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STRUCTURE ACTIVITY RELATIONSHIPS OF TETRAHYDROCANNABINOL ANALOGUES ON HUMAN CANNABINOID RECEPTORS.

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Abstract: A series of Δ^8-tetrahydrocannabinol (THC) and biphenylic derivatives were prepared and their binding affinity for both human cannabinoid receptors hCB_1 and hCB_2 evaluated.

Δ^9-Tetrahydrocannabinol, an active component of marijuana, has been used for thousands of years in the treatment of a variety of ailments.[1] It possesses a wide range of physiological effects such as analgetic, appetite stimulant, anti-inflammatory, anti-convulsive, anti-emetic, immunosupressive and intraocular pressure lowering.[1,2] In spite of this therapeutic interest, its use has been limited by its psychotropic effects.[2] The search for analogues of medicinal value without the psychotropic effects has therefore received much attention.[3]

With the recent discovery of a second peripheral cannabinoid receptor,[4] it has been proposed that the psychotropic effects of cannabinoids may be mediated by the receptor expressed in the brain (CB_1), while some of the other beneficial properties may be associated with the peripheral receptor (CB_2). Given the structural differences between CB_1 and CB_2, which have both been cloned and expressed,[4,5] it should be possible to prepare selective hCB_2 ligands that might be valuable in the treatment of certain diseases. We now report our results on the SAR of some THC analogues.

All compounds[6] were evaluated in binding studies using the displacement of radiolabelled [^{3}H] (-) CP-55940[7] to determine the ligand potencies on hCB_2 and hCB_1. The compounds in this study were Δ^8-THC analogues containing a dimethylheptyl (DMH) chain, known to demonstrate increased potency[8] relative to the pentyl chain of Δ^9-THC in the classical CB_1 linked pharmacological assays as well as at the CB_1 receptor.

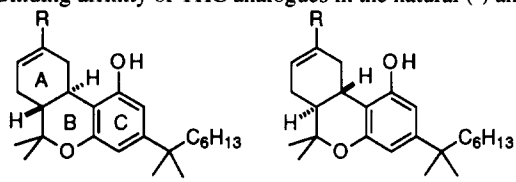
We paid special attention to the critical issue of the optical purity of cannabinoid ligands.[9] This turns out to be very important with some extremely potent ligands. Indeed, if such a ligand is present as a contaminant in its enantiomer, the K_i value obtained will be erroneous and directly linked to the optical purity of the batch under study. We were able to synthetize starting material and products at >97% optical purity using Brown's optical enrichment procedure on (+) and (-)-pinene.[10]

Table 1 shows the effect of chirality on receptor binding affinity of two pairs of enantiomers of THC analogues prepared according to reported procedure from (+) and (-)-pinene.[11] In both cases, the (-) enantiomer exhibited stronger binding than the (+) enantiomer for both the hCB_1 and hCB_2 receptor by at least 45 fold, consistent with previous reports. Unfortunately, the desired selectivity for hCB_2 over hCB_1 was found to be poor in both the (-) and in the (+) series.

A phenolic oxygen and a lipophilic chain are believed to be necessary for classical cannabinoid activity.[12] Table 2 shows the effect of modifications at the C-1 position on the affinity (K_i) at both receptors while keeping the DMH chain unaltered. For instance, transformation of the phenol **1** to the methyl ether **5**[13] resulted in a 40

fold loss in potency on hCB_2. On the other hand this loss in potency resulted in an increase in selectivity to 793 fold by virtue of the very weak binding on the hCB_1 receptor, as reported by Mechoulam *et al.*[14] An important effect on hCB_1 was also observed when the methoxy group of **5** was replaced by a hydrogen atom to give **7**.[15] The binding affinity increased from 15.9 μM to 0.25 μM, still 300 fold less potent than phenol **1**. Such a large effect on affinity caused by a small structural change may indicate that a region of the hCB_1 receptor was involved in interactions such as hydrogen bonding and/or steric effects with the ligand. Interestingly, the K_i of hCB_2 was not affected by this change. The phosphate **6**[16] was not very active, particularly on hCB_1. A K_i value of 0.44 μM was obtained on CB_2 and >20 μM for CB_1. In this particular case, polarity and steric factors certainly played an important role. We concluded from the results of Table 2 that simple modification of the phenol to a methoxy group gave a less potent but selective CB_2 cannabinoid.

Table 1: Binding affinity of THC analogues in the natural (-) and the unnatural (+) series.

