Resorcinol Derivatives: A Novel Template for the Development of Cannabinoid CB1/CB2 and CB2-Selective Agonists

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ABSTRACT

The role of the oxygen of the benzopyran substituent of Δ9-tetrahydrocannabinol in defining affinity for brain cannabinoid (CB1) receptors is not well understood; however, it is known that opening the pyran ring can result in either increased potency and affinity, as in CP 55,940 ([(−)-cis-3-[2-hydroxy-4(1,1-dimethyl-heptyl)phenyl]-trans-4-(3-hydroxy-propyl)cyclohexanol], or in an inactive cannabinoid, as in cannabidiol. In the present study, a series of bicyclic resorcins that resemble cannabidiol were synthesized and tested in vitro and in vivo. Analysis of the structure-activity relationships of these analogs revealed several structural features that were important for maintaining CB1 receptor recognition and in vivo activity, including the presence of a branched lipophilic side chain and free phenols as well as substitution of a cyclohexane as the second ring of these bicyclic cannabinoids. Many of these analogs exhibited CB2 selectivity, particularly the dimethoxyresorcinol analogs, and this selectivity was enhanced by longer side chain lengths. Hence, unlike cannabidiol, these resorcinol derivatives had good affinity for CB1 and/or CB2 receptors as well as potent in vivo activity. These results suggest that the resorcinol series represent a novel template for the development of CB2-selective cannabinoid agonists that have the potential to offer insights into similarities and differences between structural requirements for receptor recognition at CB1 and CB2 receptors.

At least five distinct classes of cannabinoids have been identified: traditional tricyclic tetrahydrocannabinols [e.g., Δ9-tetrahydrocannabinol (THC)], synthetic bicyclic cannabinoids (e.g., CP 55,940; Little et al., 1988), aminoalkylindoles (e.g., WIN 55,212; D’Ambra et al., 1992), endocannabinoids (e.g., anandamide; Devane et al., 1992), and pyrazole antagonists (e.g., SR141716A; Rinaldi-Carmona et al., 1994). Although the chemical structures of these cannabinoids differ markedly, all of them contain at least one oxygen that is hypothesized to be involved in the binding of these drugs to brain cannabinoid (CB1) receptors. Δ9-THC, the primary psychoactive constituent of the marijuana plant, and other tetrahydrocannabinols contain two oxygens: a phenolic hydroxyl at position 1 and an oxygen in a pyran ring on the opposite side of the molecule (Fig. 1). The phenolic hydroxyl group at position 1 interacts with the CB1 receptor through hydrogen bonding with a lysine residue (Lys-192) (Song and Bonner, 1996). The role of the oxygen of the benzopyran substituent of Δ9-THC is less clear; however, it is known that opening the pyran ring (as in CP 55,940) does not eliminate binding or in vivo activity (Little et al., 1988). Furthermore, in the absence of a phenolic hydroxyl, as in 1-deoxy analogs of Δ9-THC, orientation of the cannabinoid molecule with respect to the CB1 receptor may be inverted, and the pyran oxygen may substitute as a substrate for hydrogen bonding with Lys 192 (Huffman et al., 1996. 1999).

In contrast to the high binding affinity of CP 55,940 and other similar pyran ring open analogs, the natural product cannabidiol is also a pyran ring open compound, but it does not bind to CB1 or CB2 receptors nor does it have a cannabinoid profile of effects in vivo. Even the 1’,1’-dimethylheptyl analog of cannabidiol binds very poorly to the CB1 receptor (R. K. Razdan, unpublished observations). This intriguing feature of cannabidiol prompted us to examine the structure-activity relationship of resorcinol derivatives, which could be considered as cannabidiol analogs.

After our work on the resorcinol series was initiated, Hanuš et al. (1999) published the synthesis and activity of HU-308, a dimethoxyresorcinol derivative that is a CB2-selective agonist. The transmembrane regions of CB2 receptors (areas involved in ligand recognition) exhibit 68% homology

ABBREVIATIONS: THC, tetrahydrocannabinol; CP 55,940, (−)-cis-3-[2-hydroxy-4(1,1-dimethyl-heptyl)phenyl]-trans-4-(3-hydroxy-propyl)cyclohexanol; MPE, maximal possible antinociceptive effect; CB1, brain cannabinoid; THF, tetrahydrofuran; TLC, thin layer chromatography; DMH, dimethylheptyl; DM, dimethyl.
with those of CB₁ receptors (Munro et al., 1993). Showalter et al. (1996) reported a high positive correlation ($r = 0.82$) between binding affinities at these two cannabinoid receptors for cannabinoids in various classes. Given these findings, it is not surprising that some of the structural features of the tetrahydrocannabinols that enhance affinity for CB₁ receptors also increase binding to CB₂ receptors. For example, addition of a 1',1'-dimethyl group to the lipophilic C₃ side chain of Δ⁹-THC results in higher affinities for both types of cannabinoid receptors compared with a nonbranched chain of identical length (Showalter et al., 1996). Several previous studies have explored the role of oxygen in CB₂ binding. Synthesis of a series of Δ⁹-THC analogs in which the phenolic hydroxyl at position 1 was removed (deoxy-Δ⁹-THC analogs) or replaced with a methoxyl resulted in analogs with selectivity for CB₂ receptors (Gareau et al., 1996; Huffman et al., 1996, 1999). Incorporation of an oxygen into a fourth ring attached at C1 also increased CB₂ selectivity, suggesting possible differences in the interaction of oxygen in the binding pockets of CB₁ and CB₂ receptors (Reggio et al., 1997). In the present study, we examined structure-activity relationships of a series of bicyclic resorcinols in which the core chemical structure contained two hydroxyl substituents positioned with a single intervening carbon on a benzene ring. For most of the bicyclic resorcinols presented here, the second cyclic substituent is attached at the intermediate carbon.

