C3-Heteroaroyl cannabinoids as photolabeling ligands for the CB2 cannabinoid receptor

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Article info
Article history:
Received 14 April 2012
Revised 3 June 2012
Accepted 5 June 2012
Available online xxx

Keywords:
CB2
Cannabinoid
Ligand-assisted protein structure
Photolabeling
Photoactivatable group

A series of tricyclic cannabinoids incorporating a heteroaroyl group at C3 were prepared as probes to explore the binding site(s) of the CB1 and CB2 receptors. This relatively unexplored structural motif is shown to be CB2 selective with $K_i$ values at low nanomolar concentrations when the heteroaromatic group is 3-benzothiophenyl (41) or 3-indolyl (50). When photoactivated, the lead compound 41 was shown to successfully label the CB2 receptor through covalent attachment at the active site while 50 failed to label. The benzothiophenophene moiety may be a photoactivatable moiety suitable for selective labeling.

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The known phytocannabinoids have long been known to exhibit only moderate receptor binding affinities and signaling profiles in vitro, yet they exhibit substantial potency in vivo. The best known of these classical cannabinoids, $\Delta^2$-tetrahydrocannabinol ($\Delta^2$-THC) binds with nearly equal affinity to the two known G-protein coupled cannabinoid receptors, CB1 and CB2. The design of novel CB1/CB2 analogues possessing higher affinities and selectivities can be based on structural information related to the interaction of cannabinergic ligands with their respective receptors. In the absence of either X-ray crystallographic or NMR data, information on the structural features of the ligand-cannabinoid receptor binding motifs can be gained through the use of carefully designed high-affinity electrophilic or photoactivatable probes. Such compounds interact with the receptor at or near the binding site and attach covalently to one or more amino acid residues. Identification of the attachment site(s) can subsequently be accomplished using targeted mutations within the receptor or by using LC/MS/MS to characterize the ligand–receptor complex. This approach that was developed in our laboratory combines the use of receptor mutants and mass spectrometry and was designated as Ligand-Assisted Protein Structure (LAPS). The present work describes our efforts to develop a new class of photoaffinity labels thus extending current work in our laboratory aimed at characterizing ligand–cannabinoid receptor binding motifs.

Earlier work from our laboratories had shown that 3-naphthoyl and 3-naphthylmethyl tricyclic cannabinoids have moderate affinities for the CB1 receptor. More recently Moore and coworkers showed that the tricyclic $\Delta^8$-THC analogue 1 bearing a benzoyl unit at C3 is CB2 selective while we have shown that the bicyclic analogues such as 2 are also selective for CB2 (Fig. 1). We have now designed and synthesized a series of tricyclic cannabinoids bearing a heteroaromatic group with a carbonyl spacer at C3. Design of our novel compounds incorporates the northern $\beta$-hydroxyl pharmacophore as well as an arylophenyl component, a photoactivatable group capable of transforming the ligand into a GPCR covalent label. Earlier work from the laboratories of Martin and coworkers has shown that the northern $\beta$-hydroxyl enhances affinity for both receptors while imparting the molecule with enhanced polar properties and water solubility.
Our SAR approach involves the attachment of different aryl groups to the 3-keto group of the tricyclic cannabinoid moiety.

Chemistry. Utilizing a strategy that has been developed in our group,17,18 we prepared bicyclic intermediate 7 via the acid catalyzed condensation between persilylated phloroglucinol 6 and a mixture of diacetates 4 and 5 (Scheme 1) following a general approach that was applied to the synthesis of nabnilone by the Eli Lilly group.19 It should be noted that persilylating phloroglucinol was essential to improve solubility in the reaction medium so as to ensure a high yield of 7. Ketone 7 was subsequently treated with TMSOTf to promote the rearrangement-cyclization to yield tricyclic compound 8. Selective conversion of the C3 phenolic hydroxyl group to the corresponding triflate led to 9 in 57% overall yield from 7. Reduction of ketone 9 with NaBH4 led to a 95/5 mixture of C9 diastereoisomers in 97% yield. Simultaneous protection of the phenolic and aliphatic hydroxy groups in 10 as methoxymethyl ether groups (MOM) led to 11 in 93% yield.

As in earlier work,18 we wanted to prepare all compounds from 11, a common advanced intermediate, utilizing a cross coupling procedure. The carboxylative Stille coupling was an attractive option for the installation of the heteroaryl unit due to the large relative ease of preparation from their corresponding aryl bromide or iodide. Treatment of trflate 11 with a slight excess of heteroaryl stannane, PdCl2(dppf)2/CH2Cl2,0 °C, 1 h, rt, 12 h; (c) KOH, MeOH, 0 °C, 1.5 h; 68% from 4 to 5; (d) TMSOTf, Et3N, CH2Cl2, 0 °C to rt; 57% from 7; (f) NaBH4, MeOH, rt; 1 h; (g) NaH, MeI; 97%.

Removal of the methoxymethyl ether protecting groups from 12 to 26 and 30 to 33 with TMSBr led to 3 and 34–51 in moderate to good yields (Scheme 4). The low yield for deprotection of the furyl and thiophenyl compounds can be attributed to the high nucleophilicity of the electron rich aromatic ring. Reaction with the methoxymethyl bromide that is generated during deprotection may be responsible for the appearance of byproducts. Since poor yields were also observed in these cases in the presence of poly(4-vinylpyridine), it is unlikely that the poor yields of depro-
ected products can be attributed to the presence of strong acid. Other common conditions to remove methoxymethyl ethers such as methanolic HCl or ZnBr2/n-BuSH, which served us well in the past, also failed to improve the yields.

