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Design and synthesis of novel tri-aryl CB2 selective cannabinoid ligands

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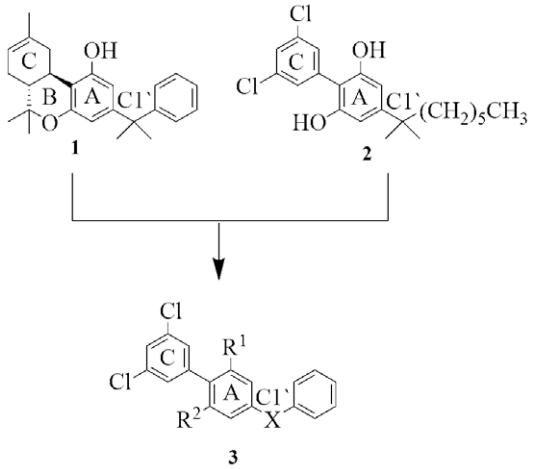
ABSTRACT

A novel series of cannabinoid ligands with a structurally unique tri-aryl core has been designed, synthesized and assayed. Receptor binding assays show that these compounds possess CB2 receptor sub-type selectivity with binding affinities ranging from 1.07 (±0.05) for **7** to 4.77 (±0.57) nM for **6**. The selectivity of the compounds was enhanced 9–600-fold for the CB2 receptor over the CB1 receptor. The results of our present study identify a novel, highly selective cannabinoid scaffold with a non-classical core.

The therapeutic potential of cannabinoids in pathological conditions ranging from inflammation[1] to cancer[2,3] has been evaluated. As a result of this, a surge in the screening of cannabinoid based compounds has occurred over the last decade. These studies have been predominantly aimed at developing compounds that can elicit the desired physiological effects of compounds such as Δ^9-THC without producing the unwanted CNS side effects mediated by the CB1 receptor.

The discovery of unique ligands has identified a number of practical therapeutic applications for the treatment of pain and inflammation. The therapeutic potential combined with the need to develop new, selective cannabinoids with reduced side-effects has prompted the development of novel structural motifs. These include compounds like the *N*-alkyl isatin acylhydrazones,[4] benzimidazoles,[5] orally available *N*-arylamide oxazolidones[6] and quinolone-3 carboxamides.[7] These compounds possess 2–3000-fold selectivity for the CB2 receptor over CB1 and exhibit partial to full agonist activity at the CB2 receptor. Other classes of CB2 selective ligands are the substituted biaryl and bicyclic cannabinoids with the presence of a geminal dimethylheptyl side chain at the C1′ position. These include resorcinol derivatives,[8] open ring cannabinol analogues[9] and bicyclic HU-308.[10] These compounds have been studied extensively and have exhibited a higher binding affinity towards the CB2 receptor subtype.

Our novel tri-aryl core reported in the present communication combines aspects of two distinct cannabinoid series. The design utilizes the C1′ aryl substitution (**1**) previously reported by our laboratory[11] and the introduction of an aryl ring (**2**) in place of the terpene ring as reported by Makriyannis and co-workers (Fig. 1).[12] The design was hypothesized on the basis that the A ring of **1** and **2** occupy the same region in the CB1 and CB2 ligand binding pockets.



4 R^1= OCH_3; R^2= OCH_3; X= CHOH
5 R^1= OH; R^2= OH; X= C=O
6 R^1= OH; R^2= OCH_3; X= C=O
7 R^1= OH; R^2= OH; X= $C(CH_3)_2$

Figure 1. Design of novel tri-aryl cannabinoid core.

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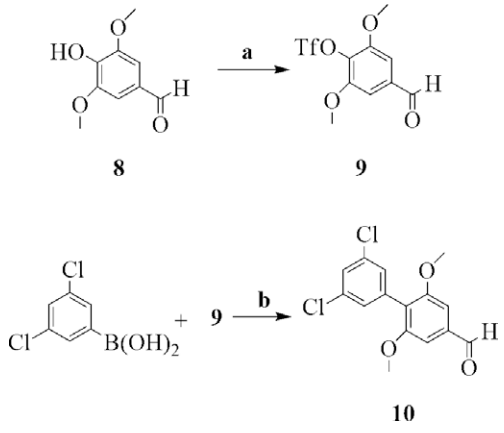
These modifications resulted in a new non-classical cannabinoid tri-aryl core **3** (Fig. 1). In an effort to improve CB2 receptor affinity the C ring of the classical cannabinoid core was substituted with a dichlorophenyl ring. This modification was based on the observation of Makriyannis and co-workers wherein an improvement of CB2 receptor affinity increased with a dichlorophenyl substituted C ring in the biaryl series.[12] In addition to the above-mentioned structural modifications three types of linkers (X) hydroxyl, carbonyl, and *gem* dimethyl in **3** were synthesized and assayed for binding affinity.