**Materials and Methods**

**Subjects.** Male Institute for Cancer Research (ICR) mice (25–32 g), obtained from Harlan (Indianapolis, IN), were housed in groups of five. All animals were kept in a temperature-controlled (20–22°C) environment with a 12-h light/dark cycle (lights on at 7 AM). Separate mice were used for testing each dose of each experimental compound in the in vivo behavioral procedures. Brain tissue for binding studies was obtained from male Sprague-Dawley rats (150–200 g) purchased from Harlan.

**Apparatus.** Measurement of spontaneous activity in mice occurred in standard activity chambers interfaced with a Digiscan animal activity monitor (Omnitech Electronics, Inc., Columbus, OH). A standard tail-flick apparatus and a digital thermometer (Fisher Scientific, Pittsburgh, PA) were used to measure antinociception and rectal temperature, respectively.

**Compounds.** Resorcinols were synthesized in our laboratories (Organix, Inc., Woburn, MA) according to the procedure specified below and were suspended in a vehicle of absolute ethanol, Emulphor-620 (Rhône-Poulenc, Inc., Princeton, NJ), and saline in a ratio of 1:1:18. Experimental compounds were administered to the mice i.v. in the tail vein at a volume of 0.1 ml/10 g.

Analogs O-1376 and O-1532 listed in Table 1 were synthesized as previously described (Mahadevan et al., 2000). Analog O-1601 was synthesized from 1-deoxy-9-carbomethoxy cannabinol dimethylheptyl analog (Mahadevan et al., 2000) by lithium/liquid ammonia reduction as described for the preparation of O-1376. The compounds listed in Tables 2 and 3 were prepared using a three-step sequence (Fig. 2). The 2-lithio derivative of 1,3-dimethoxy-5-(1'-dimethylheptyl)resorcinol was prepared using n-BuLi/hexane in THF (step 1). It was condensed with the appropriate ketone to give the tertiary alcohol (step 2), which upon treatment with trifluoroacetic acid/Et₃SiH gave the dimethoxy precursors (step 3). Demethylation with BBr₃/CH₂Cl₂ gave the target compounds (Crocker et al., 1999). The general procedure is illustrated in Fig. 2 and described below.

To a solution of the resorcinol (5 mmol) in 25 ml of dry THF was added a 2.5 M solution of n-BuLi in hexane (5.5 mmol) at 0°C with stirring in N₂. After additional stirring for 1 h at 0°C, a solution of the ketone (7.5 mmol) in 3 ml of dry THF was added all at once. The solution was stirred for 0.5 h at 0°C and then for 18 h at 23°C. The

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### TABLE 1

CB₁ and CB₂ binding affinities and pharmacological effects of phenols

<table>
<thead>
<tr>
<th>ID</th>
<th>R</th>
<th>R₁</th>
<th>$K_i$ (nM)</th>
<th>CB₁/CB₂</th>
<th>SA</th>
<th>ED₀.5</th>
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<td></td>
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</tr>
<tr>
<td>1</td>
<td>O-1376</td>
<td>CH₃</td>
<td>DMH</td>
<td>33 ± 4</td>
<td>3 ± 0.4</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>O-1532</td>
<td>CH₃</td>
<td>Dimethylbutyl</td>
<td>876 ± 18</td>
<td>113 ± 21</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>O-1601</td>
<td>CH₂OH</td>
<td>DMH</td>
<td>5 ± 0.6</td>
<td>3 ± 0.4</td>
<td>2</td>
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</table>

SA, suppression of spontaneous activity; RT, rectal temperature; TF, tail flick.
reaction was worked up by the addition of saturated NH₄Cl solution and extracted with ether. After washing (H₂O) and drying (Na₂SO₄), the solvent was evaporated to give the crude tertiary alcohol, which was used as such in the subsequent reaction. A solution of the tertiary alcohol (5 mmol) in 10 ml of dry CH₂Cl₂ was treated with CF₃COOH (27.5 mmol) followed by Et₃SiH (12.5 mmol). The solution was stirred in N₂ for 1 h more (followed by TLC) and then quenched by the addition of saturated NaHCO₃ solution. The organic layer was separated and after washing (H₂O) and drying gave the crude dimethoxy precursor of the target compound. This material was used as such for the demethylation step. Treatment of the dimethoxy precursor as a solution in dry CH₂Cl₂ at 0°C with three equivalents of 1NB Br₃ solution in CH₂Cl₂, using the standard procedure and workup, gave the crude target compound, which was purified by chromatography, generally using hexane/ethyl acetate mixtures. In the case of O-1662 (Table 2), the corresponding tertiary alcohol, upon treatment with CF₃COOH/Et₃SiH, gave the unsaturated compound (dehydrated but not reduced), which on catalytic reduction (PtO₂/C/H₂) in acetic acid gave the desired dimethoxy precursor. The final compound was purified by chromatography using a 5% Et₃NH₂/EtOAc mixture.

