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Brief Article

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Indole-2-carboxamides as Allosteric Modulators of the Cannabinoid CB_1 Receptor

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S *Supporting Information*

ABSTRACT: We synthesized new *N*-phenylethyl-1*H*-indole-2-carboxamides as the first SAR study of allosteric modulators of the CB_1 receptor. The presence of the carboxamide functionality was required in order to obtain a stimulatory effect. The maximum stimulatory activity on CB_1 was exerted by carboxamides **13** (EC_{50} = 50 nM) and **21** (EC_{50} = 90 nM) bearing a dimethylamino or piperidinyl group, respectively, at position 4 of the phenethyl moiety and a chlorine atom at position 5 of the indole.

INTRODUCTION

Cannabinoid (CB) receptors belong to the endocannabinoid system that encompasses three components: (i) CB_1 and CB_2 G-protein-coupled receptors,[1] (ii) endocannabinoids (i.e., endogenous CB receptor ligands), and (iii) proteins, enzymes, and carriers involved in endocannabinoid formation and inactivation.[2] The endocannabinoid system is thought to be involved in an ever-growing number of physiological processes and pathologies. Metabolic and eating disorders,[3] cancer,[4] pain,[5] neurodegeneration,[6] immunosuppression,[7] locomotion,[8] and liver disease[9] have demonstrated a strong correlation with the endocannabinoid system. Thus, the endocannabinoid system may provide excellent options for the development of new drugs.[10]

CB_1 receptors are found predominantly at neuronal terminals where they act as modulators of neurotransmitter release. CB_1 receptor antagonists/inverse agonists have therapeutic potential in the treatment of a variety of pathological conditions[2a] including obesity,[11] Parkinson's disease,[12] metabolic disorders, smoking cessation, and drug abuse.[13,14] On the other hand, selective CB_2 agonists may be used to treat pain and inflammation,[15] osteoporosis,[16] CB_2-receptor-expressing malignant gliomas,[17] tumors of immune origin,[18] and multiple sclerosis.[19]

Monod[20] described the term *allosteric* as the capability of ligands to affect positively or negatively the biological activity of enzymes and receptors by binding to sites (the allosteric sites) that are separate from the substrate/ligand-binding site. Interaction of an allosteric ligand induces conformational changes in the protein which affect the binding of the substrate/ligand into its orthosteric site, thereby fine-tuning its biological activity.

G-protein-coupled receptors show allosteric binding sites for endogenous and/or synthetic ligands.[21] The first evidence of an allosteric binding site at the cannabinoid CB_1 receptor was reported by Price et al in 2005.[22] To our knowledge, this site was recognized by means of three small molecules, Org 27569 (**1**), Org 29647 (**2**), and Org 27759 (**3**), which showed different efficacy versus affinity to the orthosteric site; later, the phenylurea derivative PSNCBAM-1 (**4**) was shown to exhibit similar pharmacological profile. In particular, these small molecules proved to be allosteric enhancers of agonist binding and affinity and allosteric inhibitors of agonist signaling efficacy[23,24] (Chart 1S, Supporting Information (SI)).

The identification of the CB_1 allosteric binding site prompted the search for other allosteric ligands as new therapeutic tools. Allosteric enhancers of the CB_1 receptor may lead to useful agents without the CNS side effects that are characteristic of direct receptor agonism because they would be inactive per se and potentiate the effects of endogenous ligands, which are more "site- and time-selective".[22] However, apart from the recently reported parametrization of **1**,[25] neither crystallographic/modeling nor structure−activity relationship (SAR) studies with these small molecules have been published so far. Thus, this novel as well as original and potentially safer avenue of therapeutic development has not yet been exhaustively explored. Here, we report the first synthesis of indole-2-carboxamides **8**−**26** structurally correlated to **1**. Our results show that new potent positive CB_1 allosteric modulators may be obtained by chemical modification of the *N*-phenylethyl-1*H*-indole-2-carboxamide scaffold (Table 1).

