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Evaluation of (Z)-2-((1-benzyl-1*H*-indol-3-yl)methylene)-quinuclidin-3-one analogues as novel, high affinity ligands for CB1 and CB2 cannabinoid receptors

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Abstract

A small library of *N*-benzyl indolequinuclidinone (IQD) analogs has been identified as a novel class of cannabinoid ligands. The affinity and selectivity of these IQDs for the two established cannabinoid receptor subtypes, CB1 and CB2, was evaluated. Compounds **8** (R=R²=H, R¹=F) and **13** (R=COOCH₃, R¹=R²=H) exhibited high affinity for CB2 receptors with *K_i* values of 1.3nM and 2.5 nM, respectively, and had lower affinities for the CB1 receptor (*K_i* values of 9.1nM and 85.7 nM, respectively). Compound **13** had the highest selectivity of all the compounds examined, and represents a potent cannabinoid ligand with 35-times greater selectivity for CB2R over CB1R.

Keywords

N-Benzyl indolequinuclidinone; *N*-Benzyl indolequinuclidinol; *N*-Benzyl indolequinuclidin-3-one oxime; Cannabinoid ligands; CB1 receptors; CB2 receptors

⁹-Tetrahydrocannabinol (⁹-THC, Fig. 1), the well-known primary psychoactive constituent of marijuana, exhibits a variety of potential therapeutic effects, including anti-convulsive, anti-emetic, analgesic, lowering of intraocular pressure and immune modulation.¹ However, undesirable central nervous system (CNS) effects and potential addictive properties of ⁹-THC has limited its medical use, and raised the necessity for identifying the receptors responsible for cannabinoid activity in order to develop drugs with fewer adverse side-effects. CB1 and CB2 are the two major cannabinoid-specific receptors which have been characterized from mammalian tissues.^{2,3} CB1 receptors (CB1R) are abundantly expressed in the CNS. These receptors are also expressed in peripheral nerve terminals and in many extra-neuronal sites. CB1R activation is responsible for most of the pharmacological effects of cannabinoids in the nervous system. In contrast, CB2 receptors (CB2R) are known to be expressed in other tissues^{4,5}, predominantly in cells of the immune

system.⁶ Interestingly, many types of cancer cells express relatively high densities of CB1 and CB2 receptors, and most recent studies report that cannabinoids can reduce tumor growth and progression in several animal models of cancer.^{7–9} There are three main structural classes of cannabinoid ligands. These are the ‘classical’ cannabinoid analogues of Δ^9 -THC, the ‘non-classical’ bicyclic and tricyclic cannabinoid analogues of Δ^9 -THC, the aminoalkylindoles (e.g. WIN 55212-2, Fig. 1)¹⁰ and other diverse heterocyclic structures such as the pyrazoles (e.g. SR141716A, Fig. 1)^{11–13} and indolopyridones (e.g. IPDN, Fig. 1).¹⁴ In the evaluation of synthetic or non-classical cannabinoids, the amino alkylindole WIN 55212-2 was reported as a potent non-selective cannabinoid receptor agonist with K_i values of 1.9 nM for CB1R and 0.3 nM for CB2R.¹⁴

In contrast, the pyrazole compound SR141716A was reported to selectively bind to CB1R where it acts as an antagonist/inverse agonist without producing cannabimimetic activity *in vivo*.^{12–13} The indolopyridone analogue IPDN exhibits higher affinity for CB2R ($K_i = 1.0$ nM) than for CB1R ($K_i = 16$ nM).¹⁴

The indole nucleus played an important role in the activity of the cannabinoid compound WIN 55212-2, and indolopyridone (IPDN) analogues and other analogs that incorporate a variety of different bicyclic ring systems as subunits have emerged as potent cannabinoid ligands.^{14–15} These observations prompted us to undertake a more detailed investigation into the structure–activity relationships of various indole analogs that incorporate a quinuclidinone bicyclic ring system as a structural subunit. The present study focuses on the synthesis and evaluation of some (Z)-2-((1-benzyl-1H-indol-3-yl)methylene)-quinuclidin-3-one analogues (IQD) as novel potent ligands for CB1 and CB2 cannabinoid receptors.

