro* Cannabinoid Receptor 1 Activity of Recently Detected Synthetic Cannabinoids 4F-MDMB-BICA, 5F-MPP-PICA, MMB-4en-PICA, CUMYL-CBMICA, ADB-BINACA, APP-BINACA, 4F-MDMB-BINACA, MDMB-4en-PINACA, A-CHMINACA, 5F-AB-P7AICA, 5F-MDMB-P7AICA, and 5F-AP7AICA

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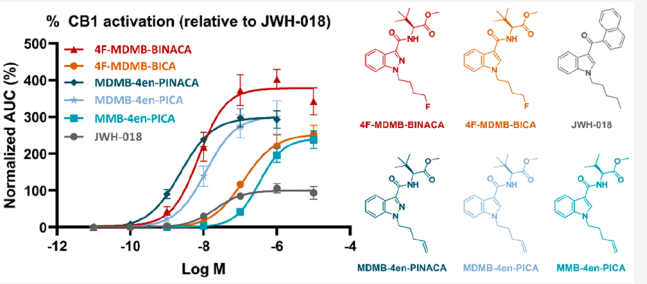
ABSTRACT: Synthetic cannabinoid receptor agonists (SCRAs) are an evolving class of new psychoactive substances (NPS) with structurally diverse compounds emerging each year. Due to the rapid pace at which these drugs enter the market, there is often little or nil information regarding the pharmacology of these substances despite widespread human use. In this study, 12 recently emerged SCRAs (reported between 2018 and 2020) were synthesized, analytically characterized, and pharmacologically evaluated using a live cell-based nanoluciferase complementation reporter assay that monitors *in vitro* cannabinoid receptor type 1 (CB1) activation via its interaction with β-arrestin 2 (βarr2). All synthesized SCRAs acted as agonists of CB1, although differences in potency (EC_{50} = 2.33−5475 nM) and efficacy (E_{max} = 37−378%) were noted, and several structure−activity relationships were identified. SCRAs featuring indazole cores (EC_{50} = 2.33−159 nM) were generally of equal or greater potency than indole analogues (EC_{50} = 32.9−330 nM) or 7-azaindole derivatives (EC_{50} = 64.0−5475 nM). Interestingly, with the exception of APP-BINACA (E_{max} = 75.7%) and 5F-A-P7AICA (E_{max} = 37.4%), all SCRAs showed greater efficacy than the historical SCRA JWH-018 to which responses were normalized (E_{max} = 142−378%). The most potent CB1 agonists in the study were ADB-BINACA (EC_{50} = 6.36 nM), 4F-MDMB-BINACA (EC_{50} = 7.39 nM), and MDMB-4en-PINACA (EC_{50} = 2.33 nM). Notably, all of these SCRAs featured an indazole core as well as a "bulky" *tert*-butyl moiety in the pendant amino acid side chain. This study confirms that recently detected SCRAs 4F-MDMB-BICA, 5F-MPP-PICA, MMB-4en-PICA, CUMYL-CBMICA, ADB-BINACA, APP-BINACA, 4F-MDMB-BINACA, MDMB-4en-PINACA, A-CHMINACA, 5F-AB-P7AICA, 5F-MDMB-P7AICA, and 5F-AP7AICA were all able to activate the CB1 receptor in vitro, albeit to different extents, and are potentially psychoactive in vivo. These results indicate that further evaluation of these widely used NPS is warranted to better understand the risks associated with human consumption of these drugs.



KEYWORDS: *cannabinoid, pharmacology, MDMB, CUMYL, BINACA, PINACA, CHMINACA, P7AICA*

INTRODUCTION

The field of new psychoactive substances (NPS) is constantly evolving, with new compounds entering the illicit drug market each year.[1] More than 290 synthetic cannabinoid receptor agonists (SCRAs) have been reported to the United Nations Office on Drugs and Crime (UNODC), representing one of the largest and most structurally diverse classes of NPS.[1,2] SCRAs were created as unregulated alternatives to cannabis, mimicking the effect of the main psychotropic constituent Δ^9-tetrahydrocannabinol (THC).[3,4] They exert their effects mainly via the cannabinoid (CB) receptors, with cannabinoid receptor type 1 (CB1) being mainly responsible for the psychoactive effects.[5] CB1 is a G protein-coupled receptor (GPCR) widely distributed in the central nervous system, while the CB2 subtype is predominantly found in immune cells.[6,7] Activation of CB1 induces an interaction of the receptor with the G protein (in this particular case $G\alpha_i$), resulting in a series of downstream signaling events.[8,9] Besides activation of the canonical G protein pathway, β-arrestin 2 (βarr2) is also recruited to the activated GPCR. This

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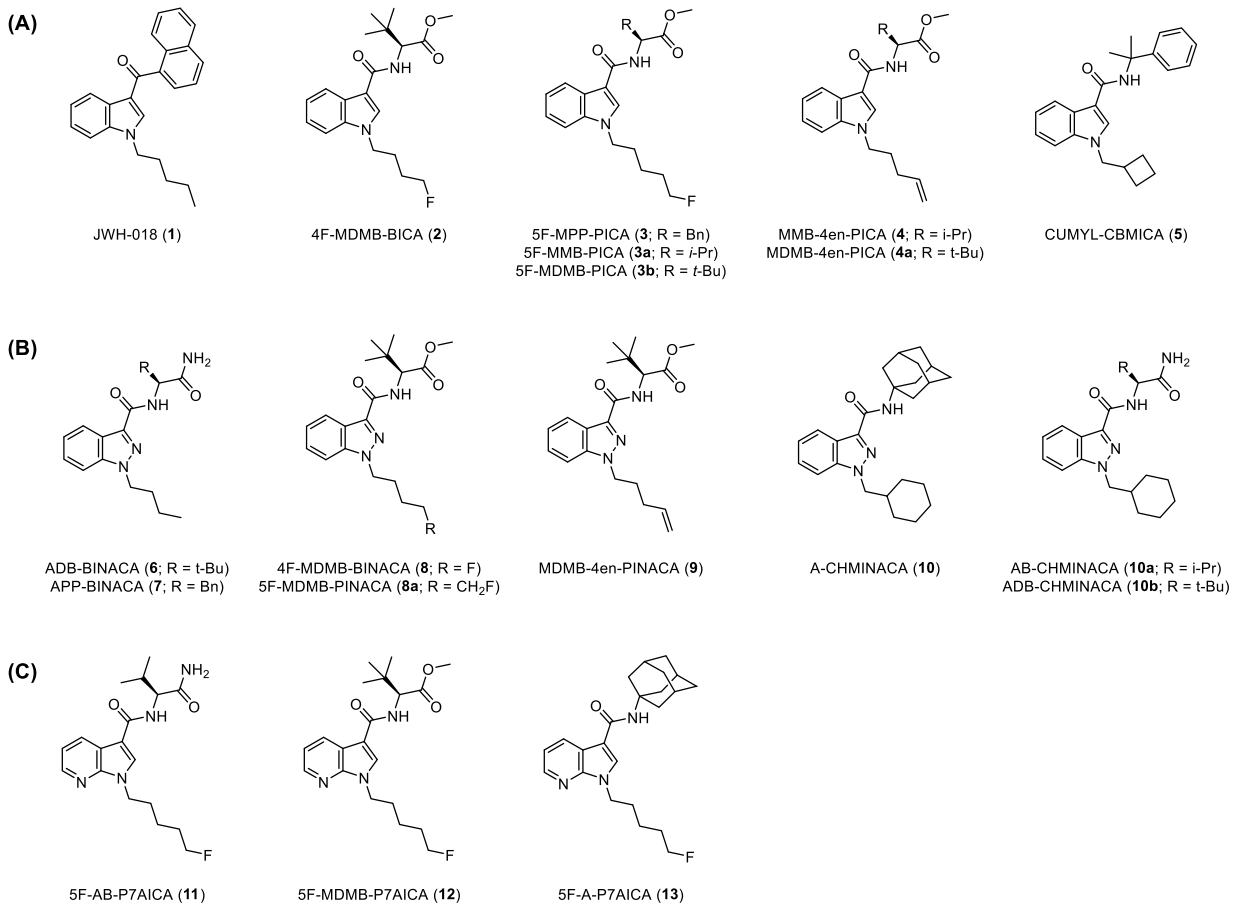

Figure 1. Chemical structures of the different SCRAs, divided based on their heterocyclic core: (A) indole SCRAs (including the reference JWH-018), (B) indazole SCRAs, and (C) 7-aza-indole SCRAs.

scaffolding protein can induce the desensitization and/or internalization of the receptor, and is responsible for a distinct set of signaling events.[8−10]

SCRAs often have greater potency and efficacy at CB receptors compared to THC, which acts as a partial agonist at both subtypes, which may partially explain the limited toxicity seen with the use of cannabis.[4] In contrast, SCRAs are linked to numerous health issues, including dependence, psychosis, seizures, organ toxicity, and death.[11−19] Many legislative approaches have been implemented at the national and international levels to control the manufacturing, trafficking, and possession of these substances.[11,20] However, increased control of specific SCRAs has had the perverse consequence of motivating the design, synthesis, and sale of novel compounds that circumvent existing controls.[2,21,22] Due to the rapid pace at which these substances enter the market, there is often little or no information regarding the pharmacology of these substances despite widespread human use.

To provide the first report of the chemistry and bioactivity of several prevalent SCRAs, a panel of 12 recent indole, indazole, and 7-azaindole SCRAs were synthesized and pharmacologically characterized in comparison to reference SCRA JWH-018 (**1**, Figure 1). The indole SCRAs include 4F-MDMB-BICA (4F-MDMB-BUTICA, 4F-MDMB-2201, **2**), 5F-MPP-PICA (MPhP-2201, 5F-MPhP-PICA, **3**), MMB-4en-PICA (AMB-4en-PICA, MMB-022, **4**), and CUMYL-CBMICA (**5**). The indazoles comprised ADB-BINACA (ADB-BUTINACA, **6**), APP-BINACA (APP-BUTINACA, PY, **7**), 4F-MDMB-BINACA (4F-MDMB-BUTINACA, **8**), MDMB-4en-PINACA ((pentyl-4en) MDMB-PINACA, **9**), and A-CHMINACA (ADAMANTYL-CHMINACA, SGT-37, AKB48CH, **10**). Finally, the 7-azaindoles consisted of 5F-AB-P7AICA (**11**), 5F-MDMB-P7AICA (7′N-5F-ADB, **12**), and 5F-A-P7AICA (**13**). Nothing is known about the pharmacology of any of these SCRAs, although the metabolic profiles of MMB-4en-PICA,[23] MDMB-4en-PINACA,[24,25] and 5F-MDMB-P7AICA[26,27] and the analytical characterization of CUMYL-CBMICA[28] and A-CHMINACA[29] were recently described. It is also worth noting that many of the novel amino acid-derived SCRAs described here were presumably inspired by the structures claimed in a patent awarded to Pfizer in 2009 for the development of cannabinoid agonists.[30]