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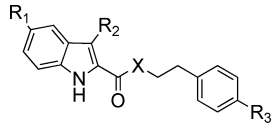
Table 1. Structure and Action of Compounds 5−26 and Reference Compound 1 as Stimulators or Inhibitors of [^{3}H]CP55940 Binding to Human Recombinant Cannabinoid Receptors[a]



5-26

					CB_1		CB_2	
Compd	R_1	R_2	R_3	X	EC_{50} (μM)	BS[b] or BI[c] (%)	IC_{50} (μM)	BS[b] or BI[c] (%)
5	Cl	Et	–N⬡	O	IC_{50} >10[d]	18.0[d]	8.26	52.7
6	Cl	Et	NO_2	O	IC_{50} >10[d]	24.7[d]	>10	16.8
7	Cl	Et	NMe_2	O	IC_{50} >10[d]	19.6[d]	5.57	65.7
8	Cl	Et	–N⬡	NH	>10	133.9	EC_{50}>10[e]	134.7[e]
9	Cl	Et	–N⬡NMe	NH	>10	132.4	6.97	66.7
10	Cl	Et	NO_2	NH	0.36	242.5	>10	0.00
11	Cl	Et	NH_2	NH	>10	144.8	>10	31.1
12	Cl	Et	NHMe	NH	0.96	183.4	9.13	55.7
13	Cl	Et	NMe_2	NH	0.05	226.8	>10	22.8
14	Cl	Et	Me	NH	0.55	188.9	>10	29.0
15	Cl	Et	Cl	NH	0.76	178.7	>10	48.9
16	Cl	Et	F	NH	>10	111.9	>10	37.7
17	Cl	COMe	–N⬡	NH	IC_{50} >10[d]	19.9[d]	>10	7.9
18	Cl	CH(OH)Me	–N⬡	NH	8.45	180.1	>10	33.1
19	Cl	Me	–N⬡	NH	0.23	242.7	EC_{50}>10[e]	110.9[e]
20	H	Et	–N⬡	NH	0.81	244.1	8.97	55.2
21	Cl	H	–N⬡	NH	0.09	206.7	>10	37.9
22	Cl	H	NO_2	NH	>10	140.7	>10	32.7
23	Cl	H	NH_2	NH	IC_{50} >10[d]	3.6[d]	>10	37.7
24	Cl	H	NHMe	NH	IC_{50} >10[d]	3.8[d]	>10	43.7
25	Cl	H	NMe_2	NH	1.53	170.9	>10	42.1
26	Cl	H	Cl	NH	>10	115.2	>10	11.1
1	Cl	Et	–N⬡	NH	0.14	243.8	>10	20.6

[a]EC_{50} or IC_{50} (means: n = 2; **8**: n = 4) were determined by nonlinear or linear regression analysis, respectively, of enhancement or inhibition of radioligand binding by increasing compound concentrations. [b]BS: % of binding stimulation at the maximum concentration tested (10 μM; 25 μM for **17**). [c]BI: % of binding inhibition at the maximum concentration tested (10 μM). [d]Weak radioligand binding inhibitor. [e]Weak radioligand binding stimulator.

CHEMISTRY

The synthesis of new compounds **5**−**26** is depicted in Scheme 1. Compounds **1**, **8**−**10**, **14**−**17**, **19**−**22**, and **26** were synthesized by the coupling reaction of the appropriate indole-2-carboxylic acid **27**−**31** with the corresponding amine **32**−**38** in the presence of the BOP reagent and triethylamine in anhydrous DMF. Compounds **5**−**7** were obtained by reaction of 27 with alcohols **39**−**41** in the presence of DMAP and DCI in anhydrous dichloromethane. Tin(II) chloride reduction of the nitro group of **10** in ethyl acetate furnished **11**, which upon treatment with dimethyl sulfate in the presence of potassium carbonate in acetone gave a mixture of the mono- (**12**) and dimethylamino (**13**) derivatives, separated by column chromatography. In a similar manner were obtained **23**−**25** starting from **22**. Acetylindole **17** was transformed into the corresponding ethanol derivative **18** by sodium borohydride reduction in aqueous tetrahydrofuran. The synthesis of the new synthetic intermediates **27**−**31** and **32**−**41** is described in Schemes 1S−4S of Supporting Information.