### Table 2

<table>
<thead>
<tr>
<th>ID</th>
<th>R</th>
<th>R1</th>
<th>R2</th>
<th>Kᵢ (nM)</th>
<th>CB₁/CB₂</th>
<th>ED₅₀</th>
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<td></td>
<td></td>
<td>CB₁</td>
<td>CB₂</td>
<td>SA</td>
</tr>
<tr>
<td>4</td>
<td>O-1424</td>
<td>DMH</td>
<td>H</td>
<td>95 ± 6</td>
<td>7 ± 0.4</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>O-1422</td>
<td>DMH</td>
<td>H</td>
<td>11 ± 2</td>
<td>1.5 ± 0.1</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>O-1656</td>
<td>DMH</td>
<td>H</td>
<td>18 ± 1</td>
<td>2 ± 0.2</td>
<td>9</td>
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<tr>
<td>7</td>
<td>O-1660</td>
<td>DMH</td>
<td>H</td>
<td>7 ± 1</td>
<td>3 ± 0.8</td>
<td>2</td>
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<tr>
<td>8</td>
<td>O-1425</td>
<td>DMH</td>
<td>H</td>
<td>153 ± 17</td>
<td>12 ± 2</td>
<td>13</td>
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<tr>
<td>9</td>
<td>O-1661</td>
<td>DMH</td>
<td>H</td>
<td>138 ± 4</td>
<td>28 ± 12</td>
<td>5</td>
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<tr>
<td>10</td>
<td>O-1662</td>
<td>DMH</td>
<td>H</td>
<td>&gt;10,000</td>
<td>5,424 ± 1,103</td>
<td>87% (30)</td>
</tr>
<tr>
<td>11</td>
<td>O-1423</td>
<td>DMH</td>
<td>H</td>
<td>97 ± 5</td>
<td>28 ± 5</td>
<td>3</td>
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<tr>
<td>12</td>
<td>O-2010</td>
<td>H</td>
<td>C₆H₁₃</td>
<td>9,515 ± 332</td>
<td>NT</td>
<td>−18% (30)</td>
</tr>
</tbody>
</table>

SA, suppression of spontaneous activity; RT, rectal temperature; TF, tail flick; NT, not tested.
compounds listed in Tables 4 and 5 were prepared (Fig. 2) from the 2-lithio derivative of 1,3-dimethoxy-5-((1H,1H,3H,7H)-dimethylheptyl)resorcinol and the appropriate ketones using BuLi, as in the preparation of the tertiary alcohol, and isolating and purifying the compounds by chromatography (ethyl acetate/hexane mixtures). Deprotection of O-2092 was carried out by treatment with 10% HCl in an ether/THF (5:4) mixture for 0.5 h at 23 °C to give a mixture of O-2115 (major) and the dehydrated compound O-2114 (minor). Sodium borohydride reduction of O-2115 furnished a mixture of diastereomeric compounds, which were separated by column chromatography on silica gel and eluting with hexane/ethyl acetate mixtures (5:1 to 3:1) to give the target compounds O-2116A and O-2117B. Separation of O-1966A and O-1967B from a diastereomeric mixture was undertaken similarly, by eluting with hexane followed by 99% hexane/1% ethyl acetate mixture. Epoxidation of O-2114 followed by NaBH₄ reduction gave the target compound O-2122. In the preparation of O-2090, the corresponding diethoxyresorcinol derivative of step 1 was used in place of the 2-lithio derivative of 1,3-dimethoxy-5-((1H,1H,3H,7H)-dimethylheptyl)resorcinol. All compounds showed appropriate 1H NMR profiles (Jeol Eclipse 300 MHz; Jeol USA, Inc., Peabody, MA) and were characterized on the basis of their 1H NMR profiles, TLC, and elemental analyses. The general profile of resorcinols (Tables 2 and 3) is illustrated by the 1H NMR profile of O-1797A: (CDCl₃) δ (H9254), 6.26 (s, 2H), 4.64 (s, 2H, D₂O exchangeable), 3.4 to 3.2 (m, 1H), 2.4 to 1.2 (m, 25H), 1.1 (d, J 5.9 Hz, 3H), and 0.86 (t, 3H). The dimethoxyresorcinols (Tables 4 and 5) showed an additional peak at δ, 3.85 region (s, 6H) for the methoxyl groups and the multiplet for the benzylic methine at δ, 3.4 to 3.2 was absent.

**Mouse Behavioral Procedures.** Prior to testing in the behavioral procedures, mice were acclimated to the experimental setting.
Binding was initiated by the addition of 150 μg of P2 membranes to test tubes containing [3H]CP 55,940 (final reaction concentration, 0.5 nM), an appropriate concentration of unlabelled CP 55,940 or test compound, and sufficient quantity of assay buffer (50 mM Tris-HCl, 1 mM EDTA, 3 mM MgCl₂, pH 7.4) to bring the total incubation volume to 0.5 ml. Nonspecific binding was determined by the addition of 1 μM unlabeled CP 55,940. Following incubation at 30°C for 1 h, binding was terminated by addition of ice-cold buffer and vacuum filtration through pretreated filters in a 12-Well sampling manifold (Millipore Corp., Bedford, MA). After washing, filters were placed into plastic scintillation vials (Packard Instrument Co., Inc., Downers Grove, IL) and shaken. The quantity of radioactivity present was determined by liquid scintillation spectrometry.