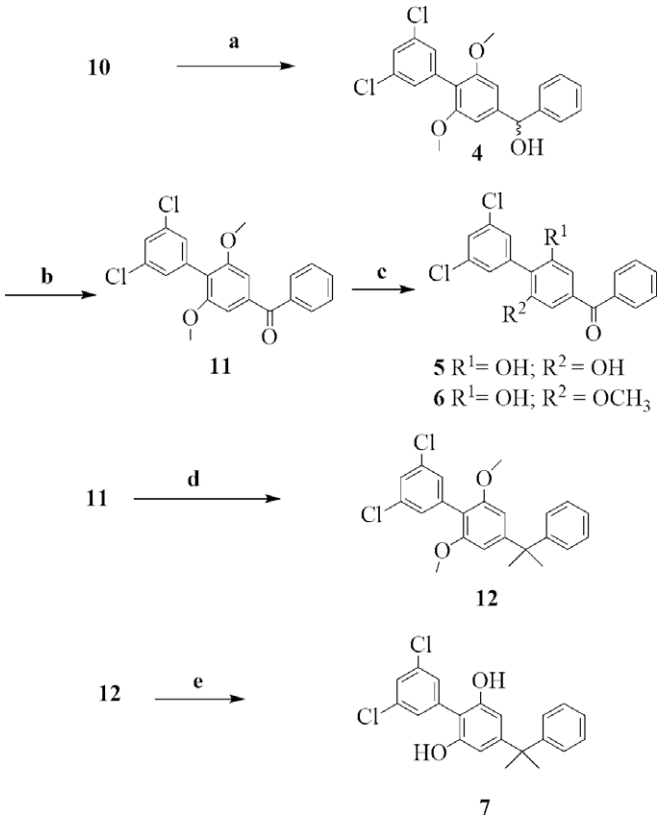
In order to synthesize the core of the tri-aryl compounds, and provide a route for future functionalization of the lead molecule, an efficient synthetic route was designed. Commercially available syringealdehyde **8** was selected to form the A ring in the tri-aryl series. The phenolic hydroxyl was activated for a Suzuki coupling by reaction with triflic anhydride to yield intermediate **9** (Scheme 1). Formation of the biaryl ring system was carried out via a microwave assisted Suzuki coupling reaction with 3,5-dichlorophenyl boronic acid, in the presence of tetrakis(triphenylphosphine) palladium, and potassium carbonate in toluene and water to yield aldehyde **10**. The resulting aldehyde was reacted with phenyl magnesium bromide (Scheme 2) to obtain the racemic alcohol **4**, which was oxidized using PCC, to yield the ketone **11**. The ketone analogs **5** and **6** were synthesized by BBr_3 deprotection of **11**. In another reaction, the ketone **11** was dimethylated using dimethylzinc and titanium chloride to yield compound **12**. This intermediate was then deprotected using BBr_3 to yield compound **7**.

Binding affinity studies were carried out using cell membranes from HEK293 cells transfected with the human CB1 receptor (Perkin Elmer, Lot #288-842-A, B_{max}: 1.7 pmol/mg protein, K_d for tritiated CP 55,940 binding: 0.186 nM) and membranes from HEK293 cells transfected with the human CB2 receptor (Perkin Elmer Lot #2312, B_{max}: 3.3 pmol/mg protein, K_d for tritiated CP 55,940 binding: 0.12 nM). Non-specific binding was determined using 10 μM WIN55, 212-2. Increasing concentrations of compounds to be tested were made ranging from 10^{-12} M to 10^{-4} M and were added in triplicate for each experiment and the individual molar IC_{50} values were determined using GraphPad Prism software. The corresponding K_i values for each drug were determined utilizing the Cheng-Prusoff equation and final data are presented as $K_i \pm$ standard deviation of $n = 3$ experiments run in triplicate.