RESULTS AND DISCUSSION

The potential properties of compounds **1** and **26** as positive allosteric modulators of the binding of orthosteric ligands to the CB_1 receptor were evaluated by measuring the stimulation of the specific binding of the nonselective CB_1/CB_2 receptor agonist [^{3}H]CP55940 (**42**) to cell membranes overexpressing the human recombinant CB_1 (Table 1). As a control, we also carried out studies with preparations of cell membranes overexpressing the human recombinant CB_2 receptor. Compounds **10**, **12**−**15**, and **19**−**21** were found to be enhancers of the binding of **42** to the CB_1 receptor, two of which (**13** and **21**) were particularly potent as indicated by their nanomolar EC_{50} values, whereas **8**, **9**, **11**, **16**, the racemate **18**, **22**, **25**, and **26** enhanced **42** binding only weakly. In contrast, **5**−**7** inhibited the binding of **42** to the CB_1 receptor. Clearly, in the case of inhibition of **42**, our assay does not allow to distinguish between orthosteric and allosteric ligands. Only **8** and **19** showed very weak activity as enhancers of **42** binding to the CB_2 receptor. Compound **10** was devoid of any binding activity

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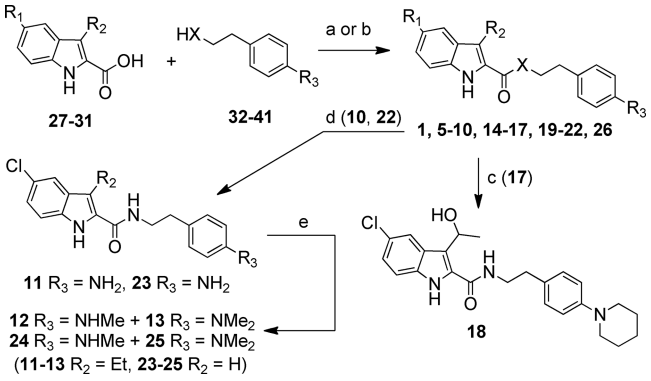
Scheme 1. Synthesis of Compounds 1 and 5−26[a,b]



[a]**1**, **5**−**26**: (see Table 1). **27**: R_1 = Cl, R_2 = Et. **28**: R_1 = Cl, R_2 = COMe. **29**: R_1 = Cl, R_2 = Me. **30**: R_1 = H, R_2 = Et. **31**: R_1 = Cl, R_2 = H. **32**: X = NH, R_3 = NO_2. **33**: X = NH, R_3 = piperidin-1-yl. **34**: X = NH, R_3 = pyrrolidin-1-yl. **35**: X = NH, R_3 = 4-methylpiperazin-1-yl. **36**: X = NH, R_3 = Me. **37**: X = NH, R_3 = Cl. **38**: X = NH, R_3 = F. **39**: X = O, R_3 = piperidin-1-yl. **40**: X = O, R_3 = NO_2. **41**: X = O, R_3 = NMe_2. [b]Reagents and reaction conditions: (a) BOP, Et_3N, DMF, rt, 16 h; (b) DCI, DMAP, CH_2Cl_2, rt, 12 h, for **5**; (c) $NaBH_4$, aqueous THF, reflux, 2 h; (d) $SnCl_2{\cdot}2H_2O$, AcOEt, 60 °C, 4 h; (e) Me_2SO_4, K_2CO_3, acetone, rt, 14 h.

at CB_2 receptors, and all other compounds not only lacked stimulating binding activity but weakly inhibited the binding of the radioligand. Also, in this case, our assay does not allow to distinguish between orthosteric and allosteric ligands.

The presence of the carboxamide functionality at the position 2 of the indole emerged as being crucial for the enhancement of the binding of **42** to the CB_1 receptor. Replacement of the carboxamide of **1** for an ester group (**5**−**7**) resulted in a dramatic drop of stimulation with the concomitant appearance of inhibitory activity. However, such a feature is necessary but not sufficient per se to confer stimulatory properties to the compounds investigated here because **17**, **23**, and **24** also did not enhance **42** binding. Replacement of the piperidin-1-yl group of **1** with the smaller pyrrolidin-1-yl (**8**) or the more polar 4-methylpiperazin-1-yl (**9**) group, or the chlorine atom of **15** with a fluorine (**16**) led to weaker enhancement of the CB_1 binding activity at all test concentrations. The stimulatory **42**-binding activity observed was fully restored by introduction of a nitro group (**10**) in the place of the piperidin-1-yl moiety (R_3), suggesting that, at position 4 of the phenyl moiety, both steric and electronic factors are involved in the observed activities. The corresponding amino derivative **11** resulted in a weaker stimulation of **42**−CB_1 receptor binding. The methyl analogue (**12**) of **11** was comparable to **10** as binding stimulator. Finally, the dimethylamino derivative **13** of **12** yielded the most potent allosteric ligand within the present series (EC_{50} = 50 nM).

