Exploring 6-Azaindole and 7-Azaindole Rings for Developing Cannabinoid Receptor 1 Allosteric Modulators

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Abstract

Introduction and Objective: Org27569 is a prototypical allosteric modulator of the cannabinoid receptor 1 (CB1). It belongs to the indole-2-carboxamide scaffold and has been intensively investigated in pharmacology and in structure–activity relationship (SAR) studies. Although azaindoles are rare in natural products and differ only by the presence of an extra ring nitrogen, they were demonstrated as valuable bioisosteres in many pharmacologically important molecules. To extend the SAR investigation of the indole-2-carboxamide class of CB1 allosteric modulators, azaindole (pyrrolopyridine) rings were used to replace the indole ring of Org27569 analogs to explore the potential of azaindole-2-carboxamides as CB1 allosteric modulators. Using 6- and 7-azaindole in lieu of the indole moiety within this class of CB1 allosteric modulators indeed improved the aqueous solubility.

Materials and Methods: We synthesized 6- and 7-azaindole-2-carboxamides and their indole-2-carboxamide counterparts. The molecules were evaluated by [3H]CP55,940 binding and [35S]GTPγS binding assays for their allosteric modulation of the CB1 receptor.

Results: The 7-azaindole-2-carboxamides lost the ability to bind to the CB1 receptor. The 6-azaindole-2-carboxamides (e.g., 3c and 3d) showed markedly reduced binding affinities to the CB1 receptor in comparison with their indole-2-carboxamide counterparts. However, they behaved similarly as indole-2-carboxamides in potentiating the orthosteric agonist binding and inhibiting the orthosteric agonist-induced G-protein coupling. The results indicated that some azaindole scaffolds (e.g., 6-azaindole) are worth further exploration, whereas the 7-azaindole ring is not a viable bioisostere of the indole ring in the Org27569 class of CB1 allosteric modulators.

Keywords: allosteric modulators; azaindole; bioisostere; cannabinoid; CB1 receptor

Introduction

The cannabinoid receptor 1 (CB1) is a G-protein-coupled receptor and has been recognized as a promising target for the treatment of many disorders, including pain, inflammation, metabolic syndromes, and neurodegenerative diseases.1 However, the CB1 receptor has been challenging as a druggable target because of central nervous system side effects associated with drug candidates that bind to the orthosteric site where the endogenous cannabinoids bind. Therefore, recent efforts have focused on developing allosteric modulators that target CB1 at sites topographically distinct from the orthosteric sites.2 Several small molecules have been revealed as CB1 allosteric modulators over the last 10 years.2–4

Org27569 is an indole-2-carboxamide and a prototypical CB1 allosteric modulator that has been intensively investigated.5–7 Following its discovery, several structure–activity relationship studies have addressed the key requirements to maintain or improve allosteric modulation effects.8 Most of the structural optimization

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of Org27569 was based on the indole moiety except two cases, in which benzofuran and benzimidazole rings were used in lieu of the indole ring. The binding affinities of the benzofuran analogs of Org27569 were significantly reduced, although the binding cooperativities with the orthosteric agonist were markedly enhanced.9 Similarly, the binding affinities of benzimidazole analogs of Org27569 were abolished, while allosteric modulation on agonist-induced GTP\textsubscript{i}/S binding was maintained.10

In recent years, azaindoles (pyrrolopyridine) have gained significant attention as the bioisosteres of the indole ring due to their ability to facilitate pharmaceutical optimization, such as increasing solubility, reducing lipophilicity, enhancing target binding, and improving ADME as well as toxicology properties.11–15

The fusion of a pyrimidine ring and a pyrrole ring can provide a pyrrolopyridine structure having four isomers (i.e., 4-, 5-, 6-, and 7-azaindoles). Among them, the 7-azaindoles have been demonstrated with luminescent and fluorescent properties, which can render the molecule containing the 7-azaindole moiety with some chromophoric functions.11,12 In the synthesis of a group of cannabinoid receptor agonists from the known cannabinoid ligand JWH-018, it was demonstrated that bioisosteric replacement of the indole ring with the azaindole moieties (e.g., 5-, 6-, and 7-azaindoles) substantially improved the physicochemical properties of the resultant compounds.16 In addition, bioisosteric replacement of the indole ring with an azaindole moiety can enhance the drug–target interaction by formation of an extra hydrogen bond and significantly increase the pharmacological effects.17,18