**CB₂ Binding Procedure.** Human CB₂ cDNA was provided by Dr. Sean Munro (MRC Laboratory of Molecular Biology, Cambridge, England) and was expressed in Chinese hamster ovary cells as previously described (Showalter et al., 1996). Briefly, transfected CB₂ Chinese hamster ovary cell lines were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) to maintain selective pressure of stable transformants and 10% fetal clone II (HyClone Laboratories, Inc., Logan, UT) plus 0.3 to 0.5 mg/ml G418 (to maintain selective pressure) under 5% CO₂ at 37°C. When confluent, cells were harvested with 1 mM EDTA in phosphate-buffered saline and centrifuged at 1000g for 5 min at 4°C. The supernatant was saved, and the P₁ pellet was resuspended in centrifugation buffer. Homogenization and centrifugation were repeated twice, and the combined supernatant fractions were centrifuged at 40,000g for 30 min at 4°C. The P₂ pellet was resuspended in centrifugation buffer (50 mM Tris HCl, 1 mM EDTA, and 3 mM MgCl₂, pH 7.4) to a protein concentration of approximately 2 mg/ml. The membrane preparation was quickly frozen in a bath solution of dry ice and 2-methylbutane (Sigma-Aldrich, St. Louis, MO), then stored at −80°C for no more than 2 weeks. Prior to performing a binding assay, an aliquot of frozen membrane was rapidly thawed, and protein values were determined by the method of Bradford (1976).

Binding was initiated by the addition of 150 μg of P2 membranes to test tubes containing 1 nM [3H]CP 55,940 (79 Ci/mmol) and a sufficient quantity of buffer to bring the total incubation volume to 1 ml. Nonspecific binding was determined by the addition of 1 μM unlabeled CP 55,940. Following incubation at 30°C for 1 h, binding was terminated by addition of ice-cold buffer and vacuum filtration through pretreated filters in a 12-Well sampling manifold (Millipore Corp., Bedford, MA). After washing, filters were placed into plastic scintillation vials (Packard Instrument Co., Inc., Downers Grove, IL) and shaken. The quantity of radioactivity present was determined by liquid scintillation spectrometry.
from 50 to 10,000 pM. Nonspecific binding was determined by the addition of 1 μM unlabeled CP 55,940. CP 55,940 and all cannabinoid analogs were prepared by suspension in assay buffer from 1 mg/ml ethanolic stock without evaporation of the ethanol (final concentration, no more than 0.4%). In competition studies, analog concentrations ranged from 0.1 nM to 10 μM. After incubation at 30°C for 1 h, binding was terminated by the addition of 2 ml of ice-cold wash buffer (50 mM Tris-HCl and 1 mg/ml bovine serum albumin) and vacuum filtration through pretreated filters in a 12-well sampling manifold (Millipore). Reaction vessels were washed once with 2 ml of ice-cold wash buffer. Filters were placed into 7-ml plastic scintillation vials (RPI Corp., Mount Prospect, IL) with 4 ml of Budget-Solve (RPI Corp.). After shaking for 30 min, the radioactivity present was determined by liquid scintillation spectrometry. Three reaction vessels were used for each drug concentration in each assay. The results represent the combined data of three independent experiments. All assays were performed in siliconized test tubes, which were prepared by air drying (12 h) inverted borosilicate tubes after two rinses with a 0.1% solution of AquaSil (Pierce Chemical, Rockford, IL). The GF/C glass-fiber filters (2.4 cm; Baxter, McGaw Park, IL) were pretreated in a 0.1% solution of pH 7.4 polyethylenimine (Sigma-Aldrich) for at least 6 h.

Data Analysis. Based on data obtained from numerous previous studies with cannabinoids, maximal cannabinoid effects in each procedure were estimated as follows: 90% inhibition of spontaneous activity, 100% MPE in the tail-flick procedure, and -6°C change in rectal temperature. ED50 was defined as the dose at which half-maximal effect occurred. For compounds that produced one or more cannabinoid effect, ED50 was calculated separately using least-squares linear regression on the linear part of the dose-effect curve for each measure in the mouse tetrad, plotted against log10 transformation of the dose. For the purposes of potency comparison, potencies were expressed as millimoles per kilogram.

