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Synthesis and Testing of Novel Phenyl Substituted Side-Chain Analogues of Classical Cannabinoids

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Abstract—A series of novel phenyl substituted side-chain analogues of classical cannabinoids were synthesized and their CB1 and CB2 binding affinities were evaluated relative to Δ^8-THC and compound **2**. CB1 and CB2 binding assays indicate that the dimethyl and ketone analogues (**3**) and (**6**) display selectivity for the CB2 receptor in comparison to Δ^8-THC and compound **2**. This study provides newer insights into the geometrical and functional group requirements of the ligand binding pockets of the CB1 and the CB2 receptors.

The cannabinoids are a diverse class of compounds that possess the ability to bind to the cannabinoid receptor-1 (CB1) and receptor-2 (CB2),[1–4] acting as agonist, antagonist or inverse agonist. Each of these activities has gained considerable attention due to their potential indication in the treatment of obesity,[5] cancer,[6] glaucoma[7] and several other pathological conditions. The therapeutic potential of CB1 and CB2 ligands has resulted in the synthesis of a diverse set of compounds that can be broadly grouped into the classical cannabinoids, non-classical cannabinoids,[8] alkyl amino indoles [9] and pyrazoles.[10]

The classical cannabinoids are, by far, the most extensively studied group in terms of structure–activity relationships (SAR) and pharmacology. The natural product, Δ^9-THC [11] (**1**) (Fig. 1) is the prototypical example for this class of cannabinoids and it is a partial agonist of the CB1 receptor.[12] Considerable efforts have been made to elucidate the SAR of these compounds with respect to modifications of the tricyclic ring and side chain substituents at the C3 position.[13] To assess the latter, researchers have introduced a variety of substituents including branched chain alkyls,[14,15] unsaturated alkyls[16–18] and alkyls containing 1′,1′-cyclic functionality.[19] All these efforts have been directed at determining the structural requirements of the ligand-binding pocket (LBP).

An understanding of the structural requirements of the ligand-binding pocket is essential for guiding the design of subtype selective ligands; however, to date neither the X-ray nor NMR solution structures of either the CB1 or

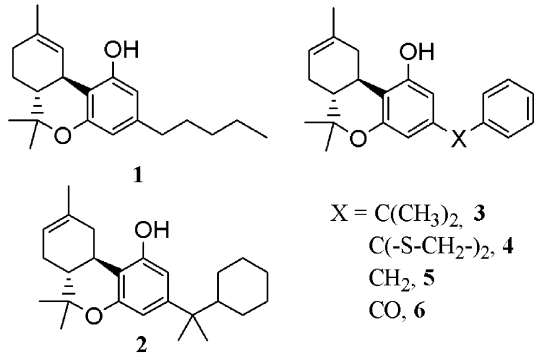

Figure 1.

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the CB2 receptors have been determined. In the absence of this data, researchers must utilize site directed mutagenesis, molecular modeling studies and traditional medicinal chemistry to provide insights into the geometric and chemical requirements of the LBP.

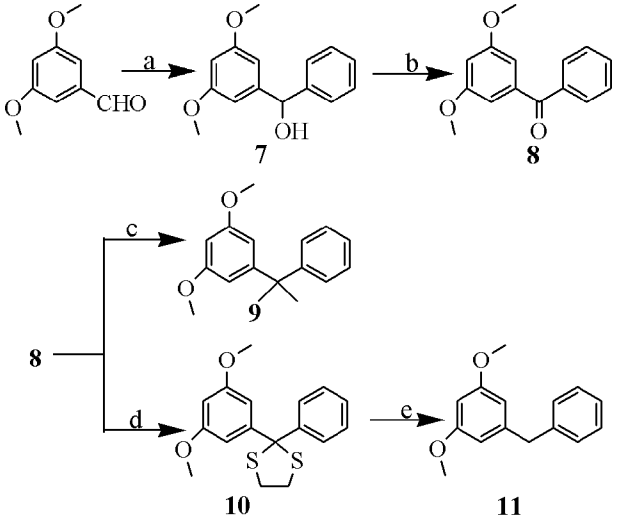
As part of our efforts to characterize the structural requirements of the CB1 and the CB2 receptors, we had previously synthesized a series of cycloalkyl side-chain analogues of Δ^8-THC.[20] The series consisted of cyclopentyl, cyclohexyl and cycloheptyl side chains with 1′, 1′-dimethyl or dithiolane functionalities. The 1′,1′-dimethyl series exhibited, on an average, a 100 fold increase in binding affinity for both the CB1 and CB2 receptors, while the dithiolanes had an average 10-fold higher affinity. Interestingly, compound (**2**) had comparable affinity to the CB1 receptor as its linear carbon equivalent congener 1′,1′-dimethyl heptyl THC (DMHT).[13]

