# **Cannabinol Derivatives: Binding to Cannabinoid Receptors and Inhibition of** Adenylylcyclase

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Several derivatives of cannabinol and the 1,1-dimethylheptyl homolog (DMH) of cannabinol were prepared and assayed for binding to the brain and the peripheral cannabinoid receptors (CB<sub>1</sub> and CB<sub>2</sub>), as well as for activation of CB<sub>1</sub>- and CB<sub>2</sub>-mediated inhibition of adenylylcyclase. The DMH derivatives were much more potent than the pentyl (i.e., cannabinol) derivatives. 11-Hydroxycannabinol (4a) was found to bind potently to both  $CB_1$  and  $CB_2$  ( $K_i$  values of 38.0  $\pm$  7.2 and 26.6  $\pm$  5.5 nM, respectively) and to inhibit CB<sub>1</sub>-mediated adenylylcyclase with an  $EC_{50}$  of 58.1  $\pm$  6.2 nM but to cause only 20% inhibition of CB<sub>2</sub>-mediated adenylylcyclase at 10  $\mu$ M. It behaves as a specific, though not potent, CB<sub>2</sub> antagonist. 11-Hydroxycannabinol-DMH (**4b**) is a very potent agonist for both CB<sub>1</sub> and CB<sub>2</sub> ( $K_i$  values of 100  $\pm$  50 and 200  $\pm$  40 pM;  $EC_{50}$  of adenylylcyclase inhibition 56.2  $\pm$  4.2 and 207.5  $\pm$  27.8 pM, respectively).

## Introduction

Two cannabinoid receptors have been described to date. Both are proteins with seven transmembranespanning domains. These receptors were originally found in rat brain and spleen, respectively, and are generally known as the central cannabinoid receptor,  $CB_1$ , and the peripheral cannabinoid receptor,  $CB_2$ .<sup>1,2</sup> CB<sub>1</sub> is mainly expressed in the central nervous system (CNS). It is also found, to a lesser extent, outside the CNS, in numerous other tissues such as vas deferens, adrenal gland, heart, lung, prostate, uterus, ovary, testis, bone marrow, thymus, and tonsils.<sup>3,4</sup> The CB<sub>2</sub> gene is not expressed in the brain, being found mostly in the immune system. In certain tissues, such as spleen, the mRNA content of CB<sub>2</sub> is particularly high. In blood cell subpopulations, it is found particularly in B-cells.<sup>3,4</sup> It has been suggested that "cannabinoids may exert specific receptor-mediated action on the immune system through the CB<sub>2</sub> receptor".<sup>4</sup>

While a considerable amount of work has been done on structure-activity relationships (SAR) as regards binding to CB<sub>1</sub>,<sup>5-7</sup> very little is known on binding to  $CB_2$ .<sup>8</sup> In the original paper on the identification of  $CB_2$ , Munro et al.<sup>9</sup> showed that cannabinol (CBN) (1a), which only binds feebly to  $CB_1$ , much less so than  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) (**2a**), binds to CB<sub>2</sub> at the same level of potency as  $\Delta^9$ -THC. The low binding to CB<sub>1</sub> is compatible with the low THC-type in vivo activity recorded for CBN when compared to  $\Delta^9$ -THC.<sup>10</sup> The binding of CBN to CB<sub>2</sub>, with a potency equivalent to that of  $\Delta^9$ -THC, suggests that cannabinol derivatives could serve as a novel starting point for SAR analysis in the cannabinoid series and could possibly lead to selectivity as regards binding to the two cannabinoid receptors.

We present here data on several cannabinol derivatives which have been tested for binding to  $CB_1$  and  $CB_2$ .





 $\mathbf{R}' = \mathbf{H}, \mathbf{R}'' = \mathbf{C}_{5}\mathbf{H}_{11}$ 1b,  $\mathbf{R}' = \mathbf{H}, \mathbf{R}'' = \mathbf{C}(\mathbf{C}\mathbf{H}_{3})_{2}\mathbf{C}_{6}\mathbf{H}_{13}$ 1c,  $R' = COCH_3$ ,  $R'' = C(CH_3)_2C_6H_{13}$ 



3a, Cannabidiol, R = C<sub>4</sub>H<sub>11</sub> 3b,  $R = C(CH_3)_2C_6H_{13}$ 



 $5a, R' = H, R'' = C(CH_3)_2C_6H_{13}$ 5b,  $R' = COCH_3$ ,  $R'' = C(CH_3)_2C_6H_{13}$ 5c,  $\mathbf{R}' = \mathbf{H}$ ,  $\mathbf{R}'' = \mathbf{C}_5 \mathbf{H}_{11}$ 

2a,  $\Delta^9$ -THC, R' = H, R'' = C<sub>5</sub>H<sub>11</sub> 2b, R' =COCH<sub>3</sub>, R'' = C<sub>5</sub>H<sub>11</sub>  $2c, R' = H, R'' = C(CH_3)_2C_6H_{13}$ 2d,  $R' = COCH_3$ ,  $R'' = C(CH_3)_2C_6H_{13}$ 



4a,  $\mathbf{R'} = \mathbf{H}$ ,  $\mathbf{R''} = \mathbf{C}_5 \mathbf{H}_{11}$ 4b, R' = H,  $R'' = C(CH_3)_2C_6H_{13}$ 4c, R' = COCH<sub>3</sub>, R'' =  $C(CH_3)_2C_6H_{13}$ 