Pearson product-moment correlation coefficients (with associated

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### Table 4

<table>
<thead>
<tr>
<th>ID</th>
<th>R</th>
<th>CH₂OH</th>
<th>Kᵢ (nM)</th>
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<th>CB₂</th>
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<td>22</td>
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<td></td>
<td>10,000</td>
<td>23 ± 4</td>
<td></td>
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<tr>
<td>23</td>
<td>O-1999</td>
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<td>10,000</td>
<td>466 ± 110</td>
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<tr>
<td>24</td>
<td>O-1964</td>
<td></td>
<td>10,000</td>
<td>911 ± 116</td>
<td></td>
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<tr>
<td>25</td>
<td>O-1965</td>
<td></td>
<td>10,000</td>
<td>10,000</td>
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<tr>
<td>26</td>
<td>O-1962</td>
<td></td>
<td>10,000</td>
<td>342 ± 22</td>
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<td>27</td>
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<td>4,581 ± 312</td>
<td>126 ± 12</td>
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<td>O-2122</td>
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<td>3,758 ± 184</td>
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<td>29</td>
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<td>8,442 ± 954</td>
<td>1,773 ± 184</td>
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<td>1,731 ± 117</td>
<td>125 ± 14</td>
<td>14</td>
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* Values from Hanus et al., 1999. Note: binding ligand, [³H]HU-243, was different from that used in present study.
significance tests) were calculated between CB₁ binding affinity (expressed as log $K_i$) and in vivo potency for each measure (expressed as log ED₅₀ in millimoles per kilogram) for all active cannabinoid compounds that bound to the CB₁ receptor. The Pearson product-moment correlation provided a measure of the strength and direction of relationship between each pair of quantitative variables. In addition, multiple linear regression was used to calculate the overall degree of relationship between CB₁ binding affinity and potency in the mouse measures for all active cannabinoids. A correlation between CB₁ and CB₂ binding affinities was calculated for all compounds that had a measurable $K_i$ for CB₁ and CB₂ binding ($K_i < 10,000$ nM). $K_i$ values for CB₁ and CB₂ binding were obtained from the Scatchard displacement analysis program of the KELL software package (Biosoft, Milltown, NJ).

### Results

The CB₁ and CB₂ binding affinities for substituted biphenyl analogs are shown in Table 1. These compounds contain a phenolic hydroxyl and lipophilic side chain in the same orientation as in cannabinol. In addition, the pyran oxygen is absent, and the analogs have substituents in the phenyl ring (ring C) of cannabinol. Two of the analogs (O-1376 and O-1601) have a dimethylheptyl side chain; each possess good CB₁ and CB₂ binding affinities and in vivo activity. O-1601, the more potent of the two active compounds, had a hydroxymethyl group in the phenyl ring. This substitution increased CB₁ affinity and in vivo potencies compared with O-1376 but did not affect affinity for CB₂ receptors. A similar effect was observed in the cannabinol series, where the substitution of a hydroxymethyl group in the phenyl ring. This substitution increased CB₁ affinity and in vivo potencies compared with O-1376 but did not affect affinity for CB₂ receptors. A similar effect was observed in the cannabinol series, where the substitution of a hydroxymethyl group in the phenyl ring.
cyclic ring-substituted-5-dimethylheptyl resorcinols. Manipulation of the size of the cyclic structure attached at position 2 of the resorcinol ring resulted in changes in binding affinities and potencies. Substitution of a cyclopentane ring (O-1424) resulted in moderate affinity for the CB1 receptor, with excellent affinity for the CB2 receptor. Although this compound was active in all three in vivo assays, potency was relatively poor. In addition, affinities across the measures were not equal; i.e., potency for reducing spontaneous activity was approximately half that for producing antinociceptive and hypothermic effects. Increasing ring size to a cyclohexane (O-1422), cycloheptane (O-1656), or adamantyl (O-1660) improved affinity 5- to 14-fold for both cannabinoid receptors and greatly increased potencies in vivo. Substitution of a sulfur for a carbon in a cyclohexane ring (O-1425) decreased CB1 affinity by 14-fold and CB2 affinity by 8-fold (compared with O-1422) as well as reducing in vivo potencies. Similarly, sulfur substitution in a cyclopentane ring (O-1661) also attenuated binding to both cannabinoid receptors. When a methylated nitrogen (O-1662) was inserted into the cyclohexane ring in the same position as the sulfur of O-1425, binding to CB1 receptors did not occur. In addition, CB2 binding was drastically decreased, and the compound was not fully active in vivo. In contrast, placing a double bond in the cyclohexane ring (O-1423) decreased affinities and potencies, but the compound remained active. However, moving the lipophilic side chain of O-1422 from C-5 to C-4 and replacing the dimethylheptyl with an n-hexyl chain (O-2010) produced a 865-fold decrease in CB1 affinity and a loss of activity in vivo.

Table 3 shows results of tests with cyclohexane-substituted resorcinols in which the position of the substituent at the cyclohexane ring attached to the core resorcinol was varied. All compounds were diastereomeric mixtures. All of these analogs had high ($K_i = 2 \text{ nM}$) to moderate ($K_i = 144 \text{ nM}$) affinity for CB1 receptors and were CB2-selective ($K_i$ range = 0.3–13 nM). Methylation at the 2-position of the cyclohexane ring (O-1658) did not dramatically alter affinity for either cannabinoid receptor or in vivo potencies compared with the corresponding cannabinoid with a nonmethylated cyclohexane (O-1422 in Table 2). Moving the methyl to position 4 of the cyclohexane ring (O-1659) decreased affinity for both cannabinoid receptors by about 5-fold and produced an even greater decrease (11- to 24-fold) in potencies in vivo. Substituting a phenyl group for the methyl at this same position (O-1663) resulted in 2- to 3-fold decreases in CB2 and CB1 affinities, respectively, and a loss of activity in vivo. In the next five analogs shown in Table 3, the methyl was attached at position 3 of the cyclohexane ring. O-1657 exhibited CB1 and CB2 affinities that were similar to those of O-1658; however, the profiles of in vivo potencies differed. Whereas the two analogs showed approximately equal potencies in suppressing spontaneous activity, O-1658 was twice as potent in producing antinociception and three times as potent in reducing body temperature. As described under Materials and Methods, compound O-1657 was separated into two distinct entities, which were designated O-1797A and O-1798B. These analogs were still mixtures. Affinities of O-1797A and O-1798B were two to three times greater than those of O-1657. Although potencies of these isomers for suppression of locomotor activity and hypothermia were not notably different from those of O-1657, antinociceptive potencies were reduced by about half. The 3S isomer of this series (O-1826) showed decreased affinity for CB1 receptors compared with O-1657; however, affinity for CB2 receptors was identical for both compounds. Not surprisingly given its decreased CB1 affinity, O-1826 was less potent than O-1657 in vivo. Substitution of a dimethylbutyl for the dimethylheptyl side chain at C5 of the resorcinol component (O-1890) decreased affinities for both cannabinoid receptors. This compound was active in vivo, although potency was notably low for all measures. In contrast, addition of a gem-dimethyl group at the 3-position of the cyclohexane ring, with retention of the dimethylheptyl side chain of the resorcinol component (O-1871), resulted in the best CB1 and CB2 affinities of this series. Given its higher CB1 binding affinity, in vivo potencies for this compound were lower than expected, although the lack of pharmacokinetics assessments tempers this conclusion somewhat.

