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Exploring the Benzimidazole Ring as a Substitution for Indole in Cannabinoid Allosteric Modulators

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Abstract

Introduction and Objectives: The traditional approach to target a particular receptor is to design compounds that bind to the same site as the endogenous ligand, the so-called "orthosteric site." However, recently the search has shifted to ligands that can interact with a different region of the receptor protein, the "allosteric site," since this approach offers potential pharmacological and therapeutic advantages. The aim of our work was to explore the benzimidazole heterocycle as a novel scaffold for cannabinoid allosterism.

Materials and Methods: We synthesized a series of novel benzimidazole-2-carboxamides, analogues of ORG27569, and performed their pharmacological characterization as CB_1R allosteric modulators using competitive [^{3}H]-CP55940 and [^{35}S]-GTPγS binding assays.

Results: The benzimidazoles **3** and **4** produced significant negative allosteric modulation (NAM) of CP55940 agonism at the mouse CB_1R, although are somewhat less potent than the CB_1R allosteric cannabinoid ORG27569.

Conclusions: Replacing the indole ring with a benzimidazole ring within the structure of ORG27569 abolished the binding of the resultant ligands to CB_1R, but the modulation on the agonist-induced GTPγS binding was maintained.

Keywords: CB_1 receptor; allosteric modulators; benzimidazole; ORG27569

Introduction

The traditional approach to target a particular receptor involves designing compounds, agonists or antagonists, that bind to the same site as the endogenous ligand, the so-called "orthosteric site." However, recently, the search has shifted to ligands that can interact with a different region of the receptor protein, the "allosteric site," since this approach offers potential pharmacological and therapeutic advantages.[1]

One possibility to overcome the limitations of the "traditional" cannabinoids, interacting with the orthosteric sites on cannabinoid receptors, is to look for compounds that can bind to a different (allosteric) site.[2,3] However, only a very limited number of chemical structures have been investigated as potential allosteric cannabinoid ligands (including endogenous and synthetical allosteric modulators).[4] Among them, worth mentioning are the indole carboxamides such as ORG27569 (Fig. 1), ORG27759, and ORG29647,[5] and some derivatives of these compounds,[6–9] the urea compound PSNCBAM-1[10] and its recently published[11] and patented[12] analogs, or the cocaine-related structure RTI-371.[13]

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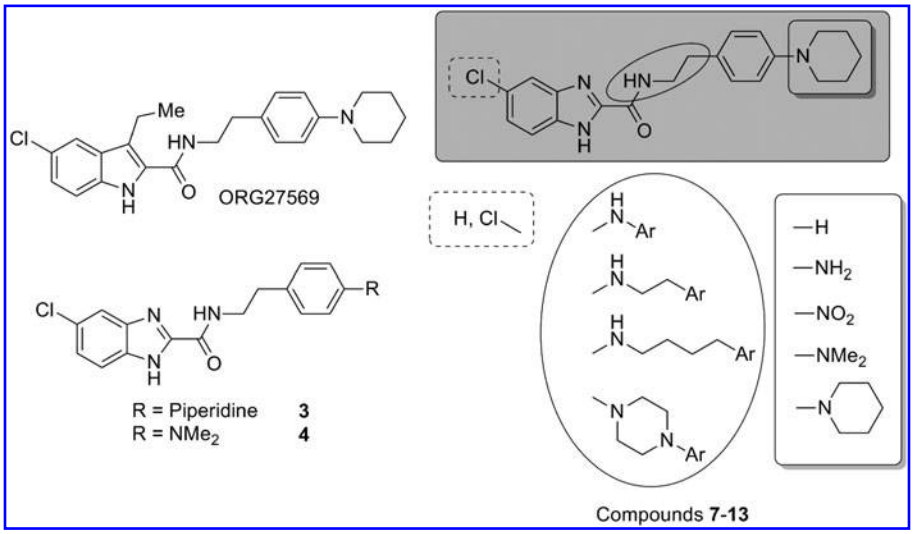

FIG. 1. Structure of ORG27569 and of the synthesized benzimidazoles.

Several analogs of ORG compounds have been extensively studied by Kendall, Lu, and coworkers,[7,14,15] but to date, the indole ring has not been replaced, with the exception of four benzofuran compounds, which had shown interesting properties.[14] Within this context, we decided to study the possibility of modifying the nature of the heterocycle in the ORG compounds, incorporating a benzimidazole ring. To explore this scaffold, structural and substituent changes have been undertaken on the benzimidazole, on the piperidine group, and on the length of the amide linker (Fig. 1). Once synthesized, the affinity of the new compounds for CB_1R and CB_2R has been measured in competitive [^{3}H]-CP55940 binding experiments, and their efficacy as allosteric modulators has been evaluated in [^{35}S]-GTPγS binding assays. Only compounds **3** and **4** showed some activity.