 $6a, R' = H, R'' = C(CH_3)_2C_6H_{13}$ 6b, R' = COCH<sub>3</sub>, R'' = C(CH<sub>3</sub>)<sub>2</sub>C<sub>6</sub>H<sub>13</sub> 6c, R' = H, R'' = C<sub>5</sub>H<sub>11</sub> 6d, R' = COCH<sub>3</sub>, R'' = C<sub>5</sub>H<sub>11</sub>

Both CB1 and CB2 binding assays were performed with transfected cells.<sup>11-13</sup> For comparison purposes, the derivatives were also assayed for CB1 binding to membranes, as previously described.<sup>14</sup>

Various laboratories, including our own, have established that the signal transduction pathway utilized by the two cannabinoid receptors proceeds through pertussis toxin-sensitive G proteins and inhibition of adenylylcyclase activity.<sup>2</sup> We recently showed that  $\Delta^9$ -THC binds to both receptors with similar affinity; however, in contrast to its capacity to serve as an agonist for the CB<sub>1</sub> receptor and to inhibit CB<sub>1</sub>-mediated adenylylcyclase,  $\Delta^9$ -THC was only able to induce a very slight inhibition of adenylylcyclase at the CB<sub>2</sub> receptor.<sup>12</sup>

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Morever  $\Delta^9$ -THC antagonizes the agonist-induced inhibition of adenylylcyclase mediated by CB<sub>2</sub>. Therefore, we concluded that  $\Delta^9$ -THC constitutes a weak antagonist for the CB<sub>2</sub> receptor.<sup>12</sup> It was thus of interest to find out whether CBN derivatives could selectively activate one of the cannabinoid receptors. Therefore, all compounds were tested for CB<sub>1</sub>- as well as CB<sub>2</sub>mediated inhibition of adenylylcyclase. The present paper is apparently the first one which examines the SAR of cannabinol derivatives for binding to the cannabinoid receptors as well as for inhibition of adenylylcyclase.

### Chemistry

Cannabinol (1a),  $\Delta^9$ -THC (2a), and cannabidiol (3a) were extracted from hashish.<sup>15,16</sup> The 1,1-dimethylheptyl (DMH) homolog (**2c**) (at position 3) of  $\Delta^9$ -THC was prepared by Lewis acid-catalyzed ring cyclization of the DMH homolog of cannabidiol (3b),<sup>17</sup> as previously described for the parallel conversion of cannabidiol (3a) into  $\Delta^9$ -THC (**2a**).<sup>16</sup> The DMH homolog of cannabinol (1b) was prepared by dehydrogenation with sulfur at 240 °C of 2d (synthesized by acetylation of 2c), leading to 1c, followed by removal of the acetate group with ethanolic sodium hydroxide. The isomeric 1,2-dimethylheptyl homolog of CBN has previously been prepared and shown to be a potent cannabimimetic.<sup>18</sup> 11-Hydroxycannabinol (4a) was prepared, as previously described, by selenium dioxide oxidation and dehydrogenation of  $\Delta^9$ -THC acetate (**2b**) followed by removal of the ester grouping.<sup>19</sup> 11-Hydroxy- $\Delta^8$ -THC (5c) and its DMH homolog (5a) were prepared as previously described.<sup>19,20</sup> Dehydrogenation of the DMH homolog of 11-hydroxy- $\Delta^8$ -THC diacetate (**5b**)<sup>20</sup> with sulfur at 240 °C led to the DMH homolog of 11-hydroxycannabinol diacetate (4c), which was converted to the diol 4b.

The same procedure was followed for the conversion of  $\Delta^{8}$ -THC-7-oic acid (**6c**)<sup>21</sup> into cannabinol-7-oic acid

(7a)<sup>22</sup> and of the DMH homolog of (-)- $\Delta^{8}$ -THC-11-oic acid (6a)<sup>23</sup> into the DMH homolog of cannabinol-11-oic acid (7c). The lactones **8a**,**b** were prepared following a route reported by the groups of Adams and Todd about 50 years ago.<sup>24</sup> Von Pechmann condensation of either olivetol (9a) or 3-(1,1-dimethylheptyl)resorcinol (9b) with ethyl 5-methyl-1-oxocyclohexane-2-carboxylate (10) led to the known tetrahydrodibenzopyrone **11a** or **11b**, respectively.<sup>24</sup> Dehydrogenation of **11a** or **11b** as described above gave **8a**<sup>24</sup> or **8b**, respectively.

## **Biological Results**

All compounds were tested for binding to CB<sub>1</sub> using African green monkey kidney (COS-7) cells transfected with the cDNA of rat CB<sub>1</sub><sup>12,25</sup> as well as rat brain synaptosomal membrane preparations.<sup>14,26</sup> Binding to CB<sub>2</sub> was performed using transfected COS-7 and Chinese hamster ovary (CHO) cells transfected with the cDNA of human CB<sub>2</sub>.<sup>9,11,12</sup> The  $K_i$  values were determined by displacement of [<sup>3</sup>H]HU-243, a probe for both cannabinoid receptors.<sup>26,27</sup> For consistency, the results discussed below are those from transfected cells except when specifically indicated. All compounds were also assayed for inhibition of CB<sub>1</sub>- and CB<sub>2</sub>-mediated adenylylcyclase.<sup>11,12</sup> The results are summarized in Table 1.