To develop CB2-selective ligands, we examined cyclic ring-substituted dimethoxyresorcinols. The CB1 and CB2 binding affinities of these analogs are shown in Tables 4 and 5. Although most of the compounds shown in Tables 4 and 5 possessed a dimethylheptyl side chain, all had poor CB1 affinity; hence, they were not tested in vivo. The bicyclic structure of O-1999 (Table 4) was almost identical to that of O-1657 (Table 3), an analog with good CB1 and CB2 affinities and potent in vivo effects. Both compounds had a dimethylheptyl side chain attached to the 5-position of a resorcinol core that was attached at position 2 to a cyclohexane ring. Each compound had a methyl group at the 3-position of the cyclohexane ring. The major structural difference between the two compounds was that O-1999 was a dimethoxy derivative of the resorcinol O-1657. This structural change from a phenol to a methoxy derivative resulted in complete loss of affinity for CB1 receptors and an almost 600-fold reduction in affinity for CB2 receptors. Similarly, the other analogs that were dimethoxy derivatives of the corresponding resorcinols had poor affinity for CB1 receptors ($K_i$ ranged from 1716 to $>10,000$) regardless of the cyclic ring substitution at position 2. In contrast, CB2 binding affinities for some of these analogs remained high, as described in more detail below.

Table 4 presents binding data for two cyclic ring-substituted dimethoxy-resorcinol-dimethylheptyl analogs that contain at least one oxygen inserted into or attached to the nonresorcinol cyclohexane ring. Compared with O-1999, which did not contain an oxygen in the cyclohexane ring, conversion of the cyclohexane ring to a pyran ring (O-1964) decreased CB2 affinity almost 2-fold without effect on CB1 binding. Further addition of a double bond at position 3 of the pyran ring resulted in O-1965, which did not bind to either cannabinoid receptor. In contrast, the introduction of a tertiary hydroxyl group at C-4 of the pyran ring (O-1962) increased CB2 affinity by 3-fold. Adding additional oxygens, such as a ketol group attached at C-4 to the point of attachment of the dimethoxyresorcinol substituent (O-2092), also increased CB2 affinity whereas adding an oxygen as an epoxide (O-2122) decreased it. The presence of a ketone group at C-4 of the cyclohexane ring and having unsaturation in the ring (O-2114) resulted in a compound with poor affinity for either cannabinoid receptor; however, if a tertiary hydroxyl group was added at the site of dimethoxyresorcinol attachment (O-2115), CB2 affinity improved. Retention of the tertiary hydroxyl, methylation at position 5, and the presence of a ketone at position 3 of the cyclohexane ring increased...
affinity for both receptors and resulted in a compound (O-2123) with the best CB₂ affinity ($K_i = 125 \text{ nM}$) in this series.

Table 5 shows CB₁ and CB₂ affinities for two cyclic ring-substituted dimethoxy-resorcinol-dimethylheptyl analogs in which the ring size and the position of the methyl or hydroxyl substituent on the cyclohexane ring are varied. The first analog (O-2072) contains one hydroxyl attached to the cyclohexane at the same position at which the resorcinol core is attached. This compound is CB₂-selective. Although it had poor affinity for CB₁ receptors, it bound with moderate affinity to CB₂ receptors. Introduction of a methyl substituent in the 3-position of the cyclohexane ring gave a diastereomeric mixture from which two distinct entities were separated by careful chromatography. These analogs (O-1966A and O-1967B) were still mixtures. This substitution resulted in a 5-fold increase in affinity for CB₂ receptors with continued poor affinity for CB₁ receptors. However, one of these isomers (O-1966A) showed the best CB₂ selectivity (225-fold) in the series and had high binding affinity for the CB₂ receptor ($K_i = 22.5 \text{ nM}$). Addition of an extra hydroxyl group to the cyclohexane ring (O-2121) reduced both selectivity and binding affinity for the CB₂ receptor comparable with those obtained with O-1967B. Removal of the methyl at position 3 and addition of a hydroxyl at position 4 resulted in two diastereomeric mixtures that could be separated, which were designated as O-2116A and O-2117B. Both of these isomers had poor affinity for CB₁ receptors, but although the B isomer also had poor affinity for CB₂ receptors, the A isomer bound to CB₂ receptors with moderate affinity. Attachment of a gem-dimethyl group to position 3 of O-2072 (i.e., O-2068B) did not significantly alter affinities for CB₁ or CB₂ receptors; however, replacement of the dimethylheptyl group of O-2068 with a methyl group (O-2139) produced loss of affinity at both receptors. Changing the dimethoxy groups of the resorcinol by adding diethoxy groups (O-2090) drastically decreased affinities for CB₁ and CB₂ receptors (compare O-2090 with O-1966A or O-1967B). Enlarging the cyclohexane ring in O-2072 to a cycloheptane ring (O-2091) resulted in little change in affinity for CB₁ receptors and an almost 2-fold increase in CB₂ affinity.