Neither acetyl (**17**) nor alcohol (**18**) produced compounds capable of efficaciously stimulating **42**−CB_1 receptor binding. The 3-methylindole **19** substantially retained the stimulatory activity of the parent compound **1**. Among **21**−**26** bearing a hydrogen atom at position 3 of the indole, only compound **21** (EC_{50} = 90 nM) exhibited greater affinity than **1** as a stimulator of **42**−CB_1 receptor binding. The indole **20** was less effective as compared to **1** as a stimulator of **42**−CB_1 receptor binding. These results have shown that the *N*-phenylethyl-1*H*-indole-2-carboxamide represents an excellent scaffold for the design of positive allosteric modulators of the human CB_1 receptor.

In summary, the carboxamide at position 2 of the indole is required in order to obtain the stimulatory effect. Both the chlorine atom (R_1) and alkyl group (R_2) at the positions 5 and 3 of the indole, respectively, were useful, but not necessary, for the stimulation of **42**−CB_1 binding. The piperidin-yl, nitro, and dimethylamino groups at the *para* position of the phenylethyl moiety (R_3) were optimal for a potent stimulation. These results show that the *N*-phenylethyl-1*H*-indole-2-carboxamide scaffold may be properly decorated to obtain potent positive allosteric ligands of the CB_1 receptor. However, and this is a limitation of the present study, whether these compounds behave as functional positive modulators, as suggested by their chemical similarity to **1**, remains to be established by using ad hoc in vitro and in vivo pharmacological studies. We also aim at investigating in depth the chemical and physical properties correlated to the stimulation of the CB_1 receptor with the synthesis of new derivatives.

CONCLUSION

We synthesized new *N*-phenylethyl-1*H*-indole-2-carboxamides as the first SAR study of allosteric modulators of the CB_1 receptor. These carboxamides were found to be selective positive modulators of the human recombinant CB_1 receptor. The stimulatory activity on CB_1 receptors required the presence of the 2-carboxamide functionality as esters **5** and **7** was demonstrated to instead inhibit binding of **42** to CB_1. These results show that new potent CB_1 stimulators may be obtained by chemical modification of the *N*-phenylethyl-1*H*-indole-2-carboxamide scaffold and represent the first attempt at investigating the SARs of CB_1 allosteric ligands using a medicinal chemistry approach. SAR and chemicophysical studies through the synthesis of new derivatives, and functional studies, are now needed.

EXPERIMENTAL SECTION

Chemistry. Combustion analysis was used as a method for establishing compound purity. Purity of the tested compounds was ≥95%. See SI for details.

General Procedure for the Synthesis of 8−10, 14−17, 19−22, and 1. Example: 5-Chloro-3-ethyl-*N*-(2-(4-(pyrrolidin-1-yl)phenyl)ethyl)-1*H*-indole-2-carboxamide (8). A mixture of **27** (0.1 g, 0.44 mmol), **34** (0.13 g, 0.66 mmol), BOP (0.29 g, 0.66 mmol), and triethylamine (0.28 g, 0.36 mL, 2.64 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) was stirred at room temperature for 16 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried, and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate−*n*-hexane 1:2 as eluent) to provide **8**. Yield 66%, mp 230−234 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 11.73 (s, 1H), 8.32 (t, J = 5.2 Hz, 1H), 8.07 (d, J = 1.8 Hz, 1H), 7.82 (d, J = 8.7 Hz, 1H), 7.60 (dd, J = 8.6, 1.9 Hz, 1H), 7.48 (d, J = 8.4 Hz, 2H), 6.91 (d, J = 8.4 Hz, 2H), 3.94−3.81 (m, 2H), 3.66−3.55 (m, 4H), 3.39 (q, J = 7.4 Hz, 2H), 3.16 (t, J = 7.3 Hz, 2H), 2.40−2.31 (m, 4H), 1.55 ppm (t, J = 7.4 Hz, 3H). MS (ESI): m/z: 596 $[M + H]^+$. IR: ν 3315, 1595 cm^{-1}. Anal. ($C_{23}H_{26}ClN_3O$ (395.93)) C, H, N, Cl. Chemicophysical characterization, preparative, and combustion analysis data of **9**, **10**, **14**−**17**, **19**−**22**, **26**, and **1** are reported in SI.