First, CBN (1a) was compared to  $\Delta^9$ -THC (2a). Under our experimental conditions, CBN was about 2-4 times less potent on binding to either CB<sub>1</sub> or CB<sub>2</sub>, in contrast to the data reported by Munro et al.,<sup>9</sup> who found that CBN and  $\Delta^9$ -THC are equipotent on CB<sub>2</sub>. These differences in recorded potencies are not unexpected, as binding data are very sensitive to experimental conditions. The CB<sub>1</sub>-mediated adenylylcyclase inhibition data seem to be closer to in vivo observations, where, as mentioned above,  $\Delta^9$ -THC is considerably more potent than CBN. We found that CBN is about 13 times less potent than  $\Delta^9$ -THC. With CB<sub>2</sub> we see a different profile. While the EC<sub>50</sub> of CBN inhibition of CB<sub>2</sub>mediated cyclase is 261.2  $\pm$  46.4 nM,  $\Delta^9$ -THC causes only 21% inhibition at 1  $\mu$ M. We have previously shown that  $\Delta^9$ -THC is a weak agonist for CB<sub>2</sub> and can actually antagonize the CB<sub>2</sub>-mediated inhibition of adenylylcyclase caused by more potent agonists.<sup>12</sup>

The next derivative to be examined was the DMH homolog of CBN (1b). We observed essentially no difference between its binding affinity to CB<sub>1</sub> or CB<sub>2</sub>. On both receptors, 1b was about 100 times more potent than CBN. It was also 100 times more potent than CBN when tested for binding to rat brain membranes. This compound also potently inhibited adenylylcyclase via both  $CB_1$  and  $CB_2$ : the  $K_i$  of inhibition by **1b** of  $CB_1$ mediated cyclase was ca. 660-fold lower, and the CB<sub>2</sub>mediated cyclase  $K_i$  was ca. 300-fold lower than that of CBN. This result demonstrates that the exchange of the pentyl group with the DMH group (at position 3) dramatically increases the affinity of the ligand to both CB<sub>1</sub> and CB<sub>2</sub> and makes it a much more potent agonist. A similar result was obtained with other cannabinol derivatives (see below). Neither CBN (1a) nor its DMH homolog 1b express any particular selectivity with regard to the inhibition of adenylyl cylase via the two receptors. However, a major metabolite of CBN, namely, 11-hydroxy-CBN (4a),<sup>19,22,28</sup> had a different profile. It binds to both receptors with  $K_i$  values ca. 5-fold lower than the K<sub>i</sub> values recorded for CBN. However, while

**Table 1.** Binding of Various Cannabinoids to Cannabinoid Receptors and Inhibition of Adenylylcyclase<sup>a,b</sup>

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	brain	COS CB <sub>1</sub> and CB <sub>2</sub>				
	binding,	binding (K <sub>i</sub> , nM)		adenylylcyclase (EC <sub>50</sub> , nM)		
compound	$CB_1$ ( $K_i$ , $nM$ )	CB <sub>1</sub>	$CB_2$	CB <sub>1</sub>	$CB_2$	
CBN (1a)	$392.2 \pm 53.5$	$211.2\pm35.0$	$126.4\pm26.0$	$120.0\pm32.1$	$261.2 \pm 46.4$	
CBN-DMH ( <b>1b</b> )	$3.3\pm0.2$	$2.0\pm0.3$	$1.5\pm0.5$	$0.18\pm0.03$	$0.79 \pm 0.021$	
$\Delta^9$ -THC ( <b>2a</b> )	$66.5\pm5.8$	$80.3 \pm 22.2$	$32.2\pm6.7$	$11.0 \pm 2.1$	21% inhib at 1 $\mu$ M	
$\Delta^9$ -THC-DMH ( <b>2c</b> )	$2.6\pm0.3$	$0.241 \pm 0.05$	$0.199 \pm 0.05$	$0.58 \pm 0.12$	$1.01\pm0.15$	
11-OH-CBN ( <b>4a</b> )	$188.9 \pm 38.3$	$38.0 \pm 7.2$	$26.6\pm5.5$	$58.1 \pm 6.2$	20% inhib at 10 $\mu$ M	
11-OH-CBN-DMH ( <b>4b</b> )	$1.8\pm0.3$	$0.1\pm0.05$	$0.2\pm0.04$	$0.056\pm0.004$	$0.208 \pm 0.028$	
11-OH-∆ <sup>8</sup> -THC ( <b>5c</b> )	$33.4\pm3.8$	$25.8\pm2.9$	$7.4 \pm 2.5$	$39.9 \pm 8.9$	19% inhib at 1 $\mu$ M	
11-OH-∆ <sup>8</sup> -THC-DMH ( <b>5a</b> )	$0.19\pm0.01$	$0.1\pm0.02$	$0.17\pm0.01$	$0.035\pm0.005$	$0.078\pm0.009$	
$\Delta^{8}$ -THC-11-oic acid-DMH ( <b>6a</b> )	$480.6\pm40.8$	$32.3\pm3.7$	$170.5\pm7.8$	$927.0\pm39.6$	$116.2\pm74.7$	
$\Delta^{8}$ -THC-11-oic acid-DMH	>10000	11% inhib at 1 $\mu$ M	9% inhib at 1 $\mu$ M	no inhib at 1 $\mu$ M	27% inhib at 1 $\mu$ M	
acetate ( <b>6b</b> )						
CBN-11-oic acid (7a)	$602.4 \pm 15.8$	$< 2\%$ inhib at 1 $\mu$ M	23% inhib at 1 $\mu$ M	10% inhib at 1 $\mu$ M	6% inhib at 1 $\mu$ M	
CBN-11-oic acid acetate (7b)	>10000	35% inhib at $1 \mu M$	19% inhib at 1 $\mu$ M	3% inhib at 1 $\mu$ M	2% inhib at 1 $\mu$ M	
CBN-DMH-11-oic acid (7c)	$6.1\pm0.7$	$6.1 \pm 1.1$	$4.8\pm1.9$	$2.4\pm0.2$	$5.4 \pm 0.3$	
CBN-DMH-11-oic acid	$556.1 \pm 40.6$	$49.8 \pm 11.5$	$17.3\pm7.4$	$13.3\pm3.4$	$39.3\pm6.1$	
lactone <b>8a</b>	>10000	14% inhib at $1 \mu M$	14% inhib at 1 µM	7% inhib at 1 µM	no inhib at $1 \mu M$	
lactone <b>8b</b>	$72.5\pm3.6$	$32.7 \pm 4.9$	$17.3 \pm 5.6$	$9.6\pm0.6$	$4.6\pm0.3$	