Materials and Methods

Chemistry

The synthesis of compounds **3** and **4** is described next. Preparation of compounds **5–13** was performed by a procedure similar to the one of compounds **1–4** (scheme and experimental synthetical details of compounds **5–13**, and general synthetical issues are covered in the Supplementary Data).

5-Chloro-2-(trichloromethyl)-1*H*-benzimidazole (**1**)

Methyl trichloroacetimidate (1.90 mL, 15.4 mmol, 1.1 equivalent [eq]) was added dropwise to a cooled solution of 4-chloro-*o*-phenylenediamine (2.00 g; 14.0 mmol, 1 eq.) in glacial acetic acid (25 mL). At the end of the addition, the dark reaction mixture was kept at room temperature for 1 h. Afterward, water (20 mL) was added to facilitate the product precipitation. The resulting residue was filtered off, washed with water, and dried under vacuum. It was purified by medium-pressure chromatography (Isolera, eluent: hexane → 3:7 *n*-hexane/EtOAc) to give 3.05 g of **1** (80%) as a pale brown solid, mp: 231–233°C. ^{1}H-NMR (300 MHz, DMSO-d6): δ 7.73 (bs, H_4, 1H); 7.68 (d, $Jo = 8.5$ Hz, H_7, 1H); 7.35 (bd, $Jo = 8.5$ Hz, H_6, 1H); ^{13}C-NMR (75 MHz, DMSO-d6): δ 151.8 C_2; 139.1 and 136.8 C_{3a} and C_{7a}; 128.2 C_5; 124.3 C_6; 117.7 and 116.1 C_4 and C_7; 88.5 CCl_3; liquid chromatography/mass spectrometry (LC/MS) (ES+) $t_R = 4.7$ min (94% purity), m/z = 271 $[M+H]^+$.

5-Chloro-1*H*-benzimidazole-2-carboxylic acid (**2**)

1 (1.50 g, 55.6 mmol, 1 eq.) was added to a solution of sodium hydroxide (100 mL; 1 N) at 0°C. The resulting solution was filtered, and the filtrate was acidified with 37% hydrochloric acid to pH 4. The precipitate was filtered off, washed twice with both water and ether. The resulting solid was then dried under vacuum to give 1.06 g (97%) of **2** as a pale brown solid, mp: 159–161°C. ^{1}H-NMR (300 MHz, DMSO-d6): δ 8.28 (s, NH, 1H); 7.66 (d, $Jm = 2.0$ Hz, H_4); 7.60 (d, $Jo = 8.6$ Hz, H_7); 7.21 (dd, $Jo = 8.6$ Hz, $Jm = 2.0$ Hz, H_6); ^{13}C-NMR

(75 MHz, DMSO-d6): δ 143.4 COOH and C_2; 139.3 and 136.6 C_{3a} and C_{7a}; 126.2 C_5; 122.1 C_6; 116.4 and 115.2 C_4 and C_7; MS (ES+), m/z = 197 $[M+H]^+$.

5-Chloro-N-[4-(piperidin-1-yl)phenethyl]-1*H*-benzimidazole-2-carboxamide (**3**)

A mixture of **2** (192 mg, 1.0 mmol, 1 eq.), PyBOP (509 mg, 1.0 mmol, 1 eq.), and NMM (323 μL, 3.0 mmol, 1 eq.) in DMF (3 mL) was stirred at room temperature for 15 min to activate the acid. Then, 2-[4-(piperidin-1-yl)phenyl]ethanamine hydrochloride (270 mg, 1.0 mmol, 1 eq.) was added and the mixture was stirred at room temperature overnight. Afterward, DMF was removed *in vacuo*. The residue was dissolved in EtOAc and washed with water (3 × 30 mL). The organic layer was dried over anhydrous $MgSO_4$, and the solvent was evaporated. The residue was purified by medium-pressure chromatography (Isolera, eluent: *n*-hexane → 3:7 hexane/EtOAc), obtaining **3** as a white solid (132 mg, 36%), mp: 173–176°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 12.78–12.68 (m, Benzimidazole NH, 1H); 8.06 (s, CONH, 1H); 7.75–7.29 (m, H_4, H_6 and H_7, 3H); 7.14 (d, *J* = 8.2 Hz, H*m*, 2H); 6.89 (d, *J* = 8.2 Hz, H*o*, 2H); 3.84–3.74 (m, $CONHCH_2$, 2H); 3.17–3.05 (m, piperidine NCH_2, 4H); 2.93 (t, *J* = 7.2 Hz, CH_2Ar, 2H); 1.78–1.67 (m, piperidine NCH_2CH_2, 4H); 1.60–1.52 (m, piperidine $NCH_2CH_2CH_2$, 2H);^{13}C-NMR (75 MHz, $CDCl_3$): δ 159.5 CONH; 151.0 C*p*; 145.9 C_2; 143.7 C_{3a}; 141.5 C_{3a} rotamer; 135.2 C_{7a}; 133.2 C_{7a} rotamer; 130.7 C_5; 129.4 C*o*; 129.0 C*ipso*; 125.6 C_6; 124.4 C_6 rotamer; 121.3 C_7; 120.2 C_7 rotamer; 117.1 C*m*; 113.6 C_4; 112.7 C_4 rotamer; 51.0 piperidine NCH_2; 41.6 $CONHCH_2$; 35.0 CH_2Ar; 25.9 piperidine NCH_2CH_2; 24.3 piperidine $NCH_2CH_2CH_2$; LC/MS (ES+) t_R = 2.9 min (99% purity), m/z = 383 $[M+H]^+$.