In addition to the frequently used 5-, 6-, and 7-azaindoles, the 4-azaindoles also was reported as a viable bioisosteric replacement of indole.19 With the goal of increasing aqueous solubility and developing a new scaffold, we designed and synthesized 6-azaindole-2-carboxamides (3c, 3d) and 7-azaindole-2-carboxamides (9a, 9b) to compare with their indole-2-carboxamide counterparts (3a and 3b) to explore the possibility of developing CB\textsubscript{1} allosteric modulators. We used dimethylaminophenyl ethylamine and piperidinylphenyl ethylamine, which have been shown in the previous series of indole-2-carboxamides to improve function\textsuperscript{8,20,21} and to synthesize the target 6- and 7-azaindole-2-carboxamides shown in Figure 1C. The aqueous solubility of some synthesized compounds were assessed by measurement of their thermodynamic solubility because kinetic solubility data frequently overestimates solubility compared to thermodynamic solubility.22

**Materials and Methods**

**Chemistry**
The synthesis of reference indole-2-carboxamides 3a and 3b was carried out by coupling of commercially available 5-chloro-indole-2-carboxylic acid with commercially available 4-(2-aminoethyl)-N,N-dimethylaniline (2a) and 2-(4-(piperidin-1-yl)phenyl)ethanamine (2b), respectively,

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**FIG. 1.** Prototypical CB\textsubscript{1} allosteric modulator Org27569 (A), referenced indole-2-carboxamide analogs of Org27569 (B), and designed 6- and 7-azaindole-2-carboxamides (C). CB\textsubscript{1}, cannabinoid receptor 1.
through the catalysis of 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) and N-methyl morpholine (NMM) in THF. Similarly, 6-azaindole-2-carboxamides 3c and 3d were synthesized from 5-chloro-6-azaindole-2-carboxylic acid. To synthesize the 7-azaindole-2-carboxamides 9a and 9b, the required intermediate methyl 5-chloro-7-azaindole-2-carboxylate 7 was obtained through Hemetsberger–Knittel indole synthesis with a protocol previously optimized by us.20 Hydrolysis of the 7-azaindole-2-carboxylate 7 was achieved by treating with a high concentration of 3c and 3d on G-protein coupling of CB1. GTPγS assays were performed essentially as described previously.20 Membranes of CB1 expressing cells were prepared, and 8 μg of membrane preparation was incubated with 0.1 μM of CP55,940 plus or minus the allosteric modulator, allosteric modulator alone, or 1 μM SR141716A alone, and 0.1 nM [35S]GTPγS (1250 Ci/mmol; PerkinElmer Life Sciences, Boston, MA), 10 μM GDP (Sigma, St. Louis, MO), and 0.1% (w/v) BSA. GTPγS binding assay buffer (50 mM Tris-HCl, pH 7.4, 3 mM MgCl2, 0.2 mM EGTA, and 100 mM NaCl) was added to 200 μL. The membranes were incubated for 1 h at 30°C. To determine nonspecific binding, 10 μM unlabeled GTPγS (Sigma) was used. To determine basal activity, membrane preparations were treated with vehicle (dimethylsulfoxide or DMSO) alone. Termination of the reaction was achieved through filtration using Whatman GF/C filter papers and washing with cold TME buffer. Bound radioactivity was measured by liquid scintillation counting.

**Receptor expression and membrane preparation**

Human embryonic kidney 293T (HEK293T) cells were grown and seeded in Dulbecco’s modified Eagle’s medium with 3.5 mg/mL glucose and 10% fetal bovine serum at 37°C and 5% carbon dioxide. To express the CB1 receptors, HEK293T cells were seeded at 1,000,000 cells/100-mm plate. The following day, cells were transfected via the calcium phosphate method with 2a and 2b, respectively, to yield the target compounds 9a and 9b. The experimental details for the synthesis of compounds 3a–d, 6–8, and 9a–9b can be found in the Supplementary Data.