<sup>a</sup> For details of procedures, see Experimental Section. <sup>b</sup> For structures, see text.



**Figure 1.** Compound **4a** antagonizes the capacity of the cannabinoid agonists **7c** (A) and **5a** (B) to inhibit the forskolin-stimulated adenylylcyclase activity in CHO cells stably transfected with cDNA of human CB<sub>2</sub>. Compounds **4a**, **7c**, and **5a** were added at the indicated concentrations; 100% represents the amount of cAMP in the absence of cannabinoids. Each point is the mean  $\pm$  standard deviation of three experiments performed in triplicate.

the EC<sub>50</sub> for the CB<sub>1</sub>-mediated inhibition of cyclase was  $58.1 \pm 6.2$  nM, the inhibition of the CB<sub>2</sub>-mediated cyclase was negligible: 20% inhibition at 10  $\mu$ M. This result indicates that 4a could serve as a functional antagonist of CB<sub>2</sub> as it binds, but does not activate, CB<sub>2</sub>. Indeed, it reduces the CB<sub>2</sub>-mediated adenylylcyclase inhibitory activity of the potent agonists 5a (HU-210)<sup>5,8b,20</sup> and 7c (Figure 1). However while the adenylylcyclase inhibition by 7c is very strongly reduced by 4a, that of 5a is considerably less. This difference may well be due to the higher inhibitory potency of 5a, compared to 7c. It should be pointed out that 4a, a major metabolite of CBN, in its differential activity between CB<sub>1</sub> and CB<sub>2</sub>, resembles  $\Delta^9$ -THC rather than CBN (see above):<sup>12</sup> both  $\Delta^9$ -THC and **4a** bind to CB<sub>1</sub> and CB<sub>2</sub> and inhibit CB<sub>1</sub>-mediated cyclase in the nanomolar range but cause only negligible inhibition of CB2mediated cyclase up to micromolar concentrations.

The DMH homolog of 11-hydroxycannabinol (**4b**) is the most potent cannabinoid in the present series. It binds to CB<sub>1</sub> and CB<sub>2</sub> with  $K_i$  values of 100  $\pm$  50 and 200  $\pm$  40 pM, respectively. Compound **4b** also inhibits adenylylcyclase *via* both CB<sub>1</sub> and CB<sub>2</sub>, at very low concentrations:  $56.2 \pm 4.2$  and  $207.5 \pm 27.8$  pM, respectively. Both the binding constants and EC<sub>50</sub> values for CB<sub>1</sub> and CB<sub>2</sub> are comparable to the values observed for HU-210 (**5a**).

Cannabinol-11-oic acid (7a),<sup>22</sup> as well as its acetate (7b), were essentially inactive on binding to either  $CB_1$ or CB<sub>2</sub>, as well as in CB<sub>1</sub>- or CB<sub>2</sub>-mediated cyclase inhibition. However, the DMH homologs, compounds 7c,d, bind to both CB1 and CB2 and inhibit adenylylcyclase via the two receptors with high potency. The nonacetylated derivative **7c** binds with  $K_i$  values of 6.1  $\pm$  1.1 and 4.8  $\pm$  1.9 nM to CB<sub>1</sub> and CB<sub>2</sub>, respectively, and inhibits cyclase CB1- and CB2-mediated activity with EC\_{50} values of 2.4  $\pm$  0.2 and 5.4  $\pm$  0.3 nM, respectively. These results were unexpected as THCtype 11-oic cannabinoid acids (including DMH homologs) such as **6a**, **c** have not been reported to cause psychotropic effects, <sup>10,21,23</sup> which are presumably mediated by CB<sub>1</sub>. The acetate **7d** is also active in all these assays, although its activity is 4-7 times lower than the nonacetylated acid (see Table 1). This difference was even larger (90 times) when the acetate 7d was compared to the nonacetylated acid 7c with regards to binding to  $CB_1$  on synaptosomal membranes. It is not clear whether the weaker activity of the acetate is due to its own lower intrinsic activity or to a certain amount of nonacetylated **7c** formed by hydrolysis during the assays.