5-Chloro-*N*-[4-(dimethylamino)phenethyl]-1*H*-benzimidazole-2-carboxamide (**4**)

A mixture of **2** (60 mg, 1.0 mmol, 1 eq.), PyBOP (158 mg, 1.0 mmol, 1 eq.), and NMM (100 μL, 3.0 mmol, 1 eq.) in DMF (3 mL) was stirred at room temperature for 15 min to activate the acid. Then, 4-(2-aminoethyl)-*N*,*N*-dimethylaniline (50 mg, 1.0 mmol, 1 eq.) was added and the mixture was stirred at room temperature overnight. Afterward, water (30 mL) was added to the reaction mixture. Then, the solid was filtered off, washed with water, and dried under vacuum, obtaining **4** as a brown solid (61 mg, 58%), mp: 187–192°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 12.83 (bs, Benzimidazole NH, 1H); 8.11 (bs, CONH, 1H); 7.75–7.45 (m, H_4 and H_7, 2H); 7.32–7.28 (m, H_6, 1H); 7.13 (d, *J* = 8.2 Hz, H*o*, 2H); 6.68 (d, *J* = 8.2 Hz, H*m*, 2H); 3.83–3.76 (m, $CONHCH_2$, 2H); 2.95–2.86 (m, CH_2Ar and NMe_2, 8H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 159.5 CONH; 149.6 C*p*; 146.1 C_2; 129.5 C*o*; 126.3 C*ipso*; 113.3 C*m*; 41.7 $CONHCH_2$; 40.9 NMe_2; 34.8 CH_2Ar; LC/MS (ES+) t_R = 3.0 min (92% purity), m/z = 343 $[M+H]^+$.

Pharmacology

Equilibrium binding assay. The equilibrium binding assays were performed as previously described.[16]

[^{35}S]-GTPγS binding assay. CP55940 was purchased from Tocris (Bristol, United Kingdom), [^{35}S]GTPγS (1250 Ci/mmol) from PerkinElmer (Seer Green, Buckinghamshire, United Kingdom), GTPγS from Roche

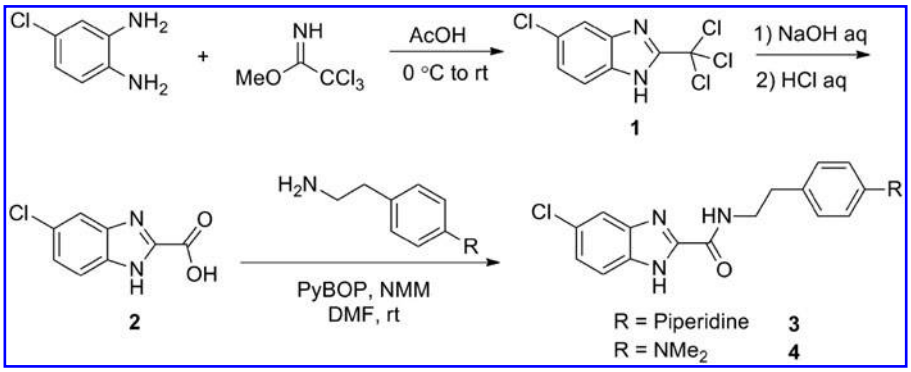

FIG. 2. Synthesis of benzimidazoles **3** and **4**.

Diagnostics (Burgess Hill, West Sussex, United Kingdom), and GDP from Sigma-Aldrich (Poole, Dorset, United Kingdom).