The synthetic strategy for the preparation of IQDs **7–17** is summarized in Scheme 1. The appropriate 1-acetylindole-3-carboxaldehyde (**1** and **2**) was prepared by *N*-acetylation of the corresponding indole-3-carboxaldehyde with acetic anhydride/ triethylamine in dichloromethane under reflux conditions. Aldol condensation of the appropriate 1-acetylindole-3-carboxaldehyde with quinuclidin-3-one hydrochloride in the presence of lithium diisopropylamide in tetrahydrofuran at -78°C afforded the (Z)-2-(*N*-acetylindol-3-ylmethylene)quinuclidin-3-one derivative (**3** and **4**). Subsequent *N*-deacetylation in refluxing 1N aqueous NaOH solution afforded the appropriate (Z)-2-(1H-indol-3-ylmethylene)-quinuclidin-3-ones (**5** and **6**).

The (Z)-2-(*N*-benzylindol-3-ylmethylene)-quinuclidin-3-one derivatives (**7–13**) were prepared in 81–87% yield by treatment of the (Z)-2-(1H-indol-3-yl methylene)quinuclidin-3-ones **5** and **6** with a variety of benzyl halides under phase-transfer catalytic (PTC) conditions using triethylbenzylammonium chloride in 50% w/v aqueous NaOH solution in dichloromethane.¹⁶ Reduction of compounds **7** and **9** with NaBH_4 in methanol afforded the corresponding (Z)-2-(*N*-benzylindol-3-yl methylene)-quinuclidin-3-ol derivatives **14** and **15** in 82–88% yield.¹⁶ Reaction of compounds **7** and **9** with hydroxyl amine hydrochloride in the presence of sodium acetate/methanol afforded the (2Z,3E)-2-((1-benzyl-1H-indol-3-yl)methylene)-quinuclidin-3-one oximes **16** and **17**.¹⁷

Confirmation of the structure and purity of these analogs was obtained from ^1H and ^{13}C -NMR spectroscopic analysis.¹⁸ The geometry of the double bond in these molecules was established from X-ray crystallographic data.^{17, 19–20}

Membrane homogenates of either whole mouse brain (50 μg) (CB1R) or Chinese hamster ovary (CHO)-hCB2 cells (25 μg) (CB2R) were incubated for 90 minutes at room temperature with 0.2 nM [^3H]CP-55,940, 5 mM MgCl_2 , and either increasing IQD concentrations (10 fM to 10 μM), 1 μM WIN-55,212-2 (to define non-specific binding) or vehicle (to determine total binding). Each ligand concentration was examined in duplicate, in a final volume of 1 mL of buffer containing 50 mM Tris, 0.05% bovine serum albumin (BSA) and 0.1 % ethanol/0.1% dimethyl sulfoxide vehicle. Reactions were terminated by rapid vacuum filtration through Whatman GF/B glass fiber filters, followed by five washes with ice-cold buffer (50 mM Tris, 0.05% BSA). Filters were immediately placed into 7 mL scintillation vials to which 4 mL of scintillation fluid was added. Bound radioactivity was determined after overnight incubation at room temperature by liquid scintillation spectrophotometry. Specific binding was expressed as total minus non-specific binding and graphed for each data point as a percentage of specific binding occurring in the absence of any competitor. The affinity of IQDs for CB1R and CB2Rs was derived by employing the Cheng-Prusoff equation²¹ to convert the observed IC_{50} values to K_i values, from 3–4 separate competition receptor binding curves for each ligand.

All the synthesized IQDs were evaluated against the two cannabinoid receptor subtypes, CB1 and CB2. Compounds **7–9**, **11** and **13** displayed high affinity (<150nM) for both CB1 and CB2 receptors, with K_i values ranging from 9.1 nM to 135 nM for CB1R and 1.3 nM to 114 nM for CB2R. Compounds **7** ($K_i=11.7$ nM), **8** ($K_i=1.3$ nM), **13** ($K_i=2.5$ nM), **14** ($K_i=55.3$ nM) and **16** ($K_i=68.6$ nM) demonstrated some degree of CB2R selectivity compared to CB1R. Compound **13**, which contains a COOCH_3 moiety at the indolic 6-position, was a potent CB2 receptor ligand and exhibited 34-fold selectivity for CB2R ($K_i=2.5$ nM) when compared to CB1R ($K_i=85.7$ nM) (Table 1). The *N*-4-fluorobenzyl analogue **8** displays high affinity for both CB1 ($K_i=9.1$ nM) and CB2 receptors ($K_i=1.3$ nM), but was less selective for the CB2 receptor than compound **13**.