**Equilibrium binding assay**

Approximately 3 μg of membrane preparation expressing CB1 was incubated with nine concentrations of allosteric modulator (1 nM-10 μM). The radio labeled tracer [3H]CP55,940 (150.2 Ci/mmol; Perkin Elmer), an orthosteric agonist of CB1, was added at 0.5 nM. Nonspecific binding was established by treating with a high concentration of unlabeled CP55,940 (10 μM; Tocris). The membranes were incubated at 30°C for 60 min, and the reaction was terminated with the addition of 300 μL of Tris-Mg2+-EDTA (TME) buffer with 5% bovine serum albumin (BSA). The mixture was harvested by filtration through a Brandel cell harvester with Whatman GF/C filter paper. Liquid scintillation counting was used to measure radioactivity.

**GTPγS binding evaluation**

To assess the impact of 3c and 3d on G-protein coupling of CB1, GTPγS assays were performed essentially as described previously. Membranes of CB1 expressing cells were prepared, and 8 μg of membrane preparation was incubated with 0.1 μM of CP55,940 plus or minus the allosteric modulator, allosteric modulator alone, or 1 μM SR141716A alone, and 0.1 nM [35S]GTPγS (1250 Ci/mmol; PerkinElmer Life Sciences, Boston, MA), 10 μM GDP (Sigma, St. Louis, MO), and 0.1% (w/v) BSA. GTPγS binding assay buffer (50 mM Tris-HCl, pH 7.4, 3 mM MgCl2, 0.2 mM EGTA, and 100 mM NaCl) was added to 200 μL. The membranes were incubated for 1 h at 30°C. To determine nonspecific binding, 10 μM unlabeled GTPγS (Sigma) was used. To determine basal activity, membrane preparations were treated with vehicle (dimethylsulfoxide or DMSO) alone. Termination of the reaction was achieved through filtration using Whatman GF/C filter papers and washing with cold TME buffer. Bound radioactivity was measured by liquid scintillation counting.

**Results and Discussion**

To synthesize the target azaindole-2-carboxamides, it requires corresponding azaindole-2-carboxylic acids (i.e., 1b and 8) as the key building blocks (Fig. 2). To
access these azaindole-2-carboxylic acids, we used the Hemetsberger–Knittl indole synthesis, which involves Knoevenagel condensation between a pyridinecarbaldehyde and the azido acetate 5. However, the condensation between 5-chloro-pyridine-3-carbaldehyde and the azido acetate 5 failed to provide the desired Knoevenagel product. In this investigation, the 6-azaindole-2-carboxylic acid 1b was obtained from a commercial source (Achemtek, Worcester, MA). In contrast, the condensation between 2-chloro-pyridine-4-carbaldehyde (4) and the azido acetate 5 successfully produced the corresponding Knoevenagel product 6, from which the required 7-azaindole-2-carboxylic acid (8) can be easily obtained. Unlike the indole-2-carboxylic acids, the coupling of the azaindole-2-carboxylic acids 1b and 8 with corresponding amines under the catalysis of HOBT or BOP and Hünig’s base leads to low yields of the amide 3c-d and 9a-b. In contrast, catalyzing the coupling reaction by DMTMM and NMM generally provided the corresponding amides.
in good yields. The synthesized compounds were assessed by equilibrium binding assay,\(^ {25,26}\) which identifies two important parameters for initial characterization of allostery: \(K_B\), the equilibrium dissociation constant that defines the affinity of an allosteric modulator for its receptor; and \(\alpha\), the cooperativity factor, which defines the magnitude and direction of impact that the allosteric modulator and orthosteric ligand have on each other when both occupy the receptor. At the receptor binding level, when \(\alpha\) is \(>1\), the ligand is a positive allosteric modulator, whereas when \(\alpha\) is \(<1\), the ligand is a negative allosteric modulator. Accordingly, when \(\alpha\) is equal to 1, it indicates no allosteric modulation on orthosteric ligand binding. The \(K_B\) and \(\alpha\) values of the synthesized compounds are presented in Table 1. In comparison with their indole-2-carboxamide counterparts 3a and 3b, the 7-azaindole-2-carboxamides 9a and 9b completely lost their binding affinity to the CB\(_1\) receptor, likely influenced by the poor solubility of these compounds. In contrast, the 6-azaindole-2-carboxamides 3c and 3d exhibited modest binding affinity to the CB\(_1\) receptor. Interestingly, 6-azaindole-2-carboxamide 3d showed comparable allosteric modulation on the binding of orthosteric agonist CP55,940, while its binding to the CB\(_1\) receptor was reduced by about 25-fold in comparison with its indole-2-carboxamide counterpart 3b. These results from 9a and 9b suggested that the 7-azaindole is not an optimal bioisostere for replacing the indole ring in the class of CB\(_1\) allosteric modulators, although it has been successfully used as an effective bioisostere in other indole-containing bioactive molecules.\(^ {14}\)