All [^{35}S]-GTPγS binding assays were carried out with mouse whole-brain membranes, prepared as described elsewhere.[17] These membranes (5 μg of protein per well) were preincubated for 30 min at 30°C with adenosine deaminase (0.5 U/mL). The membranes were then incubated with the agonist with a vehicle or modulator for 60 min at 30°C in assay buffer (50 mM Tris-HCl, 50 mM Tris-base, 5 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl, 1 mM dithiothreitol, and 0.1% BSA) in the presence of 0.1 nM [^{35}S]-GTPγS and 30 μM GDP, in a final volume of 500 μL. Binding was initiated by the addition of [^{35}S]-GTPγS. Nonspecific binding was measured in the presence of 30 μM GTPγS. The reaction was terminated by rapid vacuum filtration (50 mM Tris-HCl, 50 mM Tris-base, and 0.1% BSA) using a 24-well sampling manifold (Brandel cell harvester) and GF/B filters that had been soaked in buffer (50 mM Tris-HCl, 50 mM Tris-base, and 0.1% BSA) for at least 24 h. Each reaction tube was washed six times with a 1.2-mL aliquot of ice-cold wash buffer. The filters were oven-dried for at least 60 min and then placed in 3 mL of scintillation fluid (Ultima Gold XR). Radioactivity was quantified by liquid scintillation spectrometry.

Results and Discussion

The synthesis of compounds **3** and **4** is depicted in Figure 2. They were prepared by the coupling reaction of 5-chloro-1*H*-benzimidazole-2-carboxylic acid (**2**) with the corresponding amine in the presence of the PyBOP reagent and NMM in DMF. Acid **2** was obtained in good yield by hydrolyzing 5-chloro-2-(trichloromethyl)-1*H*-benzimidazole (**1**) with sodium hydroxide aqueous solution, followed by acidification with dilute hydrochloric acid.[18] In turn, **1** was first prepared from 4-chloro-*o*-phenylenediamine and trichloromethyl acetimidate using a weak acid.

The ability of benzimidazoles **3** and **4** to displace [^{3}H]-CP55940 from human cannabinoid CB_1R or CB_2R transfected into HEK293 EBNA cells was first evaluated at a compound concentration of 40 μM. At this concentration, none of the compounds was able to displace [^{3}H]-CP55940 from CB_1R and/or from CB_2R (data not shown). These data suggest that **3** and **4** could be discarded as orthosteric CB_1R/CB_2R binders, since these compounds (**3** and **4**) did not affect the binding affinity of the cannabinoid receptor agonist CP55940 at 40 μM (K_i CB_1R and K_i CB_2R ≥40 μM). Then, [^{35}S]-GTPγS