General Procedure for the Synthesis of 5−7. Example: 2-(4-(Piperidin-1-yl)phenyl)ethyl 5-chloro-3-ethyl-1*H*-indole-2-carboxylate (5). A mixture of **27** (0.076 g, 0.34 mmol), **39** (0.07 g, 0.34 mmol), and DMAP (0.004 g, 0.034 mmol) in anhydrous dichloromethane (5 mL) was cooled to 0 °C on an ice−water bath. DCI (0.07 g, 0.34 mmol) was added, and the mixture was stirred at room temperature for 16 h. The solid was filtered off, and the solvent was evaporated. The residue was purified by silica gel column chromatography (chloroform as eluent) to provide **5** as a white solid.

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Yield 57%, mp 110−115 °C (from aqueous ethanol). ^{1}H NMR (DMSO-d_6): δ 11.64 (s, 1H), 7.72 (d, J = 1.7 Hz, 1H), 7.43 (d, J = 8.7 Hz, 1H), 7.25 (dd, J = 8.7, 2.0 Hz, 1H), 7.16 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 4.48 (t, J = 6.9 Hz, 2H), 3.12−3.02 (m, 4H), 3.02−2.89 (m, 4H), 1.66−1.56 (m, 4H), 1.56−1.45 (m, 2H), 1.10 ppm (t, J = 7.4 Hz, 3H). MS (ESI): m/z: 411 [M + H]$^+$. IR: ν 3313, 1677 cm^{-1}. Anal. ($C_{24}H_{27}ClN_2O_2$ (410.94)) C, H, N, Cl. Chemicophysical characterization, preparative, and combustion analysis data of **5**−**7** are reported in SI.

***N*-(2-(4-Aminophenyl)ethyl)-5-chloro-3-ethyl-1*H*-indole-2-carboxamide (11).** Tin chloride dihydrate (0.45 g, 2.0 mmol) was added to a solution of **10** (0.15 g, 0.43 mmol) in ethyl acetate (5.4 mL). The reaction mixture was heated to 60 °C for 3 h. After cooling, the reaction mixture was treated with a saturated solution of sodium bicarbonate, and the precipitate was filtered off. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate−*n*-hexane 1:1 as eluent) to provide **11** as white solid. Yield 75%, mp 180−184 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 11.35 (s, 1H), 7.93 (s, 1H), 7.65 (s, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 7.7 Hz, 2H), 6.51 (d, J = 8.0 Hz, 2H), 4.87 (s, 2H), 3.44 (m, 2H), 2.97 (q, J = 7.9 Hz, 2H), 2.69 (t, J = 7.3 Hz, 2H), 1.13 ppm (t, J = 7.4 Hz, 3H). MS (ESI): m/z: 342 [M + H]$^+$. IR: ν 3422, 3346, 3285, 1619 cm^{-1}. Anal. ($C_{19}H_{20}ClN_3O$ (341.83)) C, H, N, Cl.