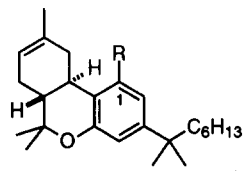


(-)-**1** R= Me (+)-**2**
(-)-**3** R= CHO (+)-**4**

	K_i (hCB_2, nM)[a]	K_i (hCB_1, nM)[a]	hCB_1/hCB_2
1-	0.49 ± 0.18	0.83 ± 0.13	1.7
2-	28.8 ± 6.4	38.8 ± 6.1	1.3
3-	2.61 ± 0.36	2.24 ± 0.05	0.9
4-	118.6 ± 30.8	97.0 ± 6.1	0.8

a) Values are mean ± S.E.M. or individual determination.

Table 2: Binding affinity of THC analogues bearing various groups at C-1.



1- R= OH **5**- R= OCH_3
6- R= $OP(O)(OC_2H_5)_2$ **7**- R= H

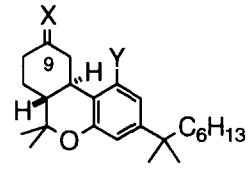
	K_i (hCB_2, nM)[a]	K_i (hCB_1, nM)[a]	hCB_1/hCB_2
1-	0.49 ± 0.18	0.83 ± 0.13	1.7
5-	20.0 ± 12.4	15850 ± 2960	793
6-	441.7 ± 74.8	>20 000, >5000	>45
7-	20.8 ± 11.2	249.7 ± 31.0	12

a) Values are mean ± S.E.M. or individual determination.

Nabilone **8**[12,17] and related compounds were prepared and the binding data are shown in Table 3. Nabilone differs structurally from Δ^9-THC and **1** by the presence of a carbonyl group at C-9. Compound **8** was very potent, but not selective with a $K_i \approx 2$ nM for both receptors. In fact, **8** is very similar to **3** with respect to binding affinity on both receptors, in spite of the carbonyl being moved one carbon atom away. The methylene analogue **10**[13] had similar potency for both receptors. On the other hand, transformation of the phenolic function of **10** to a methyl ether (**11**)[13] resulted in a loss of potency on hCB_1, leading to a high degree of selectivity (>1000), of the same order of magnitude as observed in the case of **5**. Compounds **5** and **11** actually only differ by the position of the double bond on the A ring. We concluded from this, that the position of the double bond was not critical to activity at hCB_2 in this series.

The methylation of nabilone to the ether **9**[17] resulted in a small increase in selectivity, but in a 72 fold loss in binding affinity for hCB_2 and a 284 fold loss on hCB_1. In the series of cannabinoids of Table 3, compound **11** stands out as a potent and selective hCB_2 binder.

Table 3: Binding affinity of THC analogues in the nabilone and exo-methylene series.



8, 9, 10, 11

	X	Y	K_i (hCB_2, nM)[a]	K_i (hCB_1, nM)[a]	hCB_1/hCB_2
8-	O	OH	1.84 ± 0.42	2.19 ± 0.89	1.2
9-	O	OCH_3	132.2 ± 44.3	621 ± 215	4.7
10-	CH_2	OH	0.58 ± 0.30	1.82 ± 0.11	3.1
11-	CH_2	OCH_3	19.4 ± 3.8	> 20 000, > 20 000	>1000

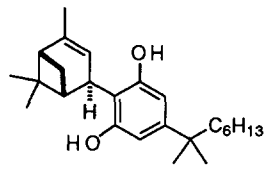
a) Values are mean ± S.E.M. or individual determination.

The radiolabelled CP-55940 used to determine the ligand potencies, is devoid of the rigid tricyclic ring system[18] common to the THC family, and we decided to test some non-tricyclic analogues. Compound **12**[19] used to prepare the tricyclic ring system, was found to be particularly potent at hCB_2 with a K_i of 41 nM (9 fold selective, Table 4). Replacement of the terpenyl moiety of **12** by a planar template that better approximates the A ring, such as a 3,5-dimethylphenyl group, greatly simplifies the molecule as well as the synthesis of potential analogues. This change resulted in stronger binding of **13** *vis-à-vis* **12** to both receptors and the net result was an improved selectivity for hCB_2. In order to evaluate the effectiveness of the phenol as hydrogen bonding donor, **13** was converted to dimethyl ether **16.** This modification led to a significant loss of the binding affinity on hCB_2 (ca. 200 fold), with a K_i of 433nM. Considering the structural simplicity of **16**, such binding is still

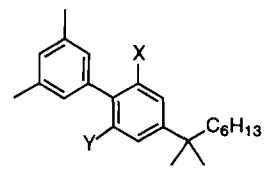
noteworthy. For the hCB_1 receptor, this modification resulted in an inactive compound.

The selectivity was lost by replacement of one OH group by a hydrogen atom (**14**). Mainly due to improved CB_1 binding. Removal of both OH groups yielded hydrocarbon **15**, which was essentially inactive at both receptors.

Table 4: Binding affinity of bicyclic and biphenylic analogues.