All the compounds synthesized with the novel tri-aryl core show significant binding affinity to the CB2 cannabinoid receptor subtype (Table 1). The receptor subtype affinity K_i values ranged from 1.07 to 4.77 nM at the CB2 receptor with 9–600-fold selectivity towards the CB2 receptor subtype.



Scheme 1. Synthetic route indicating reagents and compounds. (a) Triflic anhydride, DCM, −18 °C, 1 h; (b) $(PPh_3)_4Pd$, K_2CO_3, toluene, 120 °C, 100 W, 15 min.



Scheme 2. Synthesis of compounds **4–7**. [16]Reagents and conditions: (a) C_6H_5MgBr, THF, 0 °C, 4 h; (b) PCC, DCM, rt, 18 h; (c) BBr_3, DCM, −78 °C to rt, 12 h; (d) $TiCl_4$, dimethylzinc, DCM, −40 °C to rt, 16 h; (e) BBr_3, DCM, −78 °C to rt, 12 h.

Table 1
Binding affinity (K_i) of compounds Δ^8-THC, **1**, and **4–7** for the CB1 and CB2 receptors

Compound	CB1 K_i[a] (nM)	CB2 K_i[a] (nM)	CB1/CB2 ratio[b]
Δ^8-THC	28.5 (±3.3)	25.0 (±4.8)	1.1
1	12.3 (±0.61)	0.91 (±0.08)	13.5
4	>1000	1.66 (±0.38)	602.4
5	27.0 (±23.0)	2.94 (±1.69)	9.2
6	>1000	4.77 (±0.57)	209.6
7	93.66 (±2.33)	1.07 (±0.05)	87.5

[a] Values are means of three experiments run in triplicate, standard deviation is given in parentheses.
[b] K_i of CB1/K_i of CB2.

The tri-aryl analogs described herein contain the traditional A and C rings of the classical cannabinoid core (**1**). Although omission of the B ring from the C1′ phenyl substituted classical core does not seem to affect the binding affinity to the CB2 cannabinoid receptor, there is a pronounced effect on the affinity for the CB1 receptor as evidenced by our new analogs **4–7**. The extent to which the compounds show selectivity towards the CB2 receptor appears to depend on the substitution pattern on ring A and the type of linker X.

Previous studies have shown that a hydroxyl or carbonyl linker at the C1′ position in classical cannabinoids decreases both CB1 and CB2 receptor binding affinity.[13] Our binding affinity data show that compounds with a hydroxyl (**4**) and carbonyl linker (compounds **5** and **6**) retain affinity for the CB2 receptor when compared to the *gem* dimethyl analog **7**. Interestingly the mono-methoxy analog (**6**) shows better selectivity for the CB2 receptor when compared to the resorcinol carbonyl analog (**5**). This may be related to the fact that methylation of the C1 phenol in classical cannabinoids significantly diminishes or abolishes CB1 receptor affinity.[14] The presence of a

gem dimethyl group as a linker (compound **7**) results in a CB2 selective compound with a K_i value of 1.07 nM at the CB2 receptor compared to 93.66 nM at the CB1 receptor subtype (CB1/CB2 = 87.5).

The most significant effect is observed when the synthetic intermediate **4** was evaluated for binding affinity. This compound showed high binding affinity for the CB2 receptor with a K_i of 1.66 nM and exhibited a high 600-fold selectivity for the CB2 receptor. The introduction of a C1′ hydroxyl or carbonyl group in classical cannabinoids has not been extensively investigated; however, previous findings by our group[11,15] and by Papahatjis and coworkers,[13] indicates that these C1′ substituents reduce cannabinoid receptor affinity. Interestingly, results of our present study show that the carbonyl and hydroxyl substituent at the C1′ selectively decrease CB1 receptor affinity without altering the CB2 receptor binding. The beneficial effect of the C1′ equivalent hydroxyl, as well as the carbonyl, in CB2 receptor affinity may reflect: (1) the removal of the pyran ring provides greater conformational flexibility thus allowing for favourable receptor interactions; or (2) the tri-aryl compounds possess a different binding orientation relative to classical cannabinoids. Assuming that the hydroxyl increases CB2 binding affinity via a specific receptor interaction, it is reasonable to predict that one of the optical isomers of **4** should exhibit greater CB2 affinity and selectivity. To test this hypothesis we are currently synthesizing the optical isomers of **4**.