The high affinities of this series of compounds prompted us to question if a phenyl ring could be substituted for the cyclohexyl ring thus generating a novel series of Δ^8-THC analogues. We hypothesized that the introduction of a phenyl side chain could significantly alter the electronic properties of both the side chain and A ring while maintaining steric bulk and conformational restraint. This substitution was in part designed to study potential π–π interactions of the ligands with aromatic amino acids in the LBP.[21] To the best of our knowledge, there have been no previous reports of a 1′-phenyl substitution in the classical cannabinoid series. Therefore a series of C1′ substituted phenyl derivatives with dimethyl, dithiolane, methylene and ketone substituents at the C1′ position were synthesized (**3**–**6**).

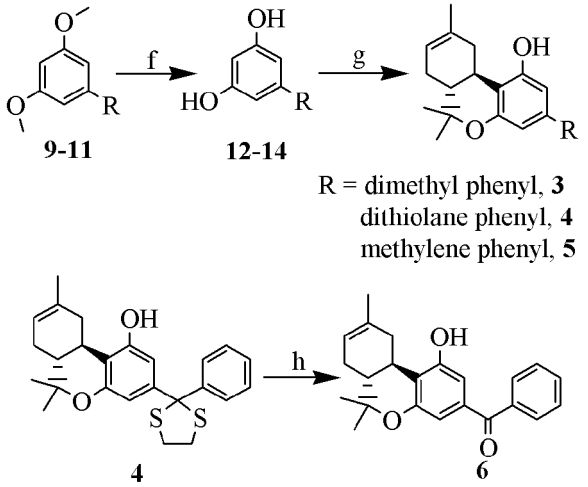
The side chains of classical cannabinoids have often been made by reaction of 3,5-dimethoxy benzonitrile with a suitable Grignard reagent and acid hydrolysis of the intermediate imine salt to the ketone.[22] Due to the reduced reactivity of aromatic Grignard reagent as compared to alkyl Grignard reagent, we used 3,5-dimethoxy benzaldehyde as the starting material and reacted this with phenyl magnesium bromide to obtain the corresponding alcohol (**7**)[23] as shown in Scheme 1. Oxidation of alcohol with PCC yielded the key intermediate ketone (**8**).[23] The dithiolane group was introduced at the C1′ position (**10**) by reacting the ketone with ethane dithiol in presence of boron trifluoride.[18] The ketone intermediate was also reacted with dimethyl zinc and titanium tetrachloride to form the dimethyl substituent at the C1′ position (**9**).[22] Desulfurisation of intermediate (**10**) with Raney–Nickel yielded the methylene intermediate (**11**).[24] The 1-substituted 3,5-dimethoxy intermediates (**9**–**11**) were deprotected with boron tribromide to yield the corresponding resorcinols (**12**–**14**).[22] Δ^8-THC analogues (**3**–**5**) were then obtained from these resorcinols (**12**–**14**) by reacting them with *cis*-Δ^2-*p*-menthene-1, 8-diol, prepared from (+)-Δ^2-carene,[25] in presence of *p*-toluene sulfonic acid. The Δ^8-THC analogue with a ketone functionality at the C1′ position (**7**) was obtained by deprotecting the analogue (**4**) with silver nitrate (Scheme 2).[26,28]

The CB1 and CB2 binding affinities of these novel Δ^8-THC analogues with phenyl side chains were determined using membrane preparations of the human receptors transfected into HEK293 EBNA cells. Receptor binding assays were carried out using tritiated CP55,940 as the competing radioactive ligand and 10 μm WIN 55212-2 was used for determining non-specific binding.[20]