Multiple regression analysis of binding affinity ($Y = \log K_i$) and potency for each mouse measure ($X_{1-3} = \log ED_{50} \text{ in mmol/kg}$) confirmed that overall potency at producing the characteristic profile of cannabinoid effects was significantly correlated with binding affinity at CB₁ receptors ($r = 0.78$; $F(3,13) = 6.9$; $p = 0.005$) for all active cannabinoids. Individual correlations between $\log K_i$ and log potency for each measure were 0.78, 0.74, and 0.75 for hypomobility, antinociception, and hypothermia, respectively ($p < 0.05$ for all three correlations). Furthermore, CB₁ binding affinity was highly correlated with CB₂ binding affinity ($r = 0.92$, $p < 0.05$) for all compounds for which both binding affinities could be calculated (i.e., $K_i < 10,000$). Scatterplots for each regression line are presented in Fig. 3.

**Discussion**

The lack of CB₁ binding affinity of cannabidiol compared with other pyran ring open analogs such as CP 55,940 prompted us to examine the structure-activity relationships of resorcinol derivatives for cannabinoid activity. Our results show that many of the structural changes that affect CB₁ receptor recognition and activation in traditional cannabinoids similarly alter binding and activity in this resorcinol series. Previous research has shown that the length and branching of a lipophilic substituent is important for CB₁ receptor recognition in all of the major cannabinoid agonist classes, including tetrahydrocannabinols and bicyclic cannabinoids (Compton et al., 1993), indole-derived cannabinoids (Wiley et al., 1998), and anandamides (Ryan et al., 1997; Seltzman et al., 1997). In the tricyclic and bicyclic series, a 1',1'-dimethylheptyl side chain is optimal (Compton et al., 1993) and is contained in most of the resorcinols presented here. Reducing the length of this substituent resulted in a concomitant elimination or decrease in CB₁ receptor recognition, as occurs in other cannabinoid series with similar structural manipulations (see references above).

Other structural features affecting CB₁ receptor recognition and activation in this series are related to the size, saturation, substitution, and methylation of the second nonresorcinol ring. In most tricyclic and bicyclic cannabinoids, the ring corresponding to the nonresorcinol ring in the current series is a cyclohexane. In the resorcinol series, reducing this size to a cyclopentane decreases CB₁ affinity and potency whereas increasing it to a cycloheptane has little effect. Similar modifications of other cannabinoids have not been reported; however, degree of saturation of, as well as the position of the double bond in the cyclohexane ring of tricyclic and bicyclic cannabinoids and in the polyolefin loop of the anandamides, has been shown to affect CB₁ receptor recognition and activity. In the resorcinol series, introduction of a single double bond (O-1423) within the ring decreased CB₁ affinity and potency to the same extent as did a reduction in the size of the ring to a cyclopentane. Greatest affinity and potency within the anandamides is achieved with four double bonds, with greater or lesser saturation resulting in a reduction in CB₁ binding and/or in vivo activity (Adams et al., 1995;
Thomas et al., 1996; Sheskin et al., 1997). Similarly, the number and position of double bonds within the cyclohexane ring of tetrahydrocannabinols and bicyclic cannabinoids affect activity. For example, moving the double bond of Δ⁸-THC to position 8 (as in Δ⁸-THC) decreases CB₁ affinity 3-fold and somewhat reduces potency (Compton et al., 1993). Unsaturation of the cyclohexane ring results in cannabinol with its greatly reduced CB₁ affinity (Showalter et al., 1996). In contrast, CP 55,940, with a completely saturated cyclohexane ring, is several fold more potent than Δ⁸-THC-dimethylheptyl, which has a single double bond in the cyclohexane ring; but Δ⁸-THC, with its single double bond, binds with better CB₁ affinity than does Δ⁸₁₁-THC, which has a completely saturated cyclohexane ring (Compton et al., 1993).