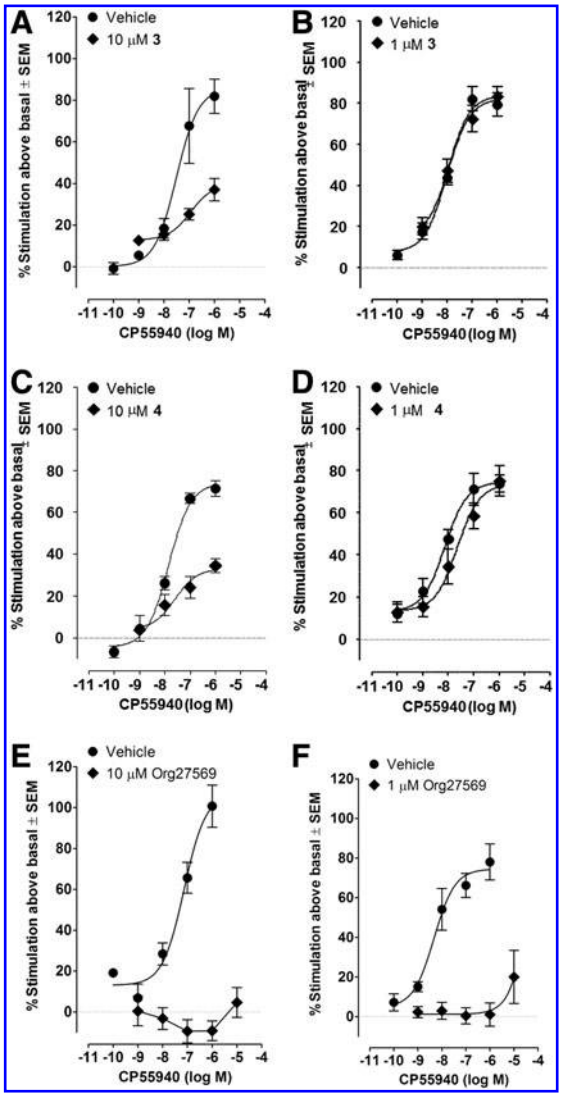

FIG. 3. Stimulation of binding of [^{35}S]-GTPγS to mouse whole-brain membranes by CP55940 in the presence of vehicle (DMSO, $n = 10$) or **3** at 10 μM ($n = 10$) **(A)**, in the presence of vehicle (DMSO, $n = 4$) or **3** at 1 μM ($n = 4$) **(B)**, in the presence of vehicle (DMSO, $n = 4$) or **4** at 10 μM ($n = 4$) **(C)**, in the presence of vehicle (DMSO, $n = 8$) or **4** at 1 μM ($n = 8$) **(D)**, in the presence of vehicle (DMSO, $n = 4$) or ORG27569 at 10 μM ($n = 4$) **(E)**, and in the presence of vehicle (DMSO, $n = 4$) or ORG27569 at 1 μM ($n = 4$) **(F)**. Each symbol represents the mean percentage stimulation above basal ± SEM.

binding assays in mouse whole-brain membranes were performed to assess the effect of the synthesized benzimidazoles on the functionality of CP55940.

In [^{35}S]-GTPγS binding assays performed with compound **3** or its vehicle (Fig. 3A), CP55940 stimulated [^{35}S]-GTPγS binding with a mean maximal effect (E_{max}) that was significantly less in the presence of **3** at 10 μM (38.0%) than in its absence (70.2%). The corresponding mean EC_{50} values of CP55940 in the presence and absence of **3** at 10 μM were 118.4 and 16.9 nM, respectively, and these EC_{50} values are not significantly different from each other. In the case of compound **4** at 10 μM (Fig. 3C), CP55940 stimulated [^{35}S]-GTPγS binding with an E_{max} that was significantly less in the presence of **4** (33.3%) than in its absence (74.1%). The corresponding mean EC_{50} values of CP55940 are not significantly different from each other: 24.5 nM in the presence of compound **4** and 14.1 nM in its absence. However, at 1 μM, none of the compounds **3** and **4** affects the E_{max}, or indeed the EC_{50} of CP55940 (Fig. 3B, D, respectively). These results indicate that benzimidazoles **3** and **4** produce significant negative allosteric modulation (NAM) of CP55940 agonism at the mouse CB_1R, although they are somewhat less potent than the CB_1R allosteric cannabinoid ORG27569, which showed efficacy on CP55940 at 1 μM in this assay (Fig. 3F). The confidence intervals of these data are covered in the Supplementary Data (Supplementary Table S1).

According to the results from molecular modeling studies of the allosteric CB_1R binding site of ORG27569 that have been published to date, the main interaction between this ligand and the receptor, in the presence of CP55940, occurs between the piperidine nitrogen and the K3.28(192) amino acid.[19] However, there are not many reported data regarding the importance of the 3-ethyl substituent or the free NH of the indole. Regarding the substitution on position 3 of the indole, a recent paper indicates that a small chain (H or Me) improved CB_1R allosteric modulation.[8] It is noteworthy that in our hands, replacement of the 3-ethyl group of ORG27569 with a nitrogen (in **3** and **4**) affects the efficacy with which GTPγS binding is allosterically modulated. Therefore, we performed the alkylation of **3** and **4,** leading to *N*-substituted compounds in position 3 of the heterocycle (data not shown). These compounds did not exhibit any CB_1R allosteric modulation in [^{35}S]-GTPγS assays (data not shown), thus indicating that the presence of a free NH in benzimidazoles seems to be crucial for CB_1R allosteric modulation.

Conclusions

In summary, the benzimidazole ring has been explored as a potential scaffold for developing cannabinoid allosteric ligands. Thus, a series of benzimidazole-2-carboxamides has been prepared. Regarding binding assays, compounds **3** and **4** significantly attenuated the ability of CP55940 to stimulate [^{35}S]-GTPγS binding, without affecting the orthosteric ligand binding affinity of [^{3}H]-CP55940 in radioligand competitive assays, suggesting that they may act as CB_1R negative allosteric modulators. In conclusion, replacement of the indole ring with a benzimidazole ring within the structure of ORG27569 abolished the binding of the resultant ligands to CB_1R, but the modulation on the agonist-induced GTPγS binding was maintained.

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

CB_1R = cannabinoid 1 receptor
CB_2R = cannabinoid 2 receptor
DMF = dimethylformamide
DMSO = dimethylsulfoxide
LC/MS = liquid chromatography/mass spectrometry
NAM = negative allosteric modulator
NMM = N-methylmorpholine
PyBOP = benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
SEM = standard error of the mean