All of the indole, indazole, and 7-azaindole SCRAs characterized herein were reported by the European Union (EU) Early Warning System (EWS) and/or NMS Laboratories between 2018 and 2020 (for tabulated detection data, please see the Supporting Information). 4F-MDMB-BICA (also known as 4F-MDMB-BUTICA, **2**) was identified in seized material in the European Union (EU) in March 2020[31,32] and the United States (US) in July 2020.[33] 4F-MDMB-BICA is the butyl homologue of 5-fluoropentyl-substituted 5F-MDMB-PICA (**3b**) and the indole analogue of indazole 4F-MDMB-BINACA (**8**), which were identified in 2016 and 2018, respectively. As both will be placed under Schedule II on the Convention on Psychotropic Substances of 1971 in November 2020, this substance could be a reaction to this. 5F-MPP-PICA (**3**, also known as MPhP-2201) was first detected in Slovenia in 2018 and was also reported in the US in 2019.[32,34,35] MMB-4en-PICA (**4**, also known as AMB-

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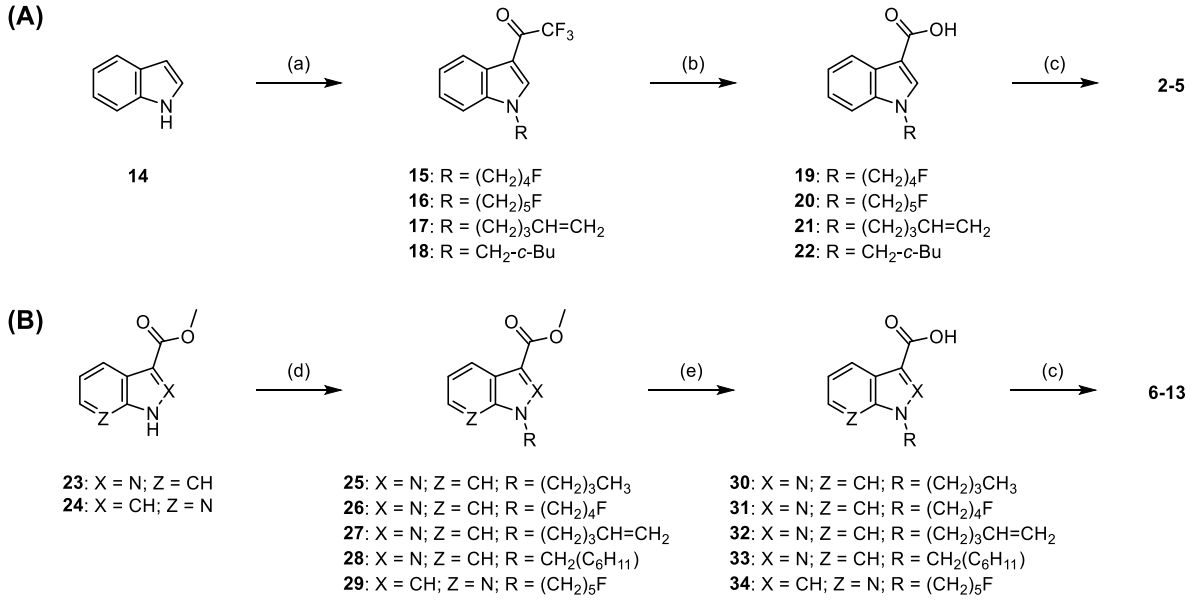

Figure 2. Reagents and conditions: (a) (i) NaH, RBr, DMF, 0 °C−rt, 1 h; (ii) $(CF_3CO)_2O$, 0 °C−rt, 2 h; (b) KOH, MeOH, PhMe, reflux, 2 h, 72−78% over 3 steps; (c) amine, EDC·HCl, HOBt·H_2O, Et_3N, DMF, rt, 18 h; 37−93%; (d) NaH, RBr, DMF, 0 °C−rt, 18 h, 39−58%; (e) 1 M aq. NaOH, MeOH, rt, 18−36 h, 82−94%.

4en-PICA and MMB022) was first identified in Europe in 2018 and represents one of the first SCRAs to incorporate the unsaturated 4-pentenyl substituent, as also present in one of the early SCRAs, JWH-022.[36] CUMYL-CBMICA (**5**) was identified in seized material in Germany in 2019 and is the first SCRA to feature a cyclobutylmethyl subunit, although such a group falls within the prophetic structures claimed in a 2014 patent.[28,37,38] ADB-BINACA (also known as ADB-BUTINACA, **6**) and APP-BINACA (also known as APP-BUTINACA, **7**) were first identified in Europe in 2019,[39,40] have been routinely found in the US since early 2019,[41] and are the butyl homologues of well-characterized SCRAs ADB-PINACA and APP-PINACA, respectively. 4F-MDMB-BINACA (4F-MDMB-BUTINACA, **8**), the butyl homologue of well-known SCRA 5F-MDMB-PINACA, has been widely detected in the UK, Europe, and the US since 2018.[42−45] MDMB-4en-PINACA (**9**) is an analogue of MMB-4en-PICA, featuring an indazole rather than an indole core and a pendant *tert*-leucinate group in place of a valinate group. MDMB-4en-PINACA was identified in mid-2018 in Europe and has appeared routinely in toxicology casework in the US since 2019.[46] A-CHMINACA (**10**) was identified in April 2018 in Germany and has also been identified in seized material in the US in 2018.[47,48] The 7-azaindoles 5F-MDMB-P7AICA (**11**) and 5F-AB-P7AICA (**12**) were identified in 2018,[49,50] while 5F-A-P7AICA (**13**) was identified in seized herbal material in Germany in 2019.[51]

All synthesized materials were analytically characterized using nuclear magnetic resonance spectroscopy (NMR), high resolution mass spectrometry (HRMS), and Fourier-transform infrared spectroscopy (FTIR) to confirm structure and purity. The synthesized compounds were pharmacologically evaluated using a live cell-based reporter assay that monitors the *in vitro* CB1 activation via its interaction with *β*arr2, an upstream signaling event, using a luminescent readout (Nanoluciferase Binary Technology). The activity of all compounds in this assay was compared to analogues featuring a single structural modification that were previously evaluated in the same system to offer structure−activity relationship insights where possible. These analogues include 5F-MMB-PICA (**3a**), 5F-MDMB-PICA (**3b**), MDMB-4en-PICA (**4a**), 5F-MDMB-PINACA (**8a**), AB-CHMINACA (**10a**), and ADB-CHMINACA (**10b**).[52−56] This allows us to better understand the pharmacology of these recent members of a structurally diverse class of NPS, which is crucial to prioritize responses by law enforcement agencies and policymakers.

RESULTS AND DISCUSSION

The different SCRAs evaluated in this study were synthesized according to the scheme shown in Figure 2 by adaptation of existing methods.[57,58] For indole SCRAs **2**−**5** (Figure 2A), indole (**14**) was alkylated with the appropriate alkyl bromide and then treated with trifluoroacetic anhydride to give intermediate *N*-alkyl-3-(trifluoroacetyl)indoles **15**−**18**. Hydrolysis of **15**−**18** under basic conditions afforded the corresponding carboxylic acids (**19**−**22**). Coupling of **19**−**22** to methyl L-valinate, methyl L-phenylalaninate, or cumylamine, respectively, provided 4F-MDMB-BICA (**2**), 5F-MPP-PICA (**3**), MMB-4en-PICA (**4**), and CUMYL-CBMICA (**5**). The synthesis of indazoles **6**−**10** and 7-azaindoles **11**−**13** required a different approach, depicted in Figure 2B. Methyl indazole-3-carboxylate (**23**) or methyl 7-azaindole-3-carboxylate (**24**) were alkylated with the appropriate alkyl bromide to give 1-alkyl ester derivatives **25**−**29**, which were subsequently saponified to the corresponding carboxylic acids **30**−**34**. Coupling of acids **30**−**34** with suitable amines using EDC yielded **6**−**13**. Commercial, enantiopure amino acid precursors were used (enantiomeric excess ≥99%), and racemization is mechanistically unlikely due to the achirality of the heterocyclic carboxylic acid precursors,[59] indicating retention of enantiomeric fidelity (*S*-enantiomers) for all chiral SCRA products.

All synthesized SCRAs were evaluated for CB1 agonist activity using a live cell-based reporter assay that monitors the *in vitro* CB1 activation via its interaction with *β*arr2.[56,60,61] The potencies (EC_{50} values) and efficacies (E_{max} values, relative to the reference JWH-018) of all SCRAs screened in this system are shown in Table 1, and full concentration response curves are

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Table 1. EC_{50} and E_{max} Values (the Latter Relative to JWH-018) Presented as a Measure of Potency and Efficacy, Respectively, Using a β-Arrestin 2 Recruitment Assay at CB1[a]

		EC_{50} (95% CI)	E_{max} (95% CI)
		indole SCRAs	
1	JWH-018	21.4 nM (10.9−47.5 nM)	100% (87.4−114 %)
2	4F-MDMB-BICA	121 nM (69.9−253 nM)	253% (224−298 %)
3	5F-MPP-PICA	32.9 nM (19.6−59.8 nM)	303% (269−354 %)
3a	*5F-MMB-PICA*	*135 nM (97.6−187 nM)*[c]	*312% (287−336%)*[c]
3b	*5F-MDMB-PICA*	*3.26 nM (2.07−5.15 nM)*[c]	*331% (311−351%)*[c]
4	MMB-4en-PICA	330 nM (174−578 nM)	241% (211−278 %)
4a	*MDMB-4en-PICA*	*11.5 nM (5.12−33.2 nM)*[b]	*302% (256−391%)*[b]
5	CUMYL-CBMICA	62.9 nM (31.1−123 nM)	153% (135−177 %)
		indazole SCRAs	
6	ADB-BINACA	6.36 nM (2.88−11.9 nM)	290% (260−322 %)
7	APP-BINACA	833 nM (508−2543 nM)	75.7% (65.7−108 %)
8	4F-MDMB-BINACA	7.39 nM (3.51−13.4 nM)	378% (342−417 %)
8a	*5F-MDMB-PINACA*	*1.78 nM (0.72−4.11 nM)*[d]	*331% (293−406%)*[d]
		0.84 nM (0.52−1.24 nM)[e]	*319% (291−354%)*[e]
9	MDMB-4en-PINACA	2.33 nM (1.32−4.19 nM	299% (272−329 %)
10	A-CHMINACA	159 nM (101−321 nM)	318% (282−360%)
10a	*AB-CHMINACA*	*6.16 nM (4.49−8.35 nM)*[d]	*324% (307−344%)*[d]
		6.1 nM (3.1−11.4 nM)[f]	
10b	*ADB-CHMINACA*	*1.49 nM (0.69−2.61 nM)*[f]	
		7-aza-indole SCRAs	
11	5F-MDMB-P7AICA	64.0 nM (29.7−126 nM)	297% (260−345 %)
12	5F-AB-P7AICA	5475 nM (3486−9148 nM)	142% (127−167 %)
13	5F-A-P7AICA	86.1 nM (44.1−306 nM)	37.4% (31.1−97.3 %)

Italics denote previously reported compounds. [a]Data are given as EC_{50}/E_{max} values (95% confidence interval (CI)). [b]Data were extracted from ref 52. [c]Data were extracted from ref 53. [d]Data were extracted from ref 54. [e]Data were extracted from ref 55. [f]Data were extracted from ref 56.