| Entry | Compd Code | Structure | \(K_B (\mu M)^{a}\) | \(\alpha^{b}\) |
|-------|------------|-----------|-----------------|----------------|
| 3a    | LDK1322    | ![Structure](image1) | 0.3             | 4.2            |
| 3b    | LDK1326    | ![Structure](image2) | 0.2             | 5.1            |
| 3c    | LDK1314    | ![Structure](image3) | 2.4             | 3.2            |
| 3d    | LDK1316    | ![Structure](image4) | 5.6             | 4.4            |
| 9a    | LDK1313    | ![Structure](image5) | NB\(^c\)        | NA\(^d\)       |
| 9b    | LDK1315    | ![Structure](image6) | NB\(^c\)        | NA\(^d\)       |

The red color indicates that the N is the newly introduced nitrogen on the original indole ring.

\(^{a}\)\(K_B\): equilibrium dissociation constant.

\(^{b}\)\(\alpha\): binding cooperativity factor.

\(^{c}\)NB: no detectable modulation.

\(^{d}\)NA: not applicable.
To investigate G-protein coupling induced by the allosteric modulators, the 6-azaindole-2-carboxamides 3c and 3d were tested for their impact on GTP\(_\text{c}\)S binding. These results, plotted as percent of basal levels of CB1 activity, are shown in Figure 3. Both 3c and 3d showed an inhibition of GTP\(_\text{c}\)S binding at 5 and 10 \(\mu\)M in the presence of 0.1 \(\mu\)M CP55,940, compared to membranes treated with 0.1 \(\mu\)M of CP55,940 alone. These data suggest that although the allosteric modulators apparently enhanced the binding of CP55,940, the G-protein coupling induced by CP55,940 was inhibited. In addition, inhibition of G-protein coupling was also seen when CB1 was treated with 3c and 3d alone. The level of G-protein coupling inhibition achievable is comparable to that observed with the inverse agonist SR141716A, although the orthosteric compound SR141716A and the allosteric modulators, 3c and 3d, likely inhibit G-protein coupling via different mechanisms.

In the solubility tests, we selected compounds 3c, 3d, and 9a because they represented the indole, 6-azaindole and 7-azaindole scaffold, respectively. We found that compound 9a has exceptionally low solubility in common organic solvents suitable for HPLC sample preparation such as methanol, acetone, and their mixtures with different stoichiometry. We tried the sampling method reported previously to prepare stock solution of 9d into a 2%–5% DMSO in Acetonitrile solution. Addition of aqueous PBS media led to precipitation of the compound. This made us unable to determine the standard curve for analysis of 9d. Hence, only compounds 3c and 3d were analyzed. The individual solubility of 3c, 3d, and 9a was also simulated using a program (Chemicalize). The simulated aqueous solubility of these three compounds was reported along with the experimental solubility of 3c, 3d, and 9a in Table 2. The results from solubility studies showed that using 6- and 7-azaindole in lieu of the indole moiety led to enhancement of aqueous solubility compared to the indole counterpart although their solubility are still poor and far below optimal solubility. This finding is in agreement with the results in an early investigation, of which 6- and 7-azaindole analogs showed solubility enhancement in comparison with indole counterpart. The binding parameters of the synthesized compounds suggested that the 6-azaindole scaffold is worth further exploration, whereas the 7-azaindole ring is not a viable bioisostere of the indole ring in the Org27569 class of CB1 allosteric modulators.

Although their binding affinity for CB1 was less than their indole-2-carboxamide counterparts, they showed similar coupling characteristics as Org27569, in which they decreased G-protein coupling when compared with treatment with CP55,940 alone, indicating that they may induce signaling in a way similar to Org27569 and its analogs. Further research is needed to fully elucidate the signaling pathways of these compounds; however, it might involve beta-arrestin coupling and signaling such as Org27569 and its previously tested analogs.