The (*Z*)-($\pm$)-2-(*N*-benzylindol-3-ylmethylene)quinuclidin-3-ols (**14** and **15**) and (2*Z*,3*E*)-2-((1-benzyl-1*H*-indol-3-yl) methylene)-quinuclidin-3-one oximes (**16** and **17**) were less potent ligands at both CB1 and CB2 receptors compared to their parent compounds (**7** and **9**) and were generally less selective. The overall affinities of quinuclidin-3-ol and quinuclidin-3-one oxime derivatives for CB1R and CB2R were reduced when compared to those of the corresponding quinuclidin-3-one derivatives (Table 2).

In summary *N*-benzylindolequinuclidinone (IQD) analogs have been identified as a novel class of cannabinoid ligands. These IQDs were evaluated against the two cannabinoid receptor subtypes, CB1 and CB2. Compounds **8** and **13** exhibited high affinity for CB2 receptors with K_i values of 1.3nM and 2.5 nM, respectively, and had lower affinities for the CB1 receptor (K_i values of 9.1nM and 85.7nM, respectively). Compound **13** had the highest selectivity of all the compounds examined, and represents a potent cannabinoid ligand with 35-times greater selectivity for CB2R over CB1R.

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18. Analytical data and yields for the most active compounds: (**8**): ¹H NMR (300 MHz, CDCl₃): δ 2.01–2.03 (m, 4H), 2.62 (p, *J*=3.0 Hz, 1H), 2.92–3.02 (m, 2H), 3.08–3.18 (m, 2H), 5.34 (s, 2H), 6.96–7.25 (m, 7H), 7.46 (s, 1H), 7.87–7.90 (m, 1H), 8.36 (s, 1H); ¹³C NMR (75 MHz, CDCl₃): δ 26.4, 40.5, 47.5, 50.0, 110.1, 111.4, 115.6, 118.8, 121.1, 121.8, 122.8, 124.3, 127.7, 128.27, 130.7, 134.3, 135.5, 135.8, 135.9, 185.2; yield: 87%; (**13**): (**13**): ¹H NMR (300 MHz, CDCl₃): δ 1.98–2.04 (m, 4H), 2.62–2.63 (p, *J*=2.7 Hz, 1H), 2.91–3.01 (m, 2H), 3.08–3.17 (m, 2H), 3.90 (s, 3H, OCH₃), 5.44 (s, 2H), 7.10–7.13 (m, 2H), 7.28–7.43 (m, 3H), 7.43 (s, 1H), 7.81–7.89 (m, 2H), 8.03 (s, 1H), 8.51 (s, 1H); ¹³C NMR (75 MHz, CDCl₃): δ 26.3, 40.4, 47.5, 50.6, 52.0, 110.6, 112.4, 117.1, 118.7, 122.0, 124.4, 126.5, 127.9, 128.9, 132.2, 135.5, 136.2, 136.9, 141.3, 167.7, 205.3; yield: 91%.
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Fig 1.
High affinity cannabinoid receptor ligands

Δ^9 -THC

WIN 55212-2

SR141716A

IPDN

Scheme 1.

Synthesis of indole quinuclidin analogs (IQDS) (**7–17**). Reagents and conditions: (a) Ac_2O , Et_3N , DCM, reflux; (b) quinuclidin-3-one hydrochloride, THF, LDA, -78°C ; (c) 1 N $\text{NaOH}_{(\text{aq})}$, reflux; (d) substituted benzyl halide, 50% $\text{NaOH}_{(\text{aq})}$, triethylbenzylammonium chloride, DCM, rt; (e) NaBH_4 , methanol, rt. (f) hydroxylamine hydrochloride, NaOAc , methanol, rt.

Table 1**K_i**values for (Z)-2-(N-benzylindol-3-ylmethylene) quinuclidin-3-ones (**7–13**) at CB1 and CB2 receptors

Compound	<u>K_i (nM)</u>	
	CB1	CB2
7	135±77	11.7±9.6
8	9.1 ± 0.6	1.3 ± 0.5
9	31.7±9.2	20.3 ± 7.4
10	629±201.3	333±156.9
11	55.3 ± 8.9	114 ± 36.0
12	953 ± 162	588 ± 237
13	85.7 ± 15.9	2.5 ± 0.5

Table 2

Effects of simple and *p*-chloro substituted *N*-benzyl indole quinuclidin-3-ol analogs (**14** and **15**) the quinuclidin-3-one oxime analogs (**16** and **17**) on binding affinity

Compound	<i>K_i</i> (nM)	
	CB1	CB2
14	540 ± 64	55.3 ± 31.0
15	85.3±42.5	113.3±2
16	357 ± 52	68.6 ± 7.3
17	95.7±2	107.3±8.7