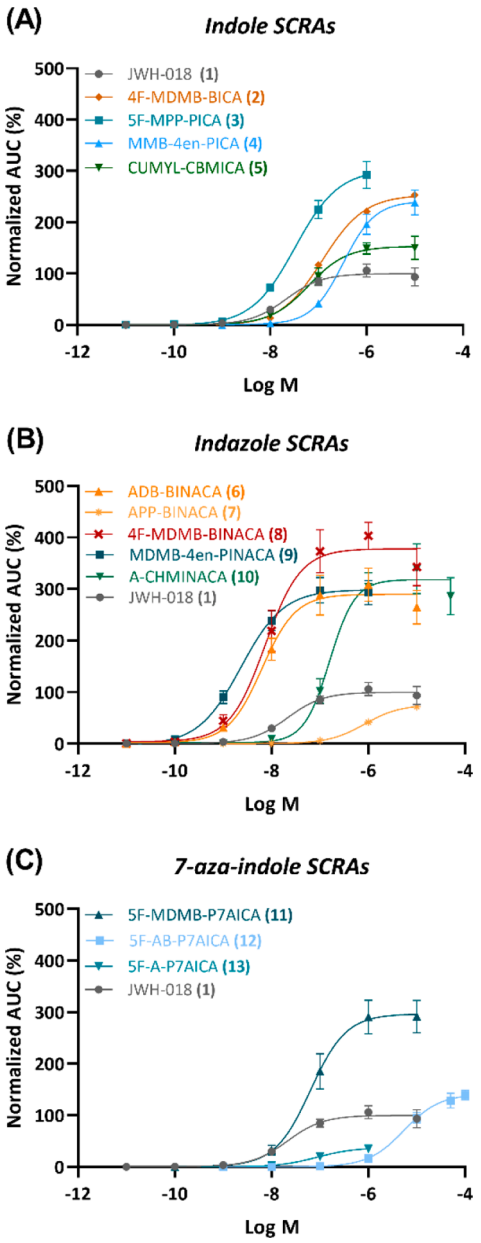

Figure 3. Concentration-dependent interaction of CB1 with β-arrestin 2 upon stimulation with the different SCRAs, divided based on their heterocyclic core: (A) indole, (B) indazole, and (C) 7-aza-indole. Data are given as mean receptor activation ± SEM ($n = 3$), normalized to the E_{max} of JWH-018 (= 100%).

shown in Figure 3. SCRA categorization was based on the heterocyclic core (see Figure 1). This region likely plays an important role in CB1 receptor activation. Existing structure−activity relationships for SCRAs show a general potency trend whereby indazoles are equally or more potent than indoles, which are in turn more potent than 7-azaindoles, subject to equivalent substitution of the N-1 position and amide group.[57,58,62,63] This trend was also observed in this study for this set of SCRAs, with the indazoles generally showing lower or equivalent EC_{50} value ranges (2.33−833 nM) compared to the indoles (32.9−330 nM), with both of these being generally greater than the 7-azaindoles (64.0−5475 nM) regardless of substituents (Table 1, shift to the left in Figure 2).

The first three SCRAs are indole-carboxamides, denoted by the -ICA suffix. MMB-4en-PICA (**4**) had a greatly reduced potency (EC_{50} = 330 nM, Table 1) compared to that of MDMB-4en-PICA (**4a**, EC_{50} = 11.5 nM) in the same functional CB1 assay.[52] The observation that a "bulky" *tert*-butyl moiety in the *tert*-leucinate MDMB-4en-PICA leads to a higher potency than the valinate MMB-4en-PICA was also seen in a previous study with the close structural analogues 5F-MMB-PICA (**3a**, EC_{50} = 135 nM) and 5F-MDMB-PICA (**3b**, EC_{50} = 3.26 nM).[53] Also, other reports found that most *tert*-leucinate functionalized SCRAs were more potent CB1 agonists than the corresponding valinate analogues,[54,55,57,62,63] suggesting that this region is important for receptor activation and therefore influences the potency.[54,64]

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The appearance of the phenylalaninate analogue 5F-MPP-PICA (**3**) in Slovenia in 2018[34] shows that SCRA manufacturers are continuing to innovate with exploration of the amino acid moiety. 5F-MPP-PICA showed a potency (EC_{50} = 32.9 nM) intermediate to those of 5F-MDMB-PICA (**3b**, EC_{50} = 3.26 nM) and 5F-MMB-PICA (**3a**, EC_{50} = 136 nM)[53] using the same functional CB1 assay. This might indicate that the phenyl group, like the *tert*-butyl moiety in *tert*-leucinate-functionalized SCRAs, is still able to interact with the TM2 and displace the N-terminal part of the CB1 receptor, although less optimally than the *tert*-butyl moiety. Truncation of the alkyl chain of 5F-MDMB-PICA gives the butyl homologue 4F-MDMB-BICA (**2**, EC_{50} = 121 nM), which showed a dramatic reduction in potency compared to that of 5F-MDMB-PICA (**3b**, EC_{50} = 3.26 nM). In terms of efficacy, 5F-MPP-PICA (**3**) showed a high E_{max} value of 303% (95% CI 269−354%), relative to the E_{max} of the reference JWH-018 (100%). This is similar to what we observed earlier for 5F-MMB-PICA (E_{max} = 312%; 95% CI 287−336%) and 5F-MDMB-PICA (E_{max} = 331%; 95% CI 311−351%),[53] and the 4-fluorobutyl homologue of the latter was similarly efficacious (**2**, E_{max} = 253%).

Overall, the potency (EC_{50}) and efficacy (E_{max}) of MMB-4en-PICA (**4**, EC_{50} = 330 nM; 241%) and 5F-MMB-PICA (**3a**, EC_{50} = 135 nM; E_{max} = 312%) were relatively similar. This suggests that substitution of the 5-fluoropentyl group for the pent-4-ene tail is well-tolerated by CB1.[52] This is not surprising as Kumar et al.[64] demonstrated, by using the structure of the CB1-G_i signaling complex bound to the highly potent agonist MDMB-FUBINACA, that the *p*-fluorobenzyl moiety of MDMB-FUBINACA is most likely involved in hydrophobic interactions with receptor elements within a conserved docking region (described as a narrow side pocket). It was suggested that this narrow pocket is an important region influencing ligand affinity rather than receptor activation, as previous studies had noted that switching the alkyl tail with a heteroaryl moiety gave compounds with similar receptor affinity and potency.[54,63,64]

CUMYL-CBMICA (**5**) was only detected for the first time in 2019[37] and represents the first example of a new SCRA comprising the cyclobutylmethyl group as a pendant moiety from the heterocyclic core. Cumylamine-derived SCRAs have been pharmacologically investigated previously and typically demonstrate potency and efficacy as CB1 agonists.[58,65−69] The incorporation of a cyclobutylmethyl group was tolerated for CB1 activation, and CUMYL-CBMICA demonstrated a potency that was comparable to those of the other indole SCRAs described here, although its efficacy was lower (EC_{50} = 62.9 nM and E_{max} = 153%).

Moving from an indole to an indazole heterocyclic core for an analogously substituted SCRA was anticipated to improve potency based on previous structure−activity relationships. ADB-BINACA (**6**) is an indazole structurally related to the previously mentioned MDMB-4en-PICA and 5F-MDMB-PICA, containing a *tert*-leucinamide group rather than a *tert*-leucinate group, and a truncated N-butyl chain rather than a 4-pentenyl or 5-fluoropentyl moiety, respectively. ADB-BINACA was also found to be a potent and efficacious CB1 agonist (EC_{50} = 6.36 nM; E_{max} = 290%) in this assay, while the related phenylalaninamide analogue APP-BINACA (**7**) showed greatly reduced potency and efficacy (EC_{50} = 833 nM; E_{max} = 75.7%).

4F-MDMB-BINACA (**8**) is a truncated analogue of the widely detected 5F-MDMB-PINACA (**8a**), containing a 4-fluorobutyl group in place of the 5-fluoropentyl substituent of the latter. Previous studies using the same functional CB1 assay showed that 5F-MDMB-PINACA is a potent CB1 agonist with EC_{50} values in the low nanomolar range (EC_{50} = 0.84−1.78 nM) and E_{max} values of greater than 300%.[54,55] Similar potency and efficacy values were also found for 4F-MDMB-BINACA (**8**, EC_{50} = 7.39 nM; E_{max} = 378%), indicating that 4F-MDMB-BINACA is a potent and efficacious CB1 agonist. The results in this study also matched the results from other independently performed studies using the same βarr2 recruitment assay (EC_{50} = 1.74−5.69 nM; E_{max} = 254−317%).[61,70,71]

MDMB-4en-PINACA (**9**) (the indazole analogue of MDMB-4en-PICA, **4a**) showed high potency and efficacy at CB1, relative to the reference compound JWH-018 in this system (EC_{50} = 2.33 nM; E_{max} = 299%). These results match those found for MDMB-4en-PINACA in other studies using the same cell system (1.11−2.47 nM; 226−239%).[52,70]

The recently detected A-CHMINACA (**10**) is an adamantyl analogue of previously screened valinamide (AB-CHMINACA, **10a**) and *tert*-leucinamide (ADB-CHMINACA, **10b**) SCRAs. In previous reports using the same βarr2 recruitment assay, both AB- and ADB-CHMINACA were found to be potent CB1 agonists, with EC_{50} values in the low nanomolar range (EC_{50} = 6.1−6.16 nM and 1.49 nM, respectively).[54,56] A-CHMINACA (**10**) was less potent (EC_{50} = 159 nM), although a similar efficacy (E_{max} = 318%) was found compared to AB-CHMINACA (E_{max} = 324%).

As increasing numbers of indole and indazole SCRAs have been subjected to control measures, SCRA manufacturers have started to modify the heterocyclic core using "scaffold hopping" in an attempt to create new SCRAs that retain *in vivo* CB1 agonist profiles while circumventing structure-based SCRA legislation.[2,3,58] Several 7-azaindole-3-carboxamide-type ("-7AICA") SCRAs have been reported in the NPS marketplace, with three of the most recent examples included in this study (Figure 1C and Table 1).

We previously showed indole 5F-MDMB-PICA (**3b**) and indazole 5F-MDMB-PINACA (**8a**) to be potent CB1 agonists, with EC_{50} values in the low nanomolar range (EC_{50} = 3.26 nM and 0.84−1.78 nM, respectively) and E_{max} values greater than 300%.[53−55] In comparison, the 7-azaindole analogue 5F-MDMB-P7AICA (**12**) had reduced potency but similar efficacy values (EC_{50} = 64.0 nM; E_{max} = 297%). Similarly, the valinamide-derived 7-azaindole 5F-AB-P7AICA (**11**) showed a dramatically reduced potency (EC_{50} = 5475 nM) as well as a reduced efficacy (E_{max} = 142%), compared to the corresponding indazole counterpart, 5F-AB-PINACA (EC_{50} = 55.4−65.5 nM; E_{max} = 217−268%), previously evaluated in the same functional CB1 assay.[53,55]

In our functional CB1 assay using βarr2 recruitment, the adamantyl 7-azaindole derivative 5F-A-P7AICA (**13**) showed only moderate potency (EC_{50} = 86.1 nM) and low efficacy (E_{max} = 37.4%) relative to the reference compound JWH-018. Again, this difference can be attributed to the difference in the heteroaromatic core (7-aza-indole vs indazole). Also, A-CHMINACA (**10**), which has the same pendant amide moiety, showed only moderate potency (EC_{50} = 159 nM), although that compound was still highly efficacious (E_{max} = 318%).