In view of these results (see Discussion), we looked again into the activity of the DMH homolog of  $\Delta^8$ -THC-7-oic acid (**6a**).<sup>23</sup> This compound, synthesized in our laboratory several years ago, was found then to reduce paw edema and leukocyte adhesion to culture dishes and was considered to be antiinflammatory. It did not cause catalepsy in mice at doses up to 1 mg/kg.<sup>23</sup> We now find that **6a** binds to CB<sub>1</sub> with a rather low  $K_i$  of  $32.3 \pm 3.7$  nM but is only a relatively weak inhibitor of CB<sub>1</sub>-mediated cyclase (EC<sub>50</sub> = 927.0 ± 39.6 nM). It binds to the CB<sub>2</sub> receptor with a  $K_i$  of  $170.5 \pm 7.8$  nM and inhibits CB<sub>2</sub>-mediated cyclase (EC<sub>50</sub> = 116.2 ± 74.7 nM). This material thus affects signaling *via* CB<sub>2</sub> more effectively than *via* CB<sub>1</sub>.

The last series to be examined were cannabinol derivatives in which the pyran ring was modified; instead of the two methyl groups on ring B, a ketone was introduced, thus forming a lactone. Compound **8a** (with a pentyl side chain)<sup>24</sup> did not bind to either CB<sub>1</sub> or CB<sub>2</sub> and did not inhibit adenylylcyclase, while the DMH derivative **8b** was highly potent, binding to both CB<sub>1</sub> and CB<sub>2</sub> with  $K_i$  values of  $32.7 \pm 4.9$  and  $17.3 \pm 5.6$  nM, respectively. It inhibited both CB<sub>1</sub>- and CB<sub>2</sub>-mediated adenylylcyclase with EC<sub>50</sub> values of  $9.6 \pm 0.6$  and  $4.6 \pm 0.3$  nM, respectively.

### Discussion

For reasons discussed in the Introduction, we assumed that CBN derivatives may show differential binding for CB<sub>1</sub> compared to CB<sub>2</sub>. The results obtained did not fulfill these expectations. Compounds binding poorly to CB<sub>1</sub> (such as 7a, 8a) had the same profile with  $CB_2$ , and this was the case also for the more potent compounds (such as 1b, 4a,b, 7c,d, 8b). However, our results proved to be of interest in another direction: we found that binding to CB<sub>2</sub> (see compound 4a) does not necessarily imply a parallel level of inhibition of CB2mediated adenylylcyclase. As mentioned above, the major CBN metabolite, 11-hydroxy-CBN (4a), binds well to both CB<sub>1</sub> and CB<sub>2</sub> ( $K_i = 38 \pm 7.2$  and  $26.6 \pm 5.5$  nM, respectively). However, while 4a inhibits adenylylcyclase strongly via  $CB_1$  (EC<sub>50</sub> = 58.1  $\pm$  6.2 nM), its inhibition of adenylylcyclase via CB2 is negligible (Table 1). Further experiments (see Biological Results and Figure 1) indeed showed that **4a** is a weak antagonist to CB<sub>2</sub>. Hence, this CBN metabolite may represent a useful target for future research and possibly a tool in CB<sub>2</sub> investigations. As **4a** is formed *in vivo* from CBN, its presence in the body may have physiological consequences associated with CB2-promoted activities, possibly in the immune system.

The replacement of the pentyl group at position 3 with the DMH group increased affinity to  $CB_1$  as well as to  $CB_2$  in all compounds tested. The dramatic increase of pharmacological activity associated with such a structural change was first noted about 50 years ago.<sup>24c,d</sup> As indicated above, the DMH homolog of 11-hydroxy-CBN (**4b**) is the most active compound in the present series as regards binding to  $CB_1$  and  $CB_2$ , as well as inhibition of adenylylcyclase (see Table 1). It may serve alongside HU-210 (**5a**) as a potent tool in cannabinoid research. Indeed, **4b** shows binding values and cyclase inhibition levels very similar to those of HU-210 (**5a**) (see Table 1).

Oxidation of the 11-CH<sub>3</sub> group in  $\Delta^9$ -THC (**2a**) or  $\Delta^8$ -THC (**12**) to a carboxyl group forming THC-7-oic acids (**13** or **6c**, respectively) leads to inactivation, as seen in behavioral assays.<sup>10,21</sup> Indeed, this route is the major



inactivation pathway of THC metabolism. The DMH homolog of  $\Delta^{8}$ -THC, 11-oic acid **6a**, has also been reported to lack THC-type activity,<sup>23</sup> although the reported test range was limited. Unexpectedly, we now find that although **6a** binds to both  $CB_1$  and  $CB_2$  in the 30-170 nM range, the EC<sub>50</sub> of adenylylcyclase inhibition *via* CB<sub>1</sub> is in the range of 927  $\pm$  39.6 nM. The CB<sub>2</sub>mediated adenylylcyclase is in the intermediate range  $(116.2 \pm 74.7 \text{ nM})$ , i.e., a certain separation between CB<sub>1</sub>- and CB<sub>2</sub>-mediated activation is noted. In the CBN-11-oic acid series, we observed a different profile: while in the pentyl series (7a,b), as expected, we recorded no binding or inhibition of cyclase, with DMH-CBN-11-oic acid (7c), we found potent binding to both  $CB_1$  and  $CB_2$  and cyclase inhibition (see Table 1). Compounds **6a** and **7c** differ in the conformation of ring A: a planar aromatic ring in 7c versus a half-chair one in **6a**. This conformational difference may represent the molecular basis for the different activities of the two cannabinoids.