12



13,14,15,16

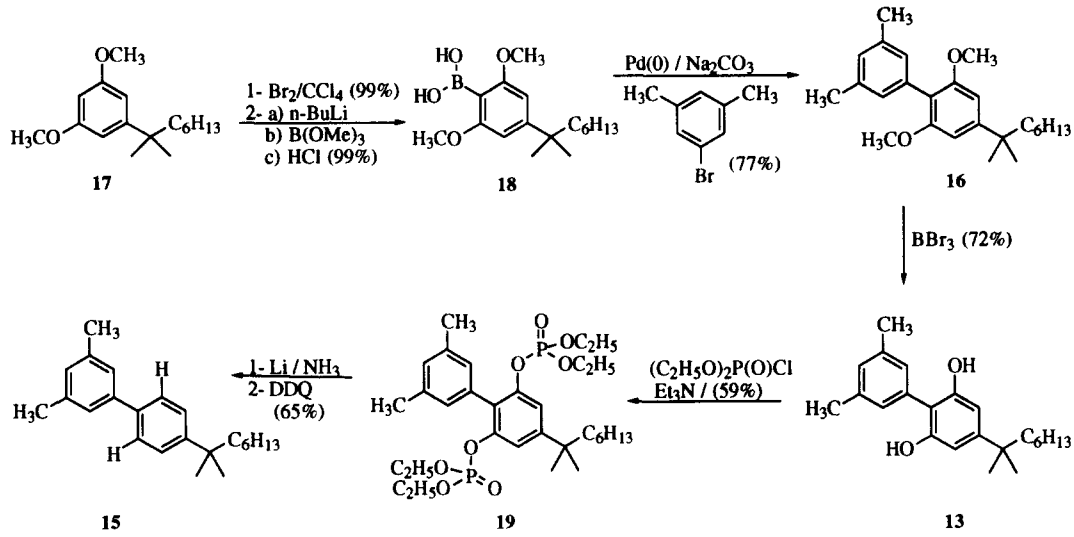
	X	Y	K_i (hCB_2, nM)[a]	K_i (hCB_1, nM)[a]	hCB_1/hCB_2
12-	-	-	41.2 ± 14.1	350.3 ± 41.4	9.0
13-	OH	OH	2.00 ± 1.08	79.1 ± 12.5	40
14-	OH	H	4.14 ± 2.51	12.5 ± 1.8	3.0
15-	H	H	2138 ± 1317	> 30 000, > 30 000	>14
16-	OCH_3	OCH_3	433 ± 203	> 20 000, > 20 000	>46

a) Values are mean ± S.E.M. or individual determination.

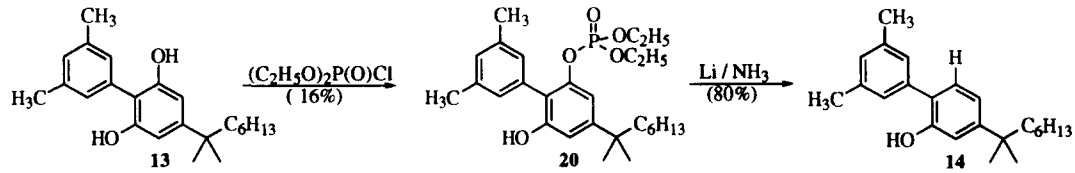
Compounds (**13-16**) were prepared according to Schemes 1 and 2. The bis-ether **17** was brominated[20] and converted to the boronic acid **18** via a metal-halogen exchange reaction with n-butyllithium. A Suzuki coupling reaction with 5-bromo-*m*-xylene gave **16**[21] in an overall yield of 75%. Demethylation[11a] of the bis-ether was performed with BBr_3 at 0° C to furnish **13** in 72% yield. Compound **15**[11a] was obtained by the formation of diphosphonate **19** followed by reduction with Li/NH_3 and reoxidation of the aromatic ring with DDQ (because of partial overreduction) in 38% yield for the 3 steps. Finally, compound **14** was prepared in a manner similar to that used for the synthesis of **15**, by formation of monophosphonate **20** in 16% yield and reduction with Li/NH_3 in 80% yield.

In conclusion, we have shown that in the naturally occuring Δ^8-THC stereochemical series, analogues such as **5** and **11** are very selective for the hCB_2 receptor regardless of the position of the exo or endo double bond. It was preferable not to have the free OH group at the C-1 position to obtain hCB_2 selective compounds. When the hydroxyl group (**1**) was replaced by a methoxy group (**5**), significant binding was retained at the hCB_2 receptor while the selectivity toward hCB_2 increased to 1000 fold. We have demonstrated, with non-cannabinoid type structures like **12-14** that the tricyclic moiety is not necessary to reach nM affinity, and that such compounds may have high selectivity.

Scheme 1



Scheme 2



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