CONCLUSION

In this study, 12 recently emerged SCRAs were synthesized and pharmacologically characterized using a live cell-based reporter assay that monitors the *in vitro* CB1 activation via its interaction with βarr2. All synthesized SCRAs acted as agonists of CB1, although there were differences in potency and efficacy. This

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study confirmed a general potency trend whereby indazole SCRAs are equally or more potent than indoles, which are in turn more potent than 7-azaindoles. Also, a "bulky" *tert*-butyl moiety in the headgroup contributes to the potency of an SCRA. It is therefore not surprising that the most potent SCRAs within this tested panel are the *tert*-leucinamide and *tert*-leucinate indazole SCRAs ADB-BINACA (**6**), MDMB-4en-PINACA (**9**), and 4F-MDMB-BINACA (**8**). These findings can help to contribute to a better understanding of the pharmacology this structurally diverse class of NPS, which is crucial to prioritizing responses by scientists, health workers, and policy makers.

MATERIAL AND METHODS

Chemicals. JWH-018 (1-pentyl-3-(1-naphthoyl)indole) was purchased from Cayman Chemical Company (Ann Arbor, MI, US). Dulbecco's modified Eagle's medium (GlutaMAX), Opti-MEM I Reduced Serum Medium, penicillin−streptomycin (5000 U/mL), and amphotericin B (250 μg/mL) were purchased from Thermo Fisher Scientific (Pittsburgh, PA, US). Fetal bovine serum (FBS) and poly-D-lysine were supplied by Sigma-Aldrich (Overijse, Belgium). The Nano-Glo Live Cell reagent, which was used for the readout of the bioassay, was procured from Promega (Madison, WI, US). All other compounds were synthesized and analytically characterized in the laboratory of Dr. Banister at the University of Sydney.

General Chemical Synthesis and Analysis Details. All reactions were performed under an atmosphere of nitrogen unless otherwise specified. All reagents and reactants were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia) and used as purchased. The enantiomeric excess (ee) of chiral amino acid precursors was 99% (as determined by gas liquid chromatography, GLC) in the certificates of analysis (COA). Deuterated solvents (CD_3OD, $CDCl_3$, and DMSO-d_6) were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, US) and used as is. Other reagents were purchased and used as is from various commercial sources.

Analytical thin-layer chromatography was performed using Merck aluminum-backed silica gel 60 F254 (0.2 mm) plates (Merck, Darmstadt, Germany), which were visualized using shortwave (254 nm) UV fluorescence. Flash chromatography was performed using a Biotage Isolera Spektra One and Biotage SNAP KP-Sil silica cartridges (Uppsala, Sweden), with gradient elution terminating at the solvent combination indicated for each compound (vide infra).

Melting point ranges (mp) were measured in open capillaries using a Stuart SMP50 Automated melting point apparatus (Cole-Palmer, Staffordshire, UK) and are uncorrected.

Nuclear magnetic resonance spectra were recorded at 298 K using a Bruker AVANCE DRX400 (400.1 MHz) spectrometer (Bruker, Bremen, Germany). The data are reported as chemical shift (δ ppm) relative to the residual protonated solvent resonance, relative integral, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, quart. = quartet, quin. = quintet, m = multiplet), coupling constants (J Hz) and assignment. Assignment of signals was assisted by correlation spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple-bond correlation (HMBC) experiments where necessary.

LC-MS/UV data were acquired using a Shimadzu (Kyoto, Japan) Nexera LC-30AD UHPLC system coupled to a Shimadzu SPD-20AV photo diode array detector and a Shimadzu LCMS-8040 triple-quadrupole mass spectrometer, equipped with an electrospray ionization (ESI) source. Compounds were dissolved in a 90:10 mixture of 0.1% formic acid in water and methanol and placed in the UHPLC autosampler that was maintained at 8 °C. Elution was performed in gradient mode with an Agilent (CA, US) Zorbax XDB-C18 column (2.1 × 50 mm, 3.5 μm particle size), held at 50 °C, with a 10 μL injection volume. The mobile phases were 0.1% formic acid in water (A) and methanol (B). Mobile phase composition was held at 10% B until 0.5 min and then steadily increasing to 100% B at 2.5 min, holding until 3 min, and then re-equilibrating at 10% B for a total run time of 4 min. UV absorbance was measured from 190 to 800 nm. UV data from blank injections were subtracted to account for mobile phase absorbance and background noise. Single stage mass spectra (MS1) were collected in positive ESI mode using a single quadrupole (Q3) scanning from m/z 100−600. All data were processed using Shimadzu LabSolutions (v 5.89) software.

High resolution mass spectrometry data were collected using an Agilent LC 1260-QTOF/MS 6550 (Santa Clara, CA, US). A methanolic extract of each pure standard was run using an electrospray ionization source in automated MS/MS (information dependent acquisition) mode. Accurate mass for the parent ion and its corresponding mass error expressed in parts per million (ppm) are reported.

FTIR spectra were collected using a Bruker Alpha II ATR FTIR spectrophotometer with the following parameters: 24 sample scans, resolution = 4, phase resolution = 32.

General Procedure A: Amidation of 1-Alkylindole-3-carboxyic Acids, 1-Alkyl-1*H*-indazole-3-carboxylic acids, and 1-(5-Fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylic Acid. To a solution of the appropriate 1-alkylindole-3-carboxyic acid (**19**−**22**, 1.00 mmol), 1-alkyl-1*H*-indazole-3-carboxylic acid (**30**−**33**, 1.00 mmol), or 1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylic acid (**34**, 1.00 mmol) in DMF (4 mL) was added the suitable amine reactant (1.10 mmol, 1.1 equiv), HOBt·H_2O (169 mg, 1.10 mmol, 1.1 equiv), and EDC·HCl (288 mg, 1.50 mmol, 1.5 equiv). The mixture was treated dropwise with Et_3N (488 427 μL, 3.50 mmol, 3.5 equiv) and allowed to stir for 24 h. The mixture was poured onto H_2O (100 mL) and extracted with EtOAc (3 × 15 mL); the combined organic layers were washed with H_2O (2 × 10 mL) and brine (1 × 10 mL) and dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The products were obtained following purification by flash chromatography.

Methyl (*S*)-2-(1-(4-Fluorobutyl)-1*H*-indole-3-carboxamido)-3,3-dimethylbutanoate (4F-MDMB-BICA, 4F-MDMB-BUTICA, 2). Subjecting 1-(4-fluorobutyl)-1*H*-indole-3-carboxylic acid (**19**, 235 mg, 1.00 mmol) and methyl (*S*)-2-amino-3,3-dimethylbutanoate hydrochloride (200 mg, 1.10 mol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane:EtOAc 90:10 to 70:30), **2** as a white solid (135 mg, 37%). mp 86−88 °C; R_f 0.52 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (s, 1H), 8.10 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 8.1 Hz, 1H), 7.25−7.18 (m, 1H), 7.18−7.09 (m, 1H), 4.49 (d, J = 8.7 Hz, 1H), 4.47 (dt, J = 47.4, 6.0 Hz, 2H), 4.31−4.19 (m, 2H), 3.66 (s, 3H), 1.97−1.85 (m, 2H), 1.76−1.58 (m, 3H), 1.03 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.0 (CO), 164.4 (CO), 136.1 (quat.), 131.6 (CH), 126.8 (quat.), 122.1 (CH), 121.2 (CH), 120.8 (CH), 110.4 (CH), 108.8 (quat.), 83.5 (d, $^1J_{CF}$ = 161.8 Hz, CH_2), 60.0 (CH), 51.5 (CH_3), 45.6 (CH_2), 33.9 (quat.), 27.3 (d, $^2J_{CF}$ = 19.6 Hz, CH_2), 26.8 (3 × CH_3), 25.8 (d, $^3J_{CF}$ = 4.9 Hz, CH_2); ν_{max} (cm^{-1}): 3313, 2953, 1731, 1537, 1511, 1459, 1386, 1271, 1213, 1157, 1123, 1033, 895, 736, 623, 559; LCMS (ESI, + ve) m/z 363.3 ($[M + H]^+$); HRMS (ESI) $C_{20}H_{27}FN_2O_3$ exact mass 362.2006, accurate mass 362.2002 (mass error −1.04 ppm).

Methyl (*S*)-2-(1-(5-Fluoropentyl)-1*H*-indole-3-carboxamido)-3-phenylpropanoate (5F-MPP-PICA, 3). Subjecting 1-(5-fluoropentyl)-1*H*-indole-3-carboxylic acid (**20**, 249 mg, 1.00 mmol) and methyl L-phenylalaninate hydrochloride (237 mg, 1.10 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane:EtOAc, 85:15 to 70:30), **3** as an off-white solid (346 mg, 84%). mp 114−116 °C; R_f 0.53 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dt, J = 7.9, 1.0 Hz, 1H), 7.69 (s, 1H), 7.37 (dt, J = 8.3, 1.0 Hz, 1H), 7.32−7.14 (m, 7H), 6.41 (d, J = 7.5 Hz, 1H), 5.17 (dt, J = 7.5, 5.5 Hz, 1H), 4.42 (dt, J = 47.2, 5.9 Hz, 2H), 4.15 (t, J = 7.1 Hz, 2H), 3.78 (s, 3H), 3.30 (qd, J = 13.8, 5.5 Hz, 2H), 1.97−1.85 (m, 2H), 1.80−1.63 (m, 2H), 1.51−1.39 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7 (CO), 164.6 (CO), 136.7 (quat.), 136.3 (quat.), 131.9 (CH), 129.6 (2 × CH), 128.7 (2 × CH), 127.3 (CH), 125.5 (quat.), 122.7 (CH), 121.7 (CH), 120.4 (CH), 110.6 (quat.), 110.3 (CH), 83.8 (d, $^1J_{CF}$ = 165.0 Hz, CH_2), 53.3 (NCH_2), 52.5 (NCH), 46.9 (CH_3), 38.3 (CH_2), 30.1 (d, $^2J_{CF}$ = 19.8 Hz, CH_2),

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ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

29.8 (CH_2), 23.0 (d, $^3J_{CF}$ = 5.0 Hz, CH_2); ν_{max} (cm^{-1}): 2947, 1688, 1601, 1534, 1465, 1357, 1257, 1166, 1134, 961, 801, 787, 756, 718, 697, 621, 567, 549; LCMS (ESI, + ve) m/z 411.3 ($[M + H]^+$); HRMS (ESI) $C_{24}H_{27}FN_2O_3$ exact mass 410.2006, accurate mass 410.2013 (mass error 1.86 ppm).