A comparison of the binding values of **6a** with those of its CB<sub>1</sub>- and CB<sub>2</sub>-mediated cyclase inhibition (see Table 1) may explain some of the previously reported properties of **6a**. The relatively high levels of **6a** needed to inhibit adenylylcyclase *via* CB<sub>1</sub> may explain the absence of catalepsy (within the limited dose range tested), in spite of its considerable binding potency, while the ca. 10 times lower levels needed to inhibit adenylyl cylcase *via* CB<sub>2</sub> may be the basis of its reported antiinflammatory activity. These observations may serve to open new leads toward the development of antiinflammatory agents in which wider separations of activity can be achieved than these reported now.

As mentioned above, all compounds presented in Table 1 were tested for binding both in transfected cells and in rat brain synaptosomal preparations. While the general potency trend is comparable in the two assays, some individual differences are striking. Compounds 6b, 7b, and 8a are inactive in both assays; 7a is inactive in transfected cells and poorly active in the membrane assay. The highly potent **1b**, **5a**, and **7c** have the same profile in both assays. However 11-hydroxy CBN-DMH (4b) has a  $K_i$  of 100.0  $\pm$  50.0 pM in transfected cells and nearly 20 times higher  $K_i$  (i.e., lower potency) in brain membranes. In the compounds within the intermediate range, the  $K_i$  values differ widely: from about 2 times (i.e., essentially equipotent) in 1a, 2a, and 8b to about 10 times or more in 7d and 6a. The reasons for these differences are not clear, but they should be taken into account when binding data in the cannabinoid series are compared.

In summary, a comparison of the binding potency of several cannabinol derivatives to  $CB_1$  and  $CB_2$  with

their capacity to inhibit adenylylcyclase has led to the discovery of a CBN metabolite (11-hydroxy-CBN, **4a**) as a specific, though not potent,  $CB_2$  antagonist and to a new very potent agonist for both  $CB_1$  and  $CB_2$  (11-hydroxy-CBN-DMH, **4b**).

## **Experimental Section**

**Chemistry.** <sup>1</sup>H NMR spectra were measured on a Varian VXR-300S spectrophotometer using TMS as the internal standard. All chemical shifts are reported in ppm. Specific rotations were detected with a Perkin-Elmer 141 polarimeter. Melting points (uncorrected) were determined on a Buchi 530 apparatus. Column chromatography was performed with ICN silica gel 60A. Organic solutions were dried over anhydrous magnesium sulfate. Elemental analyses were obtained for all new compounds (or their acetates) and were  $\pm 0.4\%$  of the theoretical values. The analyses were performed at the Elemental Analysis Laboratory of the Hebrew University.

Δ<sup>9</sup>-**Tetrahydrocannabinol**-**DMH** (**2c**). Boron trifluoride etherate (5.5 mL) was added to cannabidiol-DMH (**3b**)<sup>17</sup> (5.7 g, 15.4 mmol) in dry dichloromethane (150 mL) containing magnesium sulfate (1 g), under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 20 min. A saturated solution of sodium bicarbonate was added until the red color observed during the reaction faded. The reaction mixture was washed with water, separated, dried, and evaporated. The oil obtained was chromatographed on a silica gel column. Δ<sup>9</sup>-Tetrahydrocannabinol-DMH (**2c**) (3 g, 53%) was eluted with 4% ether in petroleum ether: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.38, 6.26 (s, 2H), 6.3 (s 1H), 3.16 (d, 1H, *J* = 11.1 Hz), 2.14 (2H), 0.8–1.8 (m); IR (neat) 3300, 2950, 2850, 1620, 1570 cm<sup>-1</sup>. Anal. (C<sub>25</sub>H<sub>38</sub>O<sub>2</sub>) C,H.

 $\Delta^9\text{-THC-DMH}$  (**2c**) is not stable. At room temperature it rapidly becomes violet; on TLC after 0.5 h, numerous new spots are observed.

 $\Delta^9$ -Tetrahydrocannabinol-DMH acetate (2d): <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  6.67, 6.50 (s, 2H), 6.0 (s, 1H), 3.2 (d, 1H), 2.28 (s, 3H), 2.14 (2H), 1.9–0.8 (m); IR (neat) 2900, 1760, 1620, 1560 cm<sup>-1</sup>.

**Dehydrogenations of 2d, 5b, 6b,d, and 11b.** The dehydrogenations were carried out by heating each compound with sulfur at 238–240 °C, under a nitrogen atmosphere, for ca. 4 h. Each mixture was extracted with ether and evaporated. The residue was chromatographed on a silica gel column using variable concentrations of ether in petroleum ether as eluent. Compound **2d** led to cannabinol-DMH acetate (**1c**); compound **5b** gave **4c**; compound **6b** gave **7d**; compound **11b** gave **8b**. For yields and spectroscopic data, see below.