The most remarkable structural features of the resorcinol series affecting CB₁ affinity, however, are the length of the lipophilic side chain at position 5 and the size of the cyclic ring substituent at position 2 of the resorcinol core. Δ⁸-THC and CP 55,940 contain two oxygens: one as a phenol (one hydroxyl in the aromatic ring) with a second oxygen incorporated into a separate ring (pyran oxygen in Δ⁸-THC) or a hydroxyl group attached as a substituent in the cyclohexane ring, as in CP 55,940. Previous research has shown that eliminating the phenolic hydroxyl of Δ⁸-THC results in deoxy-Δ⁸-THC analogs that are CB₂-selective (Huffman et al., 1999). Although some of these analogs also retain reasonable affinity for CB₁ receptors, orientation of their binding to CB₁ receptors may be inverted such that the pyran oxygen substitutes for the absent phenolic hydroxyl in hydrogen bonding (Huffman et al., 1996). In the absence of a pyran oxygen, the nature of the substituent at position 2 of the resorcinol core is important for maintenance of in vivo activity. An acyclic ring was found to be better than a heterocyclic ring, with a cyclohexane ring being optimal. In addition, the size and the position of the substituent on the cyclic ring is important to maintenance of CB₁ affinity. The presence of a methyl substituent at position 3 enhanced activity in some cases. Furthermore, the 3S analog (O-1826; Table 2) has a poorer CB₁ binding affinity (Kᵢ = 40 nM) compared with the diastereomeric mixture O-1657 (Kᵢ = 14 nM; Table 2), suggesting that CB₁ binding affinity is enhanced when the orientation of the methyl substituent at position 3 in the cyclohexane ring is 3R compared with 3S. Methylation of the phenols of the resorcinols drastically decreased or eliminated CB₁ affinity, perhaps because hydrogen donation is less likely from a methoxy group than from the free hydroxyl group of Δ⁸-THC (B. R. Martin, unpublished observations). Similarly, methoxy substitution for the phenolic hydroxyl in the methyl esters of Δ⁸- and Δ⁸₁₁-THC-dimethylheptyl resulted in analogs that were CB₂-selective and had little CB₁ affinity (Gareau et al., 1996; Huffman et al., 1999; Ross et al., 1999).

Notably, most of the dimethoxyresorcinols tested here were CB₂-selective. As suggested by the high positive correlation between CB₁ and CB₂ binding affinities, most of the structural features that affected recognition at CB₁ receptors also affected CB₂ receptor recognition, although not always to the same degree or in the same manner. These factors included length and branching of the side chain and size and degree of saturation of the nonresorcinol cyclohexane ring. In a structure-activity relationship study on a series of CB₂-selective deoxy-Δ⁸-THC analogs, Huffman et al. (1999) reported that length and branching of the C3 side chain affected CB₂ binding in a manner similar to its effect on CB₁ affinity, as it did in the present study; however, the range of chain lengths for which moderate to good CB₂ affinity was retained for the deoxy-Δ⁸-THC analogs was greater than the range for CB₁ affinity. Similar results were obtained with a series of CB₂-selective indole-derived cannabinoids in which length of the nitrogen substituent was varied (Aung et al., 2000). To date, anandamide analogs appear to be CB₁ selective, with relatively little affinity for CB₂ receptors across several types of manipulations (Showalter et al., 1996). Insufficient research is available to determine the effect of substitution on a cyclohexane ring on CB₂ affinity across cannabinoid classes.

Other structural manipulations that eliminated or drastically reduced CB₁ receptor recognition did not necessarily alter CB₂ receptor binding in an identical manner. CB₂ selectivity was most evident in the dimethoxy analogs, primarily as a consequence of severe reductions in CB₁ affinity. HU-308, the most selective CB₂ agonist to date, has a dimethoxyresorcinol core structure and does not bind to CB₁ receptors at all (Hanuš et al., 1999). In addition, greater tolerance in CB₂ (versus CB₁) receptor recognition was observed with other C2 substitutions in the resorcinols. Huffman et al. (2001) recently reported that bicyclic pyridone analogs with carbonyl substitution at C1 and a nitrogen substituent substitution at C2 of Δ⁸-THC had little affinity for CB₁ receptors. In contrast, moderate CB₂ affinity (Kᵢ ~ 53 nM) was retained. Differences in allosteric regulation of CB₁ and CB₂ receptors by ions and guanine nucleotides have been noted previously (Showalter et al., 1996). Together, the results presented here and elsewhere (see above) suggest incomplete overlap of the pharmacophores for CB₁ and CB₂ receptors.

In summary, structure-activity relationships of the resorcinol series presented here are consistent with the CB₁ and CB₂ pharmacophores of other cannabinoid classes. In this series of resorcinols, several structural features were essential for maintenance of CB₂ receptor recognition and in vivo activity, including the presence of a branched lipophilic side chain at C5, the presence of free phenols, and substitution of a cyclohexane ring at C2. An important structural feature for receptor recognition at CB₂ receptors was side chain length. The CB₂ selectivity observed with some resorcinols was maximized in the dimethoxyresorcinol analogs, and this selectivity was greatly enhanced when a tertiary hydroxyl group was present in the cyclohexane ring in the same position at which the resorcinol core is attached. In contrast, the presence of unsaturation, a ketone group, or an additional hydroxyl substitution in the cyclohexane ring adversely affected the CB₂ selectivity. Methyl ethers were optimal for CB₂ selectivity because ethyl ethers reduced selectivity.

In conclusion, although resorcinol derivatives with cyclic ring substituents at C2 are closely related to the nonactive cannabinoid cannabidiol, many of these analogs have high CB₁ and/or CB₂ binding affinity as well as potent in vivo activity. In addition, because dimethoxyresorcinols are CB₂-selective, they have potential to offer insight into similarities and differences between requirements for receptor recognition at CB₁ versus CB₂ receptors. The results presented here suggest that the resorcinol series represent a novel template for the development of CB₁- and CB₂-selective cannabinoid agonists.
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