Methyl (*S*)-2-(1-(Pent-4-en-1-yl)-1*H*-indole-3-carboxamido)-3-methylbutanoate (MMB-4en-PICA, AMB-4en-PICA, 4). Subjecting 1-(pent-4-en-1-yl)-1*H*-indole-3-carboxylic acid (**21**, 344 mg, 1.50 mmol) and methyl (*S*)-2-amino-3-methylbutanoate hydrochloride (300 mg, 1.65 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane:EtOAc, 85:15 to 60:40), **4** as a white solid (386 mg, 75%). mp 85−87 °C; R_f 0.61 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03−7.97 (1H, m), 7.74 (1H, s), 7.44−7.36 (1H, m), 7.31−7.27 (2H, m), 6.47 (1H, d, J = 8.7 Hz), 5.85−5.75 (1H, m), 5.09−5.03 (2H, m), 4.87 (1H, dd, J = 8.7, 4.8 Hz), 4.16 (2H, t, J = 7.1 Hz), 3.79 (3H, s), 2.35−2.27 (1H, m), 2.10 (2H, q, J = 7.5 Hz), 1.98 (2H, quin., J = 7.7 Hz), 1.05 (6H, d, J = 9.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3 (CO), 165.0 (CO), 137.0 (CH), 136.7 (quat.), 131.9 (CH), 125.5 (quat.), 122.6 (CH), 121.8 (CH), 120.3 (CH), 116.2 (CH_2), 110.8 (quat.), 110.5 (CH), 57.0 (CH), 52.3 (CH_3), 46.2 (NCH_2), 31.9 (CH), 30.8 (CH_2), 29.0 (CH_2), 19.3 (CH_3), 18.2 (CH_3). ν_{max} (cm^{-1}): 3331, 2962, 1739, 1629, 1508, 1466, 1388, 1279, 1229, 1175, 1146, 984, 911, 813, 780, 750, 636, 566, 541; LCMS (ESI, + ve) m/z 343.3 ($[M + H]^+$); HRMS (ESI) $C_{20}H_{26}N_2O_3$ exact mass 342.1943, accurate mass 342.1952 (mass error 2.49 ppm).

1-(Cyclobutylmethyl)-*N*-(2-phenylpropan-2-yl)-1*H*-indole-3-carboxamide (CUMYL-CBMICA, 5). Subjecting 1-(cyclobutylmethyl)-1*H*-indole-3-carboxylic acid (**22**, 115 mg, 0.50 mmol) and cumylamine (79 μL, 0.55 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane:EtOAc, 87.5:12.5 to 62.5:37.5), **5** as a white solid (96 mg, 56%). mp 179−181 °C; R_f 0.54 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (1H, d, J = 7.3 Hz), 7.68 (1H, s), 7.55−7.49 (1H, m), 7.42−7.21 (7H, m), 6.26 (1H, s), 4.12 (2H, d, J = 7.2), 2.85 (1H, sept., J = 7.5 Hz), 2.13−2.03 (2H, m), 1.99−1.89 (2H, m), 1.87 (6H, s), 1.85−1.74 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3 (CO), 147.4 (quat.), 136.8 (quat.), 131.7 (CH), 128.4 (2 × CH), 126.5 (CH), 125.0 (quat.), 124.8 (2 × CH), 122.2 (CH), 121.3 (CH), 119.6 (CH), 111.7 (quat.), 110.4 (CH), 56.0 (quat.), 51.8 (NCH_2), 35.5 (CH), 29.7 (2 x CH_3), 26.3 (2 x CH_2), 18.1 (CH_2); ν_{max} (cm^{-1}): 2932, 1617, 1540, 1386, 1363, 1279, 1246, 1204, 1169, 1112, 1071, 915, 828, 745, 697, 604, 573, 545, 509; LCMS (ESI, + ve) m/z 347.3 ($[M + H]^+$); HRMS (ESI) $C_{23}H_{26}N_2O$ exact mass 346.2045, accurate mass 346.2046 (mass error 0.11 ppm).

(*S*)-*N*-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-butyl-1*H*-indazole-3-carboxamide (ADB-BINACA, ADB-BUTINACA, 6). Subjecting 1-butyl-1*H*-indazole-3-carboxylic acid (**30**, 88 mg, 0.40 mmol) and 2-amino-3,3-dimethylbutanamide hydrochloride (70 mg, 0.42 mmol, 1.05 equiv) to general procedure A gave, following purification by flash chromatography (CH_2Cl_2:MeOH, 100:0 to 90:10), **6** as a white powder (69 mg, 52%). mp 148.2−148.4 °C; R_f 0.37 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, $CDCl_3$,) δ 8.30 (dt, J = 8.2, 1.0 Hz, 1H), 7.65 (d, J = 9.4 Hz, 1H), 7.43−7.36 (m, 2H), 7.27−7.22 (m, 1H), 5.93 (s, 1H), 5.45 (s, 1H), 4.52 (d, J = 9.4 Hz, 1H), 4.38 (t, J = 7.2 Hz, 2H), 1.96−1.87 (m, 2H), 1.44−1.28 (m, 2H), 1.14 (s, 9H), 0.95 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.8 (CO), 161.0 (CO), 140.7 (quat.), 136.3 (quat.), 126.7 (CH), 122.5 (CH), 121.9 (CH), 121.6 (quat.), 110.5 (CH), 58.6 (NCH), 48.6 (NCH_2), 34.6 (quat.), 31.5 (CH_2), 26.6 (3 × CH_3), 19.4 (CH_2), 13.5 (CH_3); ν_{max} (cm^{-1}): 2958, 1694, 1647, 1529, 1488, 1365, 185, 828, 747, 592; LCMS (ESI, + ve) m/z 331.3 ($[M + H]^+$); HRMS (ESI) $C_{18}H_{26}N_4O_2$ exact mass 330.2056, accurate mass 330.2064 (mass error 2.43 ppm).

(*S*)-*N*-(1-Amino-1-oxo-3-phenylpropan-2-yl)-1-butyl-1*H*-indazole-3-carboxamide (APP-BINACA, APP-BUTINACA, 7). Subjecting 1-butyl-1*H*-indazole-3-carboxylic acid (**30**, 218 mg, 1.00 mmol) and methyl L-phenylalaninate hydrochloride (281 mg, 1.40 mmol, 1.40 equiv) to general procedure A gave, following purification by flash chromatography (CH_2Cl_2:MeOH, 100:0 to 90:10), **7** as a white powder (337 mg, 93%). mp 91.5−92.5 °C; R_f 0.76 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (m, J = 8.2, 1.0 Hz, 1H), 7.52−7.46 (m, 1H), 7.41−7.39 (m, 2H), 7.34−7.25 (m, 5H), 7.23−7.20 (m, 1H), 5.99 (s, 1H), 5.31 (s, 1H), 4.95−4.88 (m, 1H), 4.45−4.31 (m, 2H), 3.32−3.16 (m, 2H), 1.90 (quin., J = 7.2 Hz, 2H), 1.35 (sext., J = 7.7 Hz, 2H), 0.95 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.6 (CO), 161.3 (CO), 140.5 (quat.), 137.6 (quat.), 136.4 (quat.), 129.3 (2 × CH), 128.0 (2 × CH), 126.6 (CH), 126.3 (CH), 122.4 (CH), 122.0 (CH), 121.6 (quat.), 110.4, 53.2 (CH), 48.4 (CH_2), 37.7 (CH_2), 31.4 (CH_2), 19.4 (CH_2), 13.5 (CH_3); ν_{max} (cm^{-1}): 3323, 2932, 1696, 1672, 1642, 1531, 1492, 1397, 1324, 1231, 1196, 1131, 909, 840, 780, 746, 698, 562; LCMS (ESI, + ve) m/z 365.3 ($[M + H]^+$); HRMS (ESI) $C_{21}H_{24}N_4O_2$ exact mass 364.1899, accurate mass 364.1907 (mass error 2.15 ppm).

Methyl (*S*)-2-(1-(4-Fluorobutyl)-1*H*-indazole-3-carboxamido)-3,3-dimethylbutanoate (4F-MDMB-BINACA, 4F-MDMB-BUTINACA, 8). Subjecting 1-(4-fluorobutyl)-1*H*-indazole-3-carboxylic acid (**31**, 238 mg, 1.00 mmol) and methyl (*S*)-2-amino-3,3-dimethylbutanoate hydrochloride (209 mg, 1.10 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane:EtOAc, 100:0 to 90:10), **8** as a colorless oil (255 mg, 68%). R_f 0.31 (hexane:EtOAc, 80:20); ^{1}H NMR (400 MHz, CD_3OD) δ 8.20 (d, J = 8.3, 1.1 Hz, 1H), 7.67 (d, J = 8.5, 1.1 Hz, 1H), 7.47 (dt, J = 15.5, 7.7 Hz, 1H), 7.29 (dt, J = 15.0, 7.4 Hz, 1H), 4.61−4.59 (s, 1H), 4.57 (t, J = 7.1 Hz, 2H), 4.46 (dt, J = 47.4, 6.0 Hz, 2H), 3.77 (s, 3H), 2.10 (quin., J = 7.3 Hz, 2H), 1.72 (d quin., 2H), 1.09 (s, 9H), NH proton not observed (exchangeable); ^{13}C NMR (101 MHz, CD_3OD) δ 173.1 (CO), 164.2 (CO), 142.5 (quat.), 137.5 (quat.), 128.2 (CH), 124.0 (CH), 123.9 (CH), 123.0 (quat), 111.1 (CH), 84.4 (d, $^1J_{CF}$ = 164.1 Hz, CH_2F), 61.3 (CH), 52.4 (OCH_3), 49.6 (CH_2), 35.6 (quat.), 28.8 (d, $^2J_{CF}$ = 20.1 Hz, CH_2), 27.0 (3 × CH_3), 26.9 (d, $^3J_{CF}$ = 4.8 Hz, CH_2); ν_{max} (cm^{-1}): 3414, 2961, 1730, 1666, 1523, 1491, 1369, 1331, 1211, 1166, 1005, 939, 837, 777, 753, 541; LCMS (ESI, + ve) m/z 364.2 ($[M + H]^+$); HRMS (ESI) $C_{19}H_{26}FN_3O_3$ exact mass 363.1958, accurate mass 363.1965 (mass error 1.86 ppm).

Methyl (*S*)-2-(1-(Pent-4-en-1-yl)-1*H*-indazole-3-carboxamido)-3,3-dimethylbutanoate (MDMB-4en-PINACA, 9). Subjecting 1-(pent-4-en-1-yl)-1*H*-indazole-3-carboxylic acid (**32**, 230 mg, 1.00 mmol) and methyl (*S*)-2-amino-3,3-dimethylbutanoate hydrochloride (200 mg, 1.10 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane:EtOAc, 95:5 to 85:15), **9** as a colorless oil (246 mg, 57%). R_f 0.49 (hexane:EtOAc, 80:20); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (1H, d, J = 8.2 Hz), 7.55 (1H, d, J = 9.7 Hz), 7.44−7.38 (1H, m), 7.31−7.23 (2H, m), 5.88−5.78 (1H, m), 5.12−5.01 (2H, m), 4.73 (1H, d, J = 9.7 Hz), 4.41 (2H, t, J = 6.7), 3.76 (3H, s), 2.19−2.03 (4H, m), 1.09 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3 (CO), 162.5 (CO), 141.0 (quat.), 137.2 (CH), 136.9 (quat.), 126.8 (CH), 123.1 (quat.), 123.0 (CH), 122.7 (CH), 116.1 (CH_2), 109.4 (CH), 59.6 (CH), 51.9 (CH_3), 48.8 (CH_2), 35.2 (quat.), 30.9 (CH_2), 28.9 (CH_2), 26.8 (3 × CH_3); ν_{max} (cm^{-1}): 3413, 2961, 1729, 1667, 1520, 1490, 1367, 1329, 1214, 1166, 988, 910, 833, 775, 755, 555; LCMS (ESI, + ve) m/z 358.3 ($[M + H]^+$); HRMS (ESI) $C_{20}H_{27}N_3O_3$ exact mass 357.2052, accurate mass 357.2061 (mass error 2.34 ppm).