The CB2 binding affinities of these novel analogues were in the range of 0.9–86 nm while the CB1 binding affinities ranged from 12 to 297 nM (Table 1). Interestingly, these compounds exhibited significantly different binding profile when compared to the lead compound (**2**). The dimethyl analogue (**3**) exhibited good binding affinities for both the CB1 and the CB2 receptors with a 13-fold selectivity for the CB2 receptor. This selectivity is in contrast to the lead compound (**2**) that binds both the



Scheme 1. Reagents and conditions: (a) Phenyl magnesium bromide, THF, 10% HCl/−20°C; (b) PCC, CH_2Cl_2/rt; (c) dimethyl zinc, titanium(IV) chloride, CH_2Cl_2/−40°C; (d) ethane dithiol, BF3-diethyl etherate, CH_2Cl_2/rt; (e) Raney–Nickel, EtOH/EtOAc, reflux.



Scheme 2. Reagents and conditions: (f) BBr_3, CH_2Cl_2/0°C–rt; (g) *cis*-*p*-menthene-1,8-diol, *p*-toluene sulfonic acid, benzene/80°C; (h) $AgNO_3$, MeOH/rt.

Table 1. Affinities (K_i) of compounds **3**, **4**, **5**, **6** for CB1 and CB2 receptors

Compd	CB1 K_i (nM)[a]	CB2 K_i (nM)[a]	Ratio CB1/CB2
Δ^8-THC	28.5 (±3.30)	25.0 (±4.80)	1.14
2	0.57 (±0.05)	0.65 (±0.04)	0.87
3	12.3 (±0.61)	0.91 (±0.08)	13.5
4	17.3 (±0.33)	17.6 (±1.03)	0.98
5	67.6 (±2.90)	85.9 (±0.31)	0.78
6	297 (±10.6)	23.6 (±1.76)	12.6

[a]The K_i values for Δ^8-THC and the analogues were obtained from three independent experiments each of which was run in duplicate and are expressed as the mean of three values, with the standard error of mean shown in parentheses.

subtypes with almost equal affinity. The ketone analogue (**6**) exhibited similar binding affinity for the CB2 receptor when compared to Δ^8-THC but almost a 10-fold decrease in the binding affinity for the CB1 receptor. The dithiolane analogue (**4**) exhibited no subtype selectivity, however there was a 10-fold decrease in affinity relative to the 1′-cyclohexyl congener (CB1 $K_i = 1.86$ nM and CB2 $K_i = 1.05$ nM).[20] The methylene analogue (**5**) displayed significantly reduced binding affinities for both the subtypes in comparison to Δ^8-THC.

The binding affinities of our novel 1′-phenyl substituted Δ^8-THC analogues provide some new insights into the functional group requirements of the binding pockets of the CB1 and CB2 receptors. Valuable structural information can be gleaned from our dimethyl and ketone analogues that exhibited modest selectivity for the CB2 receptor. This in combination with our previous data for the cyclohexyl Δ^8-THC analogues[20] suggests that a subsite binding pocket of the CB2 receptor can tolerate both cycloalkyl side chains and rigid aromatic side chains when compared to the CB1 receptor. The selectivity of **3** and **6** is interesting when considering that several of the short chain C3 analogues reported by Huffman et al. also exhibited significant CB2 selectivity.[27] However, a comparison between the two structural types is difficult when considering that the short chain analogues were primarily 1-deoxy and 1-methoxy compounds as compared to our 1-hydroxy analogues. Notwithstanding, the modest selectivity of our 1-hydroxy compounds might suggest the presence of favorable interactions between the phenyl side chain and aromatic amino acids that may be present in the binding pocket of the CB2 receptor. Although it is difficult to draw any direct comparisons aromatic residues have been proposed to reside in the LBP of the CB1 receptor.[21] In contrast, reduced CB1 binding affinities exhibited by these compounds relative to the cyclohexyl[20] and linear chain derivatives[18] may suggest that the compounds cannot adopt a conformation to maximize ligand-receptor interactions. The presence of a polar C1′ keto group may also diminish CB1 affinity as has been previously proposed by Papahatjis and coworkers.[18] Further studies utilizing substituted C1′ phenyl groups should provide additional insights into the SAR of this class of Δ^8-THC analogues. Combining these studies with functional assays should contribute to a better understanding about the differences in the structural requirements of the LBP of the CB1 and CB2 receptors and may aid in developing more selective compounds.