![FIG. 3. Response of 3c (LDK1314) and 3d (LDK1316) on [\(^{35}\)S]GTP\(\gamma\)S binding to membranes expressing CB1. The effects of 0.1 \(\mu\)M CP55,940 alone, or 0.1 \(\mu\)M CP55,940 with the allosteric modulators 3c or 3d, 3c, or 3d alone, or 1.0 \(\mu\)M SR141716A alone on [\(^{35}\)S]GTP\(\gamma\)S binding were measured at the concentrations indicated. Data are presented as a percentage of basal levels of [\(^{35}\)S]GTP\(\gamma\)S binding. Nonspecific binding was measured in the presence of 10 \(\mu\)M unlabeled GTP\(\gamma\)S. Each data point represents the mean ± standard error of the mean of at least three independent experiments performed in duplicate. GTP\(\gamma\)S, guanosine 5’-O-[gamma-thio]triphosphate.](image)

### Table 2. Calculated Aqueous Solubility and the Experimental Thermodynamic Solubility of Compounds 3a, 3c, and 9a

| Compound | Aqueous solubility\(^a\) | Thermodynamic solubility\(^b\) |
|----------|--------------------------|-----------------------------|
| 3a       | 3.0 \(\mu\)g/mL          | 0 \(\mu\)g/mL               |
| 3c       | 8.0 \(\mu\)g/mL          | 1.6 \(\mu\)g/mL             |
| 9a       | 6.0 \(\mu\)g/mL          | 3.0 \(\mu\)g/mL             |

\(^a\)Aqueous solubility simulated by computation. 
\(^b\)Thermodynamic solubility obtained from experiments.
postulated that the reduced pharmacologic effects of the 6-azaindole-2-carboxamides in comparison with their indole counterparts are likely due to the impact of its electron-deficient pyridine ring on the aromatic π-π stacking during interaction with the allosteric binding site. Therefore, hypothetically, introduction of strong electron-donating groups such as alkoxyl, amino and alkylamino groups on the 6-azaindole moiety may help to improve the pharmacologic effects and aqueous solubility of future compounds bearing the 6-azaindole moiety.

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Author Disclosure Statement
No competing financial interests exist.