The results of this research provide new information with respect to the ligand binding pocket of the cannabinoid receptors. The omission of the B ring, aromatization of the C ring, and substitution of an aryl ring at C3 of the A ring in the classical cannabinoid has yielded the tri-aryl core. The increased conformational flexibility of these compounds, relative to classical cannabinoids, should provide insights into the steric constraints of the CB ligand binding pocket. Furthermore, validation of the stereo-chemical requirements of the C1′ hydroxyl may help identify a new ligand-amino acid interaction in the binding pocket. The retention of binding affinity after the abovementioned changes suggests that tri-aryl based CB2 selective compounds may provide a novel therapeutic inflammatory disease intervention. A detailed SAR study is currently in progress to determine the selectivity and functional activity of new compounds and optical isomers in this series.

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16. Selected data of final compounds; HPLC solvents: (1) 10% acetonitrile:90% methanol:0.05% TFA; and 20% acetonitrile:80% methanol:0.05% TFA:**4** yield = 79.2% (0.23 g) (R_f = 0.27; 20% EtOAc/hexanes) ^{1}H NMR, 500 MHz Varian, $CDCl_3$, δ 7.42 (d, J = 10.00 Hz, 2 Hz), 7.38 (d, J = 15.00 Hz, 2H), 7.36 (d, J = 10.00 Hz, 2H), 7.29 (m, J = 24.00 Hz, 2H), 7.06 (s, 1H), 7.01 (s, 1H), 5.88 (s, 1H), 3.81 (s, 3H), MS: m/z (ESI, pos.) = 382.8 ([M$^+$ 23]); HPLC retention time 13.166 min and 11.984 min; purity 98.60%; **5** yield = 32.2% (0.08 g) (R_f = 0.45; 20% EtOAc/hexanes) ^{1}H NMR, 500 MHz Varian, $CDCl_3$, δ 7.84 (m, J = 12.0 Hz, 2H), 7.61 (t, J = 10.0 Hz, 3H), 7.50 (m, J = 19.5 Hz, 3H), 7.42 (d, J = 2.00 Hz, 1H), 7.30 (d, J = 2.00 Hz, 1H), 6.98 (s, 2H), MS: m/z (ESI, pos.) = 382.0 ([M$^+$ 23]). HPLC retention time: 14.173 min and 12.661 min; purity 98.68%; **6** yield = 29.1% (0.41 g) (R_f = 0.27; 20% EtOAc/hexanes) ^{1}H NMR, 500 MHz Varian, $CDCl_3$, δ 7.85 (m, J = 9.5 Hz, 2H), 7.61 (t, J = 15.0 Hz, 1H), 7.51 (t, J = 8.0 Hz, 2H), 7.41 (d, J = 5.50 Hz, 1H), 7.30 (d, J = 2.00 Hz, 2H), 7.03 (d, J = 1.0 Hz, 1H), 7.00 (d, J = 1.0 Hz, 1H), 5.36 (s, 1H), 3.78 (s, 3H), MS: m/z (ESI, pos.) = 382.0([M$^+$ 23]). HPLC retention time: 9.904 min and 8.362 min; purity 95.40%; **7** yield = 46.6% (0.04 g) (R_f = 0.24; 10% EtOAc/hexanes) ^{1}H NMR, 500 MHz Varian, $CDCl_3$, δ 7.40 (d, J = 1.5 Hz, 2H), 7.34 (t, J = 3.5 Hz, 1H), 7.31 (d, J = 6.0 Hz, 2H), 7.20 (m, J = 7.5 Hz, 3H), 7.12 (d, J = 7.5 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 6.77 (d, J = 2.0 Hz, 1H), 4.84 (s, 1H), 1.69 (s, 6H); MS: m/z (ESI, pos.) = 380.7 ([M$^+$ 23]). HPLC retention time: 13.397 min and 12.054 min; purity 97.1%.