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28. Selected data of final compounds: **3.** $R_f = 0.42$ (methylene chloride/hexane 50:50), $R_f = 0.6$ (ethyl acetate: petroleum ether 10:90), ^{1}H NMR ($CDCl_3$) δ 7.14 ppm (m, 5H), 6.36 ppm (d, $J = 1.8$ Hz, 1H), 5.91 ppm (d, $J = 2.1$ Hz, 1H), 5.35 ppm (d, $J = 6$ Hz, 1H), 4.44 ppm (s, 1H), 3.1 ppm (m, 1H), 2.61 ppm (m, 1H), 2.05 ppm (m, 1H), 1.75 ppm (m, 3H), 1.62 ppm (s, 3H), 1.54 ppm (m, 6H), 1.31 ppm (s, 3H), 1.04 ppm (s, 3H); MS: (ESI, Neg), m/z 361 ($[M\text{-}1]^-$). HRMS (FAB), m/z calcd for $C_{25}H_{30}O_2$, 362.2246, experimental 362.2239.
4. $R_f = 0.22$ (methylene chloride/hexane 50:50), $R_f = 0.58$ (ethyl acetate: petroleum ether 20:80), ^{1}H NMR ($CDCl_3$) δ 7.54 ppm (m, 2H), 7.18 ppm (m, 3H), 6.65 ppm (d, $J = 2.1$ Hz, 1H), 6.39 ppm (d, $J = 2.1$ Hz, 1H), 5.35 ppm (d, $J = 4.2$ Hz, 1H), 4.61 ppm (s, 1H), 3.32 ppm (m, 4H), 3.1 ppm (m, 1H), 2.62 ppm (m, 1H), 2.06 ppm (m, 1H), 1.76 ppm (m, 3H), 1.62 ppm (s, 3H), 1.29 ppm (s, 3H), 1.03 ppm (s, 3H); MS: (ESI, Neg), m/z 423 ($[M\text{-}1]^-$). HRMS (FAB), m/z calcd for $C_{25}H_{28}O_2S_2$, 424.1531, experimental 424.1533.
5. $R_f = 0.34$ (methylene chloride/hexane 50:50), $R_f = 0.42$ (ethyl acetate/petroleum ether 10:90), ^{1}H NMR ($CDCl_3$) δ 7.22 ppm (m, 3H), 7.13 ppm (m, 2H), 6.22 ppm (m, 1H), 5.98 ppm (m, 1H), 5.35 ppm (d, $J = 6$ Hz, 1H), 4.52 ppm (s, 1H), 3.74 ppm (s, 2H), 3.1 ppm (m, 1H), 2.62 ppm (m, 1H), 2.07 ppm (m, 1H), 1.75 ppm (m, 3H), 1.62 ppm (s, 3H), 1.29 ppm (s, 3H), 1.03 ppm (s, 3H); MS: (ESI, Neg), m/z 333 ($[M-1]^-$). HRMS (FAB), m/z calcd for $C_{23}H_{26}O_2$, 334.1933, experimental 334.1928.
6. $R_f = 0.2$ (methylene chloride/hexane 60:40), $R_f = 0.47$ (ethyl acetate/petroleum ether 20:80), ^{1}H NMR ($CDCl_3$) δ 7.82 ppm (m, 2H), 7.58 ppm (m, 1H), 7.48 ppm (m, 2H), 6.92 ppm (d, $J = 1.8$ Hz, 1H), 6.83 ppm (d, $J = 1.5$ Hz, 1H), 5.48 ppm (m, 2H), 3.34 ppm (m, 1H), 2.81 ppm (m, 1H), 2.18 ppm (m, 1H), 1.88 ppm (m, 3H), 1.74 ppm (s, 3H), 1.41 ppm (s, 3H), 1.12 ppm (s, 3H); MS: (ESI, Neg), m/z 347 ($[M-1]^-$). HRMS (FAB), m/z calcd for $C_{23}H_{24}O_3$, 348.1725, experimental 348.1717.