**Cannabinol-DMH acetate (1c):** obtained in 24% yield, mp 84–86 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.8, 7.25, 7.13 (s, 3H), 6.85 (d, 1H, J = 2.1 Hz), 6.67 (d, 1H, J = 2.1 Hz), 2.36 (s, 3H), 2.32 (s, 3H), 1.6–0.8 (m); IR (neat) 2930, 1780, 1620, 1560 cm<sup>-1</sup>. Anal. (C<sub>27</sub>H<sub>36</sub>O<sub>3</sub>) C,H.

**11-Hydroxycannabinol-DMH acetate (4c):** obtained in 44% yield from **5b**; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  8.00, 7.25 (s, 3H) 6.85 (d, 1H, J = 1.5 Hz), 6.68 (d, 1H, J = 1.5 Hz), 5.10 (s, 2H), 2.34 (s, 3H), 2.10 (s, 3H), 1.6–0.85 (m); IR (neat) 2930, 1780, 1740, 1620, 1540 cm<sup>-1</sup>.

**Cannabinol-11-oic acid acetate (7b):** obtained in 22% yield from **6d**, mp 149–151 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  8.81 (s, 1H), 8.02 (d, 1H, J = 8.1 Hz), 7.38 (d, 1H, J = 8.4 Hz), 6.78, 6.64 (s, 2H), 2.42 (s, 3H), 1.7–0.8 (m); IR (neat) 2960, 1760, 1670, 1610, 1570 cm<sup>-1</sup>. Anal. (C<sub>23</sub>H<sub>26</sub>O<sub>5</sub>) C, H.

**Cannabinol-11-oic acid-DMH acetate (7d):** obtained in 20% yield from **6b**; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  8.8 (s, 1H), 8.02 (d, 1H, J = 8.1 Hz), 7.38 (d, 1H, J = 8.4 Hz), 6.86, 6.75 (s, 2H), 2.42 (s, 3H), 1.7–0.8 (m); IR (neat) 2960, 1780, 1680, 1620, 1570 cm<sup>-1</sup>. Anal (C<sub>27</sub>H<sub>34</sub>O<sub>5</sub>) C,H.

**Cannabinol-DMH (1b)**: Hydrolysis of **1c** in ethanolic sodium hydroxide solution gave compound **1b**, mp 95–98 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  8.11, 7.22, 7.08, 6.36, 6.4 (s, 5H), 2.33 (s, 3H), 1.5–0.7 (m).

**11-Hydroxycannabinol-DMH (4b):** Compound **4c** (526 mg, 1.13 mmol) in dry ether (10 mL) was added to lithium aluminum hydride (123 mg) in dry ether (8 mL). The mixture was boiled under reflux for 2 h. The oil obtained after workup

was chromatographed on a silica gel column (60 g). Elution with 20% ether in petroleum ether gave, after crystallization from pentane, compound **4b** (340 mg, 78%): mp 128–130 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  8.45, 7.255, 7.253, 6.60, 6.40 (s, 5H), 4.75 (s, 2H), 1.65–0.8 (m, 2H). Anal. (C<sub>25</sub>H<sub>34</sub>O<sub>3</sub>) C,H.

**Cannabinol-11-oic acid-DMH (7c).** Compound **7d** (60 mg) was disolved in 0.6 mL of ethanol. A solution of 60 mg of sodium hydroxide in 0.4 mL of water was added under nitrogen. The solution was stirred at room temperature for 30 min. The solution was acidified with 10% HCl (5 mL); the mixture was extracted with ether, dried with MgSO<sub>4</sub>, and evaporated. The material obtained was separated on a preparative TLC plate (elution with ether:petroleum ether, 7:3), leading to 41 mg of **7c**, 76% yield: <sup>1</sup>H NMR 9.2 (s, 1H), 8.0 (d, 1H), 7.38 (d, 1H), 6.61 (s, 1H), 6.45 (s, 1H), 1.7–0.8 (m).

**Cannabinol-11-oic acid (7a): 7b** was hydrolyzed in ethanolic sodium hydroxide solution as described for compound **7c** to yield **7a**, 82% yield; <sup>1</sup>H NMR  $\delta$  9.2 (s, 1H), 8.0 (d, 1H), 7.38 (d, 1H), 6.62 (s, 1H), 6.47 (s, 1H), 1.8–0.8 (m).

**Compound 11b.** A solution of 3-DMH-resorcinol (**9b**) (3.9 g, 16.5 mmol), ethyl 4-methyl-2-oxocyclohexanecarboxylate (**10**) (4 g, 21.7 mmol), and POCl<sub>3</sub> (3.06 mL) in dry benzene (15 mL) was boiled under reflux, under a nitrogen atmosphere, for 3 h. The solution was washed with NaHCO<sub>3</sub> followed by water. After drying and evaporation the oil was chromatographed on a silica gel column. Compound **11b**<sup>24c</sup> (40%) was eluted with 10% ether in petroleum ether: mp 158–160 °C (from pentane); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  6.83, 6.63 (s, 2H), 3.4 (dd, 2H), 2.8 (dd, 2H), 2.7–0.8 (m); IR (Nujol) 1680, 1610sh, 1580 cm<sup>-1</sup>; MS M<sup>+</sup> 356.