***N*-(Adamantan-1-yl)-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide (A-CHMINACA, 10).** Subjecting 1-(cyclohexylmethyl)-1*H*-indazole-3-carboxylic acid (**33**, 77 mg, 0.30 mmol) and adamantan-1-amine (49 mg, 0.32 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane:EtOAc, 95:5 to 91:9), **10** as a colorless oil (96 mg, 82%) R_f 0.95 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (dt, J = 8.2, 1.0 Hz, 1H), 7.39−7.36 (m, 2H), 7.25−7.20 (m, 1H), 6.80 (s, 1H), 4.17 (d, J = 7.2 Hz, 2H), 2.24−2.12 (m, 9H), 2.07−1.93 (m, 1H), 1.81−1.57 (m, 11H), 1.29−1.13 (m, 3H), 1.09−0.98 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 164.0 (quat.), 143.0 (quat.), 138.7 (quat.), 127.8 (CH), 123.6 (CH), 123.5 (quat.), 123.2 (CH), 111.2 (CH), 56.3 (NCH_2), 53.1 (quat.), 42.7 (3 × CH_2), 40.1 (CH), 37.5 (3 × CH_2), 31.9 (2 × CH_2), 31.0 (3 × CH), 27.4 (CH), 26.8 (2 × CH_2); ν_{max} (cm^{-1}): 2905, 2849, 1738, 1662, 1524, 1491, 1449, 1358, 1291, 1276, 1226, 1175, 1137, 1046, 1004, 858, 777, 748; LCMS (ESI+) m/z 392.3 ($[M + H]^+$); LCMS (ESI, + ve) m/z 392.3 ([M + H]); HRMS

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ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

(ESI) $C_{25}H_{33}N_3O$ exact mass 391.2624, accurate mass 391.2621 (mass error −0.68 ppm).

(*S*)-*N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxamide (5F-AB-P7AICA, 11). Subjecting 1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylic acid (**34**, 125 mg, 0.50 mmol) and (*S*)-2-amino-3-methylbutanamide hydrochloride (84 mg, 0.55 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane:EtOAc, 60:40 to 0:100), **11** as a white solid (103 mg, 59%). mp 188.5−189.5 °C; R_f 0.17 (hexane:EtOAc, 20:80); ^{1}H NMR (400 MHz, CD_3OD) δ 8.48 (d, J = 8.0, 1.4 Hz, 1H), 8.33 (d, J = 4.9, 1.4 Hz, 1H), 8.23 (s, 1H), 7.25 (dd, J = 8.0, 4.8, 1.1 Hz, 1H), 4.49−4.33 (m, 5H), 2.21 (m, 1H), 1.97 (quin., J = 7.3 Hz, 2H), 1.82−1.67 (m, 2H), 1.51−1.41 (m, 2H), 1.08 (d, J = 6.7 Hz, 6H), NH and NH_2 not observed (exchangeable); ^{13}C NMR (101 MHz, CD_3OD) δ 176.8 (CO), 166.8 (CO), 148.6 (quat.), 144.5 (CH), 132.7 (CH), 131.2 (CH), 120.8 (quat.), 118.5 (CH), 109.5 (quat.), 84.6 (d, $^1J_{CF}$ = 163.9 Hz, CH_2), 59.7 (NCH), 45.9 (NCH_2), 32.1 (CH), 31.1 (d, $^2J_{CF}$ = 19.8 Hz, CH_2), 30.9 (CH), 23.6 (d, $^3J_{CF}$ = 5.2 Hz, CH_2), 19.9 (CH_2), 18.9 (2 × CH_3); ν_{max} (cm^{-1}): 3297, 2945, 1620, 1516, 1426, 1314, 1267, 1163, 1135, 980, 800, 777, 747, 665, 571; LCMS (ESI, + ve) m/z 349.3 ($[M + H]^+$); HRMS (ESI) $C_{18}H_{25}FN_4O_2$ exact mass 348.1962, accurate mass 348.1968 (mass error 1.85 ppm).

Methyl (*S*)-2-(1-(5-Fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxamido)-3,3-dimethylbutanoate (5F-MDMB-P7AICA, 12). Subjecting 1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylic acid (**34**, 250 mg, 1.00 mmol) and methyl (*S*)-2-amino-3,3-dimethylbutanoate hydrochloride (200 mg, 1.10 mmol, 1.1 equiv) to general procedure A gave, following purification by flash chromatography (hexane:EtOAc, 90:10 to 80:20), **12** as a white solid (270 mg, 72%). mp 62−64 °C; R_f0.59; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (dd, J = 4.8, 1.5 Hz, 1H), 8.33 (dd, J = 8.0, 1.5 Hz, 1H), 7.83 (s, 1H), 7.23 (dd, J = 7.9, 4.7 Hz, 1H), 6.42 (d, J = 9.4 Hz, 1H), 4.76 (d, J = 9.3 Hz, 1H), 4.52−4.29 (m, 4H), 3.77 (s, 3H), 2.02−1.90 (m, 2H), 1.83−1.65 (m, 2H), 1.53−1.41 (m, 1H), 1.08 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9 (CO), 164.2 (CO), 147.6 (quat.), 143.9 (CH), 131.0 (CH), 128.9 (CH), 118.3 (quat.), 117.8 (CH), 109.2 (quat.), 83.9 (d, $^1J_{CF}$ = 164.8 Hz, CH_2), 59.8 (NCH), 52.1 (OCH_3), 45.1 (NCH_2), 35.3 (quat.), 30.1 (d, $^2J_{CF}$ = 19.8 Hz, CH_2), 30.0 (CH_2), 26.9 (3 × CH_3), 22.8 (d, $^3J_{CF}$ = 5.2 Hz, CH_2); ν_{max} (cm^{-1}): 3314, 2955, 1739, 1612, 1568, 1538, 1516, 1426, 1397, 1268, 1217, 1157, 1133, 1035, 991, 862, 800, 775, 635, 564; LCMS (ESI, + ve) m/z 378.3 ($[M + H]^+$); HRMS (ESI) $C_{20}H_{28}FN_3O_3$ exact mass 377.2115, accurate mass 377.2121 (mass error 1.79 ppm).

***N*-(Adamantan-1-yl)-1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]-pyridine-3-carboxamide (5F-A-P7AICA, 13).** Subjecting 1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylic acid (**34**, 119 mg, 0.476 mmol) and adamantan-1-amine (88 mg, 0.58 mmol, 1.2 equiv) to general procedure A gave, following purification by flash chromatography (hexane:EtOAc, 85:15 to 75:25), **13** as a white solid (94 mg, 51%). mp 129.6−130.5 °C;R_f0.59 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43−8.39 (m, 1H), 8.31−8.26 (m, 2H), 7.19−7.15 (m, 2H), 4.49−4.44 (m, 1H), 4.35 (td, J = 6.1, 1.4 Hz, 1H), 4.27 (t, J = 7.1 Hz, 2H), 2.07 (d, J = 11.8 Hz, 9H), 1.86 (quin., J = 7.2 Hz, 2H), 1.74−1.60 (m, 8H), 1.33 (quin., J = 7.8 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.3 (CO), 147.1 (quat.), 143.0 (CH), 130.6 (CH), 129.6 (CH), 119.0 (quat.), 116.9 (CH), 109.3 (quat.), 83.6 (d, $^1J_{CF}$ = 161.7 Hz, CH_2), 51.1 (quat.), 44.4 (NCH_2), 36.2 (CH_2), 29.3 (d, $^2J_{CF}$ = 19.1 Hz, CH_2), 29.3 (CH_2), 29.0 (CH), 22.0 (d, $^3J_{CF}$ = 5.4 Hz, CH_2); ν_{max} (cm^{-1}): 2903, 2849, 1620, 1535, 1425, 1398, 1358, 1277, 1252, 1170, 1140, 976, 854, 800, 777, 750, 546; LCMS (ESI, + ve) m/z 384.3 ($[M + H]^+$); HRMS (ESI) $C_{23}H_{30}FN_3O$ exact mass 383.2373, accurate mass 383.2382 (mass error 2.33 ppm).

General Procedure B: Synthesis of 1-Alkylindole-3-carboxylic Acids. To a cooled (0 °C) mixture of NaH (50.0 mmol, 2.0 equiv) in DMF (40 mL) was added a solution of indole (**14**, 25.0 mmol, 1.0 equiv) in DMF (5 mL) dropwise. The mixture was warmed to ambient temperature, stirred for 0.5 h, cooled to 0 °C, and treated dropwise with the appropriate alkyl bromide (26.25 mmol, 1.05 equiv). The mixture was stirred for 1 h at ambient temperature, cooled to 0 °C, and treated portionwise with trifluoroacetic anhydride (62.5 mmol, 2.5 equiv). The solution was stirred at ambient temperature for 2 h and quenched by pouring onto vigorously stirred ice−water (1 L). The precipitate was collected by vacuum filtration, washed with H_2O (3 × 100 mL), and allowed to air-dry for 24 h. The crude material was dissolved in PhMe (40 mL) and added dropwise to a refluxing solution of KOH (82.5 mmol, 3.3 equiv) in MeOH (20 mL). The solution was heated at reflux for 2 h, cooled to ambient temperature, and H_2O (100 mL) was added. The layers were separated, and the organic layer was extracted with 1 M aq. KOH (3 × 20 mL). The combined aqueous layers were acidified to pH 1 with 10 M aq. HCl and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and solvent evaporated under reduced pressure. The crude solids were purified by recrystallization from *i*-PrOH.

1-(4-Fluorobutyl)-1*H*-indole-3-carboxylic Acid (19). Subjecting indole (**14**, 2.93 g, 25.0 mmol) and 1-bromo-4-fluorobutane (2.82 mL, 26.25 mmol, 1.05 equiv) to general procedure B gave **19** as an off white solid (3.62 g, 67%). mp 132−134 °C; R_f 0.56 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (s, 1H), 8.08 (s, 1H), 8.05−7.99 (m, 1H), 7.58 (dd, J = 7.8, 1.2 Hz, 1H), 7.28−7.14 (m, 2H), 4.43 (dt, J = 47.4, 6.0 Hz, 2H), 4.29 (t, J = 7.0 Hz, 2H), 1.86 (quin., J = 7.1 Hz, 2H), 1.68−1.51 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.7 (CO), 136.3 (quat.), 135.3 (CH), 126.5 (quat.), 122.3 (CH), 121.3 (CH), 120.9 (CH), 110.8 (CH), 106.5 (quat.), 83.4 (d, $^1J_{CF}$ = 161.7 Hz, CH_2), 45.6 (CH_2), 27.1 (d, $^2J_{CF}$ = 19.6 Hz, CH_2), 25.5 (d, $^3J_{CF}$ = 5.0 Hz, CH_2); ν_{max} (cm^{-1}): 2948, 1655, 1524, 1490, 1469, 1430, 1395, 1274, 1228, 1158, 1115, 1093, 1052, 1014, 997, 934, 781, 771, 753, 724, 616, 591; LCMS (ESI, + ve) m/z 236.2 ($[M + H]^+$); HRMS (ESI) $C_{13}H_{14}FNO_2$ exact mass 235.1009, accurate mass 235.1004 (mass error −2.01 ppm).