Structure–Activity Relationships. Earlier work from our laboratory, as well as from the Moore and co-workers, explored the role of aryl groups as substitutions at the C-3 position in the classical cannabinoid in lieu of the traditionally used alkyl side chain. It was shown that introduction of a 3-benzoyl substituent in this class of cannabinoids with a series of analogues carrying the aryl group at the C-3 position. These structural modifications were aimed at identifying novel ligands and photoaffinity probes for the CB2 cannabinoid receptor with improved overall profiles. Our work has led to the discovery of a novel effective covalent probe for this receptor. The SAR of all novel arylphenone analogues was evaluated by measuring their respective affinities for the rat CB1 (rCB1), mouse CB2 (mCB2) and human CB2 (hCB2) receptors (Table 1). All synthesized novel analogues exhibited reduced affinities for both CB1 and CB2 receptors compared to their aromatic ring. The global minimum energy conformer for each compound is shown in stick representation.

All novel 9β-OH analogues were shown to have reduced binding affinities for both receptors when compared to the Δ9-THC tricyclic structure results in a compound (1) with high affinity for CB2. We have now extended the limited available SAR in this class of cannabinoids with a series of analogues carrying the cannabinoid receptor-favorable 9β-OH group, as well as different heteroaryl groups at the 3-position. These structural modifications were aimed at identifying novel ligands and photoaffinity probes for the CB2 cannabinoid receptor with improved overall profiles. Our work has led to the discovery of a novel effective covalent probe for this receptor. The SAR of all novel arylphenone analogues was evaluated by measuring their respective affinities for the rat CB1 (rCB1), mouse CB2 (mCB2) and human CB2 (hCB2) receptors (Table 1). All synthesized novel analogues exhibited reduced affinities for both CB1 and CB2 receptors compared to their aromatic ring. The global minimum energy conformer for each compound is shown in stick representation.

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>rCB1 (nM)</th>
<th>mCB2 (nM)</th>
<th>hCB2 (nM)</th>
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<tbody>
<tr>
<td>3</td>
<td>968</td>
<td>247</td>
<td>587</td>
</tr>
<tr>
<td>34</td>
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<tr>
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<td>406</td>
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a Binding affinities for CB1 and CB2 were determined using rat brain (CB1) or membranes from HEK293 cells expressing mouse or human CB2 and [3H]CP-55,940 as the radioligand following previously described procedures. K values for these compounds were obtained from one experiment (8 point) run in triplicate when experiments using the two point data in triplicate showed K values below 1000 nM.
Conclusions. In this SAR study we explored the value of cannabinoid analogues as photolabeling reagents for the CB2 receptors. We tested some of our compounds for their abilities to interact covalently with the mCB2 receptor. The experiment was carried out using membrane preparations obtained from a HEK293 cell line expressing mCB2. We used methodology developed in our laboratory with cannabinergic ligands carrying different photoactivatable groups, while employing conditions reported earlier for labeling the NK-1 receptor with ligands incorporating a benzopheno none moiety. Of the heteroaryl benzophenones tested, the two benzothiophenones (40 and 41) exhibited the highest affinity to photolabel the mCB2 receptor (77% and 67% respectively; Fig. 3). The meta-trifluoro analogue 49 also labeled the receptor, however, less effectively. Conversely, the 3-indolyl analogue (50) failed to label mCB2. These very successful results confirmed the value of the 3-arylphenone moieties as useful photolabels for the CB2 receptors.

Acknowledgments

Acknowledgment is made to The National Institute on Drug Abuse (DA07215, 2P01 DA09158) for generous support of this research.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.013

References and notes

21. A conformational search of the aryl substituent was performed using optimized potentials for liquid simulations (OPLS) force field, the cannabinergic tri cyclic moiety was held fixed. Conformers with greater than 0.5Å root-mean square deviation (rmsd) within 6 kcal mol⁻¹ of the global minimum were retained. All calculations were performed in Macromodel. See: (a) Jorgensen, W. L.; Tirado-riilles, J. J. Am. Chem. Soc. 1988, 110, 1657; (b) Kaminski, G. A.; Friesen, R. A.; Tirado-riilles, J.; Jorgensen, W. L. J. Phys. Chem. B 2002, 106, 6547; (c) MacroModel version 9.7; Schrödinger, LLC: New York, NY, 2009.
25. Analogue 49 was used to label hCB2. The extent of labeling was 30%.

Figure 3. Compound 41 inhibits the specific binding of [3H]CP-55,940 to mCB2 receptor. HEK293 cell membranes expressing wild type mouse cannabinoid receptor 2 (mCB2) were suspended in TME buffer (25 mM Tris-Base, 5 mM MgCl₂, 1 mM EDTA, pH 7.4) with 0.1% BSA, containing 0.34 μM 41 (i.e., 10-fold Kᵢ of 41 for WT mCB2). A membrane devoid of 41 was used as a parallel control. Incubations of both samples were performed in silanized glass tubes for 30 min at a 37 °C water bath. Subsequently, the samples were irradiated for 1 h using Black-Ray long wavelength ultraviolet lamp at 365 nm in ice-cold silanized Petri dishes. The membranes were washed once with 1% BSA TME buffer to remove unbound ligands, and once with TME buffer (no BSA) to remove BSA. Saturation binding assays were carried out after photo-labeling using [3H]CP-55940 as a radioligand. The membrane sample with 41 (342 nM; 10 × Kᵢ) exhibited a 67% reduction in its Bmax when compared to control.