**Compound 8b:** dehydrogenation of **11b** with sulfur as described above gave compound **8b** (72%), mp 184–185 °C (from pentane); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  8.82 (s, 1H), 8.3 (d, 1H), 7.38 (d, 1H), 6.94, 6.72 (s, 2H), 2.54 (s, 3H), 1.6–0.8 (m, 19H). Anal. (C<sub>23</sub>H<sub>28</sub>O<sub>3</sub>) C,H.

**Receptor Binding Assay. a. Binding to Synaptosomal Membranes.** Synaptosomal brain preparations were made from whole rat brain as described previously.<sup>26,27</sup> Binding of [<sup>3</sup>H]HU-243 (50.4 Ci/mmol) was assayed in triplicate as described.<sup>26,27</sup> In brief, each reaction mixture of 1 mL in siliconized Eppendorf tubes contained 2.4–3.8  $\mu$ g of synaptosomal membrane protein, 28–48 fmol of [<sup>3</sup>H]HU-243, and various concentrations of competing unlabeled cannabinoids. Tubes were incubated at 30 °C for 90 min and centrifuged at 13 000 rpm, and the tips of the tubes containing the pelleted membranes were cut and counted for their radioactivity.

**b.** Binding to Transfected COS-7 Cells. Two days after transfection (with  $5 \mu g/100 \text{ mm}$  of dish plasmids encoding CB<sub>1</sub> or CB<sub>2</sub>) the cells were washed with phosphate-buffered saline, scraped, pelleted, and stored at -80 °C. Cell pellets were homogenized in binding buffer (50 mM Tris-HCl, 5 mM MgCl<sub>2</sub>, and 2.5 mM EDTA, pH 7.4), and 50  $\mu$ g protein aliquots were assayed for binding of [<sup>3</sup>H]HU-243 as described above, except that the final concentration of [<sup>3</sup>H]HU-243 was 300 pM. For more detailed information see refs 11 and 12.

Specific binding was defined as the difference between the amount of radioactivity bound to the pelleted membranes in the absence and presence of 50 nM unlabeled HU-243 (for a) or 1  $\mu$ M unlabeled **5a** (HU-210) (for b) and was typically 70–80% of the total bound. The  $K_i$  values for the various cannabinoids and related compounds were calculated from the competition data according to the formula:  $K_i = IC_{50}/1 + ([^3H]-HU-243/K_d).^{29}$  The  $K_d$  values for HU-243 binding were  $45^{26}$  and 61 pM<sup>11</sup> for CB<sub>1</sub> and CB<sub>2</sub>, respectively.

**Cell Cultures.** Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 8% fetal calf serum, 2 mM glutamine, nonessential amino acids, 100 units/mL penicillin, and 100  $\mu$ g/mL streptomycin in a humidified atmosphere consisting of 5% CO<sub>2</sub> and 95% air at 37 °C. CHO cells stably transfected with the cDNA of human CB<sub>2</sub> receptor<sup>9</sup> were described earlier.<sup>11</sup> COS-7 cells in 100 mm dishes were transiently transfected<sup>12</sup> with plasmids encoding rat CB<sub>1</sub><sup>25</sup> or human CB<sub>2</sub><sup>9</sup> (5  $\mu$ g each) and, when indicated, with 2  $\mu$ g of the plasmid containing the cDNA of adenylylcyclase type V.<sup>12</sup> Twenty-four hours later, the cells were trypsinized and cultured in 24-well plates. After an additional 24 h, the cells

#### Cannabinol Derivatives

were assayed for adenylylcyclase activity. Transfection efficiency, determined by transfection with the cDNA for  $\beta$ -galactosidase, was 40-80%.

Adenylylcyclase Assay. The assay were performed as described.<sup>11,12,27</sup> Cells cultured in 24-well plates were incubated for 3 h with 0.25 mL/well fresh growth medium containing 5  $\mu$ Ci/mL [<sup>3</sup>H]adenine. This medium was replaced with Dulbecco's modified Eagle's medium containing 20 mM Hepes (pH 7.4), 1 mg/mL fatty acid-free bovine serum albumin, 0.5 mM 1-methyl-3-isobutylxanthine, and 0.5 mM RO-20-1724. Cannabinoids and forskolin (1  $\mu$ M) were added, and the cells were incubated at 37 °C for 10 min. The reaction was terminated with 1 mL of 2.5% perchloric acid containing 0.1 mM unlabeled cAMP. Aliquots of 0.9 mL were neutralized with 100  $\mu L$  of 3.8 M KOH and 0.16 M  $K_2 CO_3$  and applied to a two-step column separation procedure.<sup>12,27</sup> The [<sup>3</sup>H]cAMP was eluted into scintillation vials and counted. Background levels (cAMP accumulation in the absence of forskolin) were subtracted from all values and represented less than 10% of forskolin-stimulated cAMP accumulation.

Statistical Analysis. Data were anlyzed using the Student's t-test. Inhibition curves were generated with the Sigma Plot 4.11 program, and the EC<sub>50</sub> values were determined using an equation from the ALLFIT program.<sup>30</sup>

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