1-(5-Fluoropentyl)-1*H*-indole-3-carboxylic Acid (20). Subjecting indole (**14**, 5.86 g, 50.0 mmol) and 1-bromo-5-fluoropentane (6.53 mL, 52.5 mmol, 1.05 equiv) to general procedure B gave **20** as an off-white solid (9.76 g, 78%). mp 117−118 °C; R_f 0.55 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30−8.21 (m, 1H), 7.93 (s, 1H), 7.43−7.34 (m, 1H), 7.35−7.27 (m, 2H), 4.43 (dt, J = 47.2, 5.9 Hz, 2H), 4.20 (t, J = 7.1 Hz, 2H), 2.02−1.88 (m, 2H), 1.82−1.63 (m, 2H), 1.55−1.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8 (CO), 136.8 (quat.), 135.5 (CH), 127.1 (quat.), 123.1 (CH), 122.3 (CH), 122.1 (CH), 110.1 (CH), 106.6 (quat.), 83.8 (d, $^1J_{CF}$ = 165.0 Hz), 47.1 (NCH_2), 30.1 (d, $^2J_{CF}$ = 19.8 Hz), 29.7 (CH_2), 23.0 (d, $^3J_{CF}$ = 5.0 Hz). All physical and spectral properties were consistent with those previously reported.[72]

1-(Pent-4-en-1-yl)-1*H*-indole-3-carboxylic Acid (21). Subjecting indole (**14**, 2.93 g, 25.0 mmol) and 5-bromo-1-pentene (3.10 mL, 26.3 mmol, 1.05 equiv) to general procedure B gave **21** as an off-white solid (4.14 g, 72%). mp 123−124 °C; R_f 0.13 (hexane:EtOAc, 80:20); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28−8.23 (1H, m), 7.93 (1H, s), 7.40−7.37 (1H, m), 7.33−7.29 (2H, m), 5.86−5.76 (1H, m), 5.09 (1H, d, J = 8.2 Hz), 5.06 (1H, s), 4.19 (2H, t, J = 7.0 Hz), 2.12 (2H, q, J = 6.9 Hz), 2.01 (2H, quin., J = 7.0 Hz), OH not observed (exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8 (CO), 136.9 (CH), 136.8 (quat.), 135.7 (CH), 127.2 (quat.), 123.0 (CH), 122.3 (CH), 122.1 (CH), 116.3 (CH_2), 110.2 (CH), 106.5 (quat.), 46.4 (NCH_2), 30.8 (CH_2), 28.9 (CH_2). All physical and spectral properties were consistent with those previously reported.[73]

1-(Cyclobutylmethyl)-1*H*-indole-3-carboxylic Acid (22). Subjecting indole (**14**, 2.93 g, 25.0 mmol) and (bromomethyl)cyclobutane (2.90 mL, 26.3 mmol, 1.05 equiv) gave **22** as an off-white solid (3.98 g, 74%), mp 125−127 °C; R_f0.57 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26−8.18 (1H, m), 7.89 (1H, s), 7.42−7.33 (1H, m), 7.33−7.24 (2H, m), 4.15 (2H, d, J = 7.4 Hz), 2.88 (1H, sept., J = 7.4 Hz), 2.15−2.03 (2H, m), 1.99−1.74 (4H, m), OH not observed (exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8 (CO), 137.1 (quat.), 135.5 (CH), 127.0 (quat.), 122.9 (CH), 122.3 (CH), 122.0 (CH), 110.3 (CH), 106.4 (quat.), 52.2 (NCH_2), 35.5 (CH), 26.4 (2 × CH_2), 18.3 (CH_2); LCMS (ESI, + ve) m/z 230.1 ($[M + H]^+$); HRMS (ESI) $C_{14}H_{15}NO_2$ exact mass 229.1103, accurate mass 229.1103 (mass error 0.09 ppm).

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ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

General Procedure C: Synthesis of Methyl 1-Alkyl-1*H*-indazole-3-carboxylates and Methyl 1-(5-Fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylate. To a cooled (0 °C) solution of methyl 1*H*-indazole-3-carboxylate or methyl 1*H*-pyrrolo[2,3-*b*]-pyridine-3-carboxylate (1.0 equiv) in DMF (10 mL) was added NaH (1.05 equiv) portionwise. The mixture was warmed to ambient temperature, stirred for 1 h, cooled to 0 °C, treated dropwise with the appropriate alkyl bromide (1.05 equiv), and stirred at ambient temperature for 48 h. The reaction was quenched by pouring onto H_2O (100 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with H_2O (2 × 50 mL), brine (1 × 50 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The products were obtained following purification by flash chromatography.

Methyl 1-Butyl-1*H*-indazole-3-carboxylate (25). Subjecting methyl 1*H*-indazole-3-carboxylate (**23**, 2.63 g, 15.0 mmol) and 1-bromobutane (1.69 mL, 15.75 mmol, 1.05 equiv) to general procedure C gave, following purification by flash chromatography (hexane:EtOAc, 100:0 to 10:90), **25** as a colorless oil (1.79 g, 51%). R_f 0.46 (hexane:EtOAc, 80:20); ^{1}H NMR (400 MHz, CD_3OD) δ 8.14 (d, J = 8.3 Hz, 1H), 7.67 (d, J = 8.6 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 4.51 (t, J = 7.0 Hz, 2H), 4.01 (s, 3H), 1.93 (quin., J = 7.1 Hz, 2H), 1.32 (sext., J = 7.7 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.5 (CO), 142.1 (quat.), 135.4 (quat.), 128.2 (CH), 124.5 (quat.), 124.4 (CH), 122.9 (CH), 111.3 (CH), 52.3 (NCH_2), 50.4 (OCH_3), 32.9 (CH_2), 20.9 (CH_2), 13.9 (CH_3). All physical and spectral properties were consistent with those previously reported.[66]

Methyl 1-(4-Fluorobutyl)-1*H*-indazole-3-carboxylate (26). Subjecting methyl 1*H*-indazole-3-carboxylate (**23**, 881 mg, 5.00 mmol) and 1-bromo-4-fluorobutane (590 μL, 5.25 mmol, 1.05 equiv) to general procedure C gave, following purification by flash chromatography (hexane:EtOAc, 95:5 to 90:10), **26** as a colorless oil (675 mg, 54%). R_f 0.62 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, CD_3OD) δ 8.13 (dt, J = 8.2, 1.0 Hz, 1H), 7.68 (dt, J = 8.6, 0.8 Hz, 1H), 7.51−7.46 (m, 1H), 7.36−7.31 (m, 1H), 4.56 (t, J = 7.0 Hz, 2H), 4.43 (dt, J = 47.4, 5.9 Hz, 2H), 4.01 (s, 3H), 2.11−2.02 (m, 2H), 1.74−1.60 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.5 (quat.), 142.1 (quat.), 135.6 (quat.), 128.3 (CH.), 124.4 (CH), 122.9 (quat.), 111.3 (CH), 84.3 (d, $^1J_{CF}$ = 164.2 Hz, CH_2), 52.3 (NCH_2), 50.1 (CH_3), 28.7 (d, $^2J_{CF}$ = 20.1 Hz, CH_2), 26.9 (d, ^3J = 4.8 Hz, CH_2); LCMS (ESI, + ve) m/z 251.2 ($[M + H]^+$); HRMS (ESI) $C_{13}H_{15}FN_2O_2$ exact mass 250.1118, accurate mass 250.1116 (mass error −0.55 ppm).

Methyl 1-(Pent-4-en-1-yl)-1*H*-indazole-3-carboxylate (27). Subjecting methyl-1*H*-indazole-3-carboxylate (**23**, 4.40 g, 25.0 mmol) and 5-bromo-1-pentene (4.4 mL, 37.5 mmol, 1.5 equiv) to general procedure C gave, following purification by flash chromatography (hexane:EtOAc, 98:2), **27** as a colorless oil (3.36 g, 55%). R_f 0.45 (hexane:EtOAc, 80:20); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (1H, d, J = 8.2 Hz), 7.49−7.40 (2H, m), 7.35−7.28 (1H, m), 5.88−5.72 (1H, m), 5.04 (1H, d, J = 9.5 Hz), 5.00 (1H, s), 4.48 (2H, t, J = 7.0 Hz), 4.03 (3H, s), 2.11−2.04 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2 (CO), 140.7 (quat.), 137.1 (CH), 134.8 (quat.), 126.9 (CH), 123.9 (quat.), 123.2 (CH), 122.4 (CH), 116.0 (CH_2), 109.7 (CH), 52.1 (OCH_3), 49.3 (NCH_2), 30.8 (CH_2), 29.0 (CH_2); LCMS (ESI, + ve) m/z 245.1 ($[M + H]^+$); HRMS (ESI) $C_{14}H_{16}N_2O_2$ exact mass 244.1212, accurate mass 244.1209 (mass error −1.18 ppm).