5-Chloro-3-ethyl-*N*-(2-(4-methylaminophenyl)ethyl)-1*H*-indole-2-carboxamide (12) and 5-chloro-*N*-(2-(4-dimethylaminophenyl)ethyl)-3-ethyl-1*H*-indole-2-carboxamide (13). A mixture of **11** (0.070 g, 0.20 mmol), potassium carbonate (0.070 g, 0.51 mmol), and dimethylsulfate (0.025 g, 0.019 mL, 0.20 mmol) in anhydrous acetone (5 mL) was stirred at room temperature for 16 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate−*n*-hexane 1:2 as eluent) as eluent to give **13** as white solid. Yield 29%, mp 150−154 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 11.32 (s, 1H), 7.93 (t, J = 5.4 Hz, 1H), 7.65 (d, J = 1.9 Hz, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.19 (dd, J = 8.7, 2.0 Hz, 1H), 7.09 (d, J = 8.6 Hz, 2H), 6.69 (d, J = 8.7 Hz, 2H), 3.55−3.41 (m, 2H), 2.97 (q, J = 7.4 Hz, 2H), 2.85 (s, 6H), 2.75 (t, J = 7.5 Hz, 2H), 1.13 ppm (t, J = 7.5 Hz, 3H). MS (ESI): m/z: 370 [M + H]$^+$. IR: ν 3438, 3251, 1630 cm^{-1}. Anal. ($C_{21}H_{24}ClN_3O$ (369.89)) C, H, N, Cl. Further elution with the same eluent furnished **12** as white solid. Yield 31%, mp 110−114 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 11.32 (s, 1H), 7.91 (t, J = 5.4 Hz, 1H), 7.65 (d, J = 1.7 Hz, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.19 (dd, J = 8.7, 2.0 Hz, 1H), 6.99 (d, J = 8.3 Hz, 2H), 6.49 (d, J = 8.4 Hz, 2H), 5.43 (q, J = 5.3 Hz, 1H), 3.51−3.41 (m, 2H), 2.96 (q, J = 7.4 Hz, 2H), 2.71 (t, J = 7.4 Hz, 2H), 2.65 (d, J = 5.1 Hz, 3H), 1.13 ppm (t, J = 7.5 Hz, 3H). MS (ESI): m/z: 356 [M + H]$^+$. IR: ν 3279, 1634 cm^{-1}. Anal. ($C_{20}H_{22}ClN_3O$ (355.86)) C, H, N, Cl.

5-Chloro-3-(1-hydroxyethyl)-*N*-(2-(4-(piperidin-1-yl)phenyl)-ethyl)-1*H*-indole-2-carboxamide (18). A solution of **17** (0.03 g, 0.06 mmol) and sodium borohydride (0.023 g, 0.06 mmol) in tetrahydrofuran (2 mL) and water (0.09 mL) was heated to reflux temperature for 2 h. After cooling, the reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and evaporated. The residue was purified by a short silica gel column chromatography (ethyl acetate−*n*-hexane 1:1 as eluent) to provide **18** as a white solid. Yield 92%, mp 132−134 °C (from ethyl acetate−hexane). ^{1}H NMR (DMSO-d_6): δ 11.54 (s, 1H), 9.41 (t, J = 5.4 Hz, 1H), 7.78 (d, J = 1.6 Hz, 1H), 7.41 (d, J = 8.7 Hz, 1H), 7.18 (dd, J = 8.7, 1.9 Hz, 1H), 7.11 (d, J = 8.1 Hz, 2H), 6.86 (d, J = 7.7 Hz, 2H), 6.18 (s, 1H), 5.46−5.33 (m, 1H), 3.64−3.42 (m, 2H), 3.16−3.01 (m, 4H), 2.77 (t, J = 7.2 Hz, 2H), 1.71−1.56 (m, 4H), 1.56−1.47 (m, 2H), 1.37 ppm (d, J = 6.5 Hz, 3H). MS (ESI): m/z: 426 [M + H]$^+$. IR: ν 3232, 1635 cm^{-1}. Anal. ($C_{24}H_{28}ClN_3O_2$ (425.95)) C, H, N, Cl.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Structures of **1**−**4**, synthesis of **27**−**31** and **32**−**41**, elemental analysis of **5**−**26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CB, cannabinoid; CB_1, CB receptor type 1; CB_2, CB receptor type 2; SAR, structure−activity relationship; BOP, benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate; DMAP, *N*,*N*-dimethyl-4-aminopyridine; DCI, *N*,*N*′-dicyclohexylcarbodiimide; Xphos, 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl; BS, percent of binding stimulation at the maximum concentration tested is also reported; BI, percent of binding inhibition at the maximum concentration tested is also reported

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