References
1. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev. 2006;58:389–462.
2. Nguyen T, Li JX, Thomas BF, et al. Allosteric modulation: an alternate approach targeting the cannabinoid CB1 receptor. Med Res Rev. 2017;37:441–474.
3. Morales P, Goya P, Jagerovic N, et al. Allosteric modulators of the CB1 cannabinoid receptor: a structural update review. Cannabis Cannabinoid Res. 2016;1:22–30.
4. Kulkarni AR, Garai S, Janero DR, et al. Design and synthesis of cannabinoid 1 receptor (CB1R) allosteric modulators: drug discovery applications. Methods Enzymol 2017;593:281–315.
5. Price MR, Baille GL, Thomas A, et al. Allosteric modulation of the cannabinoid CB1 receptor. Mol Pharmacol. 2005;68:1484–1495.
6. Baille GL, Horswill J, Anavi-Goffer S, et al. CB1 receptor allosteric modulators display both agonist and signaling pathway specificity. Mol Pharmacol. 2012;83:322–338.
7. Ahn KH, Mahmoud MM, Kendall DA. Allosteric modulator ORG27569 induces CB1 cannabinoid receptor high affinity agonist binding state, receptor internalization, and Gi protein-independent ERK1/2 kinase activation. J Biol Chem. 2012;287:12070–12082.
8. Piscitelli F, Ligresti A, La Regina G, et al. Indole-2-carboxamides as allosteric CB1R modulators of the cannabinoid CB1 receptor. J Med Chem. 2012;55:5627–5631.
9. Mahmoud MM, Ali HI, Ahn KH, et al. Structure–activity relationship study of indole-2-carboxamides identifies a potent allosteric modulator for the cannabinoid receptor 1 (CB1). J Med Chem. 2013;56:7965–7975.
10. Hernandez-Folgado L, Stevenson LA, Morales P, et al. Exploring the benzimidazole ring as a substitution for indole in cannabinoid allosteric modulators. Cannabis Cannabinoid Res. 2016;1:196–201.
11. Smirnov AV, English D, Rich R, et al. Photophysics and biological application of 7-azaindole and its analogs. J Phys Chem B 1997;101:2758–2769.
12. Zhao SB, Wang S. Luminescence and reactivity of 7-azaindole derivatives and complexes. Chem Soc Rev. 2010;39:3142–3156.
13. Mérour J, Buron F, Plé K, et al. The azaindole framework in the design of kinase inhibitors. Molecules. 2014;19:19935–19979.
14. Merour J-Y, Joseph B. Synthesis and reactivity of 7-azaindoles (1H-Pyrrolo[2, 3-b]pyridine. COC. 2003;5:471–506.
15. Rodríguez J, Ferraud M. Azaindoles as medicinally relevant scaffolds. SPC Chem Mag. 2005;10:16–17.
16. Blaazer AR, Lange JH, van der Neut MA, et al. Novel indole and azaindole (pyrrolopyridine) cannabinoid (CB) receptor agonists: design, synthesis, structure–activity relationships, physicochemical properties and biological activity. Eur J Med Chem. 2011;46:5086–5098.
17. Echaller A, Bettaieb K, Ferandin Y, et al. Merirolins (3-(pyrimidin-4-yl)-7-azaindoles): synthesis, kinase inhibitory activity, cellular effects, and structure of a CDK2/cyclin A/merirolin complex. J Med Chem. 2008;51:737–751.
18. Riether D, Harcken C, Razavi H, et al. Nonsteroidal dissociated glucocorticoid agonists containing azaindoles as steroid A-ring mimetics. J Med Chem. 2010;53:6681–6698.
19. Jeanty M, Suzenet F, Delagrange P, et al. Design and synthesis of 1-(2-alkanamidoethy1)-6-methoxy-7-azaindole derivatives as potent melanin agonists. Bioorg Med Chem Lett. 2011;21:2316–2319.
20. Khurana L, Ali HI, Olszewska T, et al. Optimization of chemical functionalities of indole-2-carboxamides to improve allosteric parameters for the cannabinoid receptor 1 (CB1). J Med Chem. 2014;57:3040–3052.
21. Nguyen T, German N, Decker AM, et al. Structure–activity relationships of substituted 1H-indole-2-carboxamides as CB1 receptor allosteric modulators. Bioorg Med Chem. 2015;23:2195–2203.
22. Saal C, Petereit AC. Optimizing solubility: kinetic versus thermodynamic solubility temptations and risks. Eur J Pharm Sci. 2012;47:589–595.
23. Chen C, Okayama H. High-efficiency transformation of mammalian cells by plasmid DNA. Mol Cell Biol. Mol. 1987;7:2745–2752.
24. Qiao CJ, Ali HI, Ahn KH, et al. Synthesis and biological evaluation of indole-2-carboxamides bearing photoactivatable functionalities as novel allosteric modulators for the cannabinoid CB1 receptor. Eur J Med Chem. 2016;121:517–529.
25. Scott CE, Kendall DA. Assessing allosteric modulation of CB1 at the receptor and cellular levels. Methods Enzymol 2017;593:317–342.
26. Christopoulos A, Kenakin T. G protein-coupled receptor allosterism and complexing. Pharmacol Rev. 2002;54:323–374.
27. Ahn KH, Mahmoud MM, Samala S, et al. Profiling two indole-2-carboxamides for allosteric modulation of the CB1 receptor. J Neurochem. 2013;124:584–589.
28. Ahn KH, Mahmoud MM, Shim JY, et al. Distinct roles of β-arrestin 1 and β-arrestin 2 in ORG27569-induced biased signaling and internalization of the cannabinoid receptor 1 (CB1). J Biol Chem. 2013;288:9790–9800.

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Abbreviations Used
ADME = absorption, distribution, metabolism, and excretion
BOP = (benzotriazol-1-yl)-oxy(dimethylamino)phosphonium hexafluorophosphate
BSA = bovine serum albumin
CB1 = cannabinoid receptor 1
DMSO = dimethylsulfoxide
DMMTM = 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinum chloride
EGTA = ethylene glycol-bis(β-aminoethyl ether)-N,N′,N′-tetraacetic acid
GTP = guanosine 5′-O-(gamma-thio)triphosphate
HCl = hydrochloric acid
HOBt = 1-Hydroxybenzotriazole hydrate
MgCl₂ = magnesium chloride
NaCl = sodium chloride
NMM = N-methyl morpholine
PBS = phosphate-buffered saline
SAR = structure–activity relationship
THF = tetrahydrofuran