Methyl 1-(Cyclohexylmethyl)-1*H*-indazole-3-carboxylate (28). Subjecting methyl-1*H*-indazole-3-carboxylate (**23**, 884 mg, 5.02 mmol) and (bromomethyl)cyclohexane (1.33 g, 7.53 mmol, 1.5 equiv) to general procedure C gave, following purification by flash chromatography (hexane:EtOAc, 94:6), **28** as a white powder (539 mg, 39%). mp 64−66 °C; R_f 0.96 (hexane:EtOAc, 20:80); ^{1}H NMR (400 MHz, CD_3OD) δ 8.14 (d, 1H), 7.67 (d, 1H), 7.51−7.45 (m, 1H), 7.36−7.30 (m, 1H), 4.34 (d, J = 7.3 Hz, 2H), 4.01 (s, 3H), 2.10−1.97 (m, 1H), 1.77−1.62 (m, 3H), 1.59−1.51 (m, 2H), 1.30−1.16 (m, 3H), 1.14−1.02 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 164.5 (quat.), 142.6 (quat.), 135.4 (quat.), 128.2 (CH), 124.4 (quat.), 124.3 (CH), 122.8 (CH), 111.6 (CH), 56.6 (CH_2), 52.3 (CH_3), 40.1 (CH_2), 31.8 (CH_2), 27.3 (CH_2), 26.8 (CH_2). All physical and spectral properties were consistent with those previously reported.[66]

Methyl 1-(5-Fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylate (29). Subjecting methyl 1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylate (**24**, 881 mg, 5.00 mmol) and 1-bromo-5-fluoropentane (684 μL, 5.25 mmol, 1.05 equiv) to general procedure C gave, following purification by flash chromatography (hexane:EtOAc, 100:0 to 60:40), **29** as a white solid (761 mg, 58%). mp 37−39 °C; R_f 0.69 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, CD_3OD) δ 8.39 (dd, J = 7.9, 1.6 Hz, 1H), 8.32 (dd, J = 4.8, 1.6 Hz, 1H), 8.15 (s, 1H), 7.25 (dd, J = 7.9, 4.8 Hz, 1H), 4.38 (dt, J = 47.5, 6.0 Hz, 2H), 4.34 (t, J = 7.2 Hz, 2H), 3.89 (s, 3H), 1.92 (quin., J = 7.4 Hz, 2H), 1.78−1.62 (m, 2H), 1.46−1.35 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 166.5 (quat.), 144.7 (quat.), 136.4 (CH), 131.2 (CH), 120.7 (quat.), 119.0 (CH), 106.3 (quat.), 84.6 (d, $^1J_{CF}$ = 163.9 Hz, CH_2), 51.6 (CH_3), 46.1 (NCH_2), 31.0 (d, $^2J_{CF}$ = 19.8 Hz), 30.8 (CH_2), 23.6 (d, $^3J_{CF}$ = 5.3 Hz, CH_2). All physical and spectral properties were consistent with those previously reported.[58]

General Procedure D: Synthesis of 1-Alkyl-1*H*-Indazole-3-carboxylic Acids and 1-(5-Fluoropentyl)-1*H*-pyrrolo[2,3-*b*]-pyridine-3-carboxylic Acid. To a solution of the appropriate methyl 1-alkyl-1*H*-indazole-3-carboxylate (**25**−**28**) or methyl 1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylate (**29**) in MeOH (50 mL) was added 1 M aq. NaOH, and the solution stirred for 48 h. The solvent was evaporated *in vacuo*, and the resulting aqueous residue was adjusted to pH 2 using 1 M aq. HCl and then extracted with EtOAc (3 × 50 mL). The organic layers were dried ($MgSO_4$), and the solvent removed *in vacuo* to give the products.

1-Butyl-1*H*-indazole-3-carboxylic Acid (30). Subjecting methyl 1-butyl-1*H*-indazole-3-carboxylate (**25**, 1.60 g, 6.88 mmol) to general procedure D gave **30** as a white solid (1.34 g, 90%). mp 102−105 °C; R_f 0.29 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.99 (s, 1H), 8.08 (dt, J = 8.2, 0.9 Hz, 1H), 7.80 (d, J = 8.6 Hz, 1H), 7.46 (ddd, J = 8.5, 6.9, 1.1 Hz, 1H), 7.30 (ddd, J = 7.9, 6.9, 0.9 Hz, 1H), 4.50 (t, J = 7.0 Hz, 2H), 1.88−1.79 (m, 2H), 1.24 (sext., J = 7.4 Hz, 2H), 0.86 (d, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.5 (CO), 140.4 (quat.), 134.6 (quat.), 126.5 (CH), 123.0 (quat.), 122.8 (CH), 121.5 (CH), 110.6 (CH), 48.6 (NCH_2), 31.4 (CH_2), 19.4 (CH_2), 13.5 (CH_3). All physical and spectral properties were consistent with those previously reported.[66]

1-(4-Fluorobutyl)-1*H*-indazole-3-carboxylic Acid (31). Subjecting methyl 1-(4-fluorobutyl)-1*H*-indazole-3-carboxylate (**26**, 500 mg, 2.00 mmol) to general procedure D gave **31** as a white solid (441 mg, 94%). mp 107−109 °C; R_f 0.43 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.01 (s, 1H), 8.09 (d, J = 8.2 Hz, 1H), 7.82 (d, J = 8.6 Hz, 1H), 7.48 (ddd, J = 8.5, 6.9, 1.1 Hz, 1H), 7.31 (ddd, J = 8.0, 6.9, 0.8 Hz, 1H), 4.55 (t, J = 7.0 Hz, 2H), 4.45 (dt, J = 47.4, 6.0 Hz, 2H), 2.00−1.90 (m, 2H), 1.70−1.55 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.4 (CO), 140.4 (quat.), 134.8 (quat.), 126.5 (CH), 123.0 (quat.), 122.8 (CH), 121.5 (CH), 110.6 (CH), 83.4 (d, $^1J_{CF}$ = 161.8 Hz, CH_2), 48.4 (NCH_2), 27.1 (d, $^2J_{CF}$ = 19.6 Hz, CH_2), 25.3 (d, $^3J_{CF}$ = 5.2 Hz, CH_2); LCMS (ESI, + ve) m/z 237.2 ($[M + H]^+$); HRMS (ESI) $C_{12}H_{13}FN_2O_2$ exact mass 236.0961, accurate mass 236.0959 (mass error −0.85 ppm).

1-(Pent-4-en-1-yl)-1*H*-indazole-3-carboxylic Acid (32). Subjecting methyl 1-(pent-4-en-1-yl)-1*H*-indazole-3-carboxylate (**27**, 1.84 g, 7.56 mmol) to general procedure D gave **32** as a white solid (1.43 g, 6.23 mmol, 82%). mp 94−97 °C; R_f 0.22 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (1H, d, J = 8.2 Hz), 7.53−7.42 (2H, m), 7.38−7.34 (1H, m), 5.89−5.73 (1H, m), 5.05 (1H, d, J = 9.5 H), 5.02 (1H, s), 4.51 (2H, t, J = 6.0 Hz), 2.15−2.07 (4H, m), OH not observed (exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5 (CO), 141.0 (quat.), 137.0 (CH), 134.2 (quat.), 127.1 (CH), 124.0 (quat.), 123.6 (CH), 122.5 (CH), 116.1 (CH_2), 109.9 (CH), 49.4 (NCH_2), 30.8 (CH_2), 28.9 (CH_2); LCMS (ESI, + ve) m/z 231.1 ($[M + H]^+$); HRMS (ESI) $C_{13}H_{14}N_2O_2$, exact mass 230.1055, accurate mass 230.1061 (mass error 2.67 ppm).

1-(Cyclohexylmethyl)-1*H*-indazole-3-carboxylic Acid (33). Subjecting methyl 1-(cyclohexylmethyl)-1*H*-indazole-3-carboxylate (**28**, 498 mg, 1.84 mmol) to general procedure D gave **33** as a white

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ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

solid (393 mg, 83%). R_f 0.33 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.00 (s, 1H), 8.09 (d, J = 8.2 Hz, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.51−7.42 (m, 1H), 7.35−7.26 (m, 1H), 4.35 (d, J = 7.1 Hz, 2H), 1.99−1.87 (m, 1H), 1.69−1.61 (m, 2H), 1.61−1.58 (m, 1H), 1.49 (d, J = 12.4 Hz, 2H), 1.23−1.08 (m, 3H), 1.08−0.97 (m, 2H). All physical and spectral properties were consistent with those previously reported.[66]

1-(5-Fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylic Acid (34). Subjecting methyl 1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxylate (**29**, 343 mg, 1.30 mmol) to general procedure D gave **34** as a white solid (298 mg, 92%); R_f 0.43 (hexane:EtOAc, 50:50); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 8.36−8.30 (m, 2H), 8.29 (s, 1H), 7.26 (dd, J = 7.9, 4.7 Hz, 1H), 4.39 (dt, J = 47.5, 6.0 Hz, 3H), 4.33 (t, J = 7.1 Hz, 3H), 1.87 (quin., J = 7.3 Hz, 2H), 1.73−1.58 (m, 2H), 1.35−1.25 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.2 (CO), 147.4 (quat.), 143.4 (CH), 135.3 (CH), 129.2 (CH), 118.7 (quat.), 117.7 (CH), 105.3 (quat.), 83.6 (d, $^1J_{CF}$ = 161.6 Hz, CH_2), 44.2 (NCH_2), 29.3 (d, $^2J_{CF}$ = 19.3 Hz, CH_2), 29.1 (CH_2), 21.9 (d, $^3J_{CF}$ = 5.4 Hz, CH_2). All physical and spectral properties were consistent with those previously reported.[58]

Determination of the *in Vitro* Biological Activity at the CB1. A live cell-based reporter assay that monitors the *in vitro* CB1 activation via its interaction with β-arrestin 2 using the NanoLuc Binary Technology (NanoBiT), was applied to assess the biological activity of the compounds. Details regarding the development of the stable cell line used here have been reported elsewhere.[56,60]

The modified human embryonic kidney (HEK) 293T cells were routinely maintained at 37 °C, 5% CO_2, under humidified atmosphere in Dulbecco's modified Eagle's medium (GlutaMAX) supplemented with 10% heat-inactivated FBS, 100 IU/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B. For experiments, cells were plated on poly-D-lysine coated 96-well plates at 5×10^4 cells/well and incubated overnight. Next, the cells were washed twice with Opti-MEM I Reduced Serum Medium to remove any remaining FBS, and 100 μL Opti-MEM I and 25 μL of the Nano-Glo Live Cell reagent was added to each well. Subsequently, the plate was placed into a TriStar^2 LB 942 multimode microplate reader (Berthold Technologies GmbH & Co., Germany). Luminescence was monitored during the equilibration period until the signal stabilized (15 min). Next, 10 μL per well of test compounds present as concentrated (13.5-fold, as 10 μL was added to generate a final volume of 135 μL) stock solutions in Opti-MEM I was added. The luminescence was continuously monitored for 120 min. Solvent controls were included in all experiments.

The results are represented as mean area under the curve (AUC) ± standard error of mean (SEM), obtained in minimum three independent experiments, with duplicates run in every experiment. All results were normalized to the E_{max} of JWH-018 (= 100%), our reference compound. Curve fitting of concentration−response curves via nonlinear regression (four−parameter logistic fit) was employed to determine EC_{50} (a measure of potency) and E_{max} (a measure of efficacy) values, using GraphPad Prism software (San Diego, CA, US).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschemneuro.0c00644.

^{1}H and ^{13}C NMR spectra and LC-UV chromatograms for novel compounds (PDF)

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Author Contributions

A.C. designed and executed the *in vitro* functional assays under the supervision of C.S. E.S., E.P., A.F., J.L.L., and R.K. synthesized and analytically characterized all compounds under the supervision of S.D.B. R.E. obtained all high resolution mass spectra under the supervision of R.G. A.C., E.S., S.D.B., and C.S. conceived the experiments and wrote the manuscript, and all authors reviewed and edited drafts of the manuscript. All authors approved the final version of the paper.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

βarr2, β-arrestin 2; CB1, cannabinoid receptor 1; COA, certificate of analysis; CI, confidence interval; ee, enantiomeric excess; EU, European Union; EWS, Early Warning System; FBS, fetal bovine serum; GLC, gas liquid chromatography; GPCR, G protein-coupled receptor; HEK, human embryonic kidney; NPS, new psychoactive substances; SCRA, synthetic cannabinoid receptor agonist; SEM, standard error of mean; THC, Δ^9-tetrahydrocannabinol; UK, United Kingdom; UNODC, United Nations Office on Drugs and Crime; US, United States

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