

# Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB<sub>1</sub> and CB<sub>2</sub> receptor binding

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Received 10 August 1999; received in revised form 16 November 1999; accepted 18 November 1999

## Abstract

The N-1 alkyl side chain of the aminoalkylindole analogues (AAI) has been implicated as one of a three-point interaction with the cannabinoid CB<sub>1</sub> receptor. In this study, the morpholinoethyl of WIN 55,212-2 was replaced with carbon chains of varying lengths ranging from a methyl to heptyl group. Additional groups were added to the naphthoyl and the C2 positions of the molecule. These structural changes revealed that high affinity binding to the CB<sub>1</sub> and CB<sub>2</sub> receptors requires an alkyl chain length of at least three carbons with optimum binding to both receptors occurring with a five carbon side chain. An alkyl chain of 3–6 carbons is sufficient for high affinity binding; however, extension of the chain to a heptyl group results in a dramatic decrease in binding at both receptors. The unique structure of the cannabimimetic indoles provides a useful tool to define the ligand-receptor interaction at both cannabinoid receptors and to refine proposed pharmacophore models. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Cannabinoid; Cannabinoid receptors; Radioligand binding; Affinity; Cannabimimetic indoles; Aminoalkylindoles

## 1. Introduction

In the 35 years since the isolation and elucidation of the structure of  $\Delta^9$ -THC (Gaoni and Mechoulam, 1964) considerable effort has gone into modifying the structure of cannabinoids as well as in developing compounds structurally diverse from the classical tricyclic structures. Several nontraditional cannabinoids have been discovered, including a series of 3-arylcyclohexanols such as CP-55 940, analogues of the endogenous ligand anandamide, and various aminoalkylindole (AAI) compounds (D'Ambra et al., 1992; Melvin et al., 1995; for review see Martin et al., 1995; Khanolkar and Makriyannis, 1999). According to the three-point interaction receptor model (Fig. 1), the hydroxyl group at C-1, the lipophilic side chain at C-3 and the orientation

of the C-9 substituent (Edery et al., 1971; Binder and Franke, 1982; Razdan, 1986; Thomas et al., 1991) are essential to the bioactivity of  $\Delta^9$ -THC. For purposes of aligning the prototypical AAI, WIN 55,212-2, and  $\Delta^9$ -THC in a common pharmacophore, it has been proposed that the corresponding overlapping regions of the two respective molecules are the naphthyl ring and the cyclohexene ring, the carbonyl oxygen and the phenolic hydroxyl, and the morpholine unit and the C-3 pentyl side chain as depicted in Fig. 1 (Huffman et al., 1994).

Although WIN 55,212-2, related AAIs and other indole-derived compounds bear no obvious structural similarities to traditional cannabinoids, they have been shown to bind to brain cannabinoid receptors (CB<sub>1</sub>). The AAIs have been shown to produce a profile of behavioral effects characteristic of those observed with  $\Delta^9$ -THC and other classical and bicyclic cannabinoids that include antinociception, ring immobility, suppression of spontaneous activity, and hypothermia in mice

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(Compton et al., 1992). These pharmacological effects of  $\Delta^9$ -THC and WIN 55,212-2 are blocked by the CB<sub>1</sub> receptor antagonist, SR 141716A (Rinaldi-Carmona et al., 1994; Perio et al., 1996). Cannabimimetic indoles are also associated with potent activity at inhibiting electrically induced contractions of the mouse vas deferens (Pertwee et al., 1995). In autoradiographic studies, the distribution of AAI binding sites was similar to that reported for classically identified cannabinoid binding sites (Jansen et al., 1992).

The cDNA corresponding to the central (CB<sub>1</sub>) receptor was cloned, isolated, and identified as a member of the G-protein linked super family of receptors (Matsuda et al., 1990). In vitro labeling of sections of the adult human brain with [<sup>3</sup>H] CP-55 940, a high affinity ligand, followed by quantitative receptor autoradiography revealed a heterogenous distribution of cannabinoid receptors throughout the brain (Herkenham et al., 1990; Devane et al., 1992). The cloning and isolation of the CB<sub>2</sub> receptor from cDNA of the human promyelocytic leukemic HL60 cells by Munro et al. (1993) was followed by the determination of the distribution of these receptors in the cells of the immune system. The protein product from this clone showed 44% amino acid identity to the human CB<sub>1</sub> receptor with the degree of identity rising to 68% for the transmembrane regions thought to be involved in ligand and specificity (Munro et al., 1993). Transfection of the cDNA expression vector into Chinese hamster ovary cells and consequent binding assays allows the determination of ligands which are selective for CB<sub>1</sub> or CB<sub>2</sub> receptors (Munro et al., 1993; Huffman et al., 1996; Showalter et al., 1996).

The discovery that cannabinoids exert their pharmacological actions via at least two types of receptors, along with the discovery of endogenous cannabinoid ligands, prompted the development of a new generation of cannabimimetic analogues for probing differences in the pharmacophore of these receptor subtypes. These analogues are essential in elucidating the physiological and pharmacological role of cannabinoid receptors as well as the different structural features necessary for binding to either the CB<sub>1</sub> or the CB<sub>2</sub> receptor. In previous studies, we demonstrated that elimination of the oxygen bridge and aminoalkyl groups in WIN 55,212-2 resulted in indoles that were equally effective as cannabinoid agonists (Huffman et al., 1994; Pertwee et al., 1995; Wiley et al., 1998). The objective of the present investigation was to characterize the consequences of selected structural alterations at key positions of indoles and to determine whether receptor subtype selectivity could be achieved.

## 2. Methods

### 2.1. Drugs

$\Delta^9$ -THC was obtained from the National Institute on Drug Abuse. All indole-derived compounds were synthesized in the laboratory of Dr John Huffman (Clemson University, Clemson, SC). [<sup>3</sup>H] CP-55 940 was purchased from Dupont-NEN (Wilmington, DE).

### 2.2. Cell culture

Human CB<sub>2</sub> cDNA was provided by Dr Sean Munro, (MRC Lab, Cambridge, UK). The human CB<sub>2</sub> cDNA was expressed in chinese hamster ovary (CHO) cells as previously described (Showalter et al., 1996). Briefly, transfected CB<sub>2</sub> CHO cell lines were maintained in Dulbecco's modified Eagle's Media (GIBCO BRL, Grand Island, NY) to maintain selective pressure of stable transformants and 10% fetal clone II (Hyclone Laboratories, Inc., Logan, UT) under 5% CO<sub>2</sub> at 37°C. The cells were then harvested when confluent.

### 2.3. Membrane preparation and binding

Transfected CB<sub>2</sub> CHO cells were grown to confluence in 125 cm<sup>3</sup> flasks and harvested in 1 mM EDTA in phosphate-buffered saline. The cells were then centrifuged at 1500 × g for 5 min at room temperature and resuspended in centrifugation Solution 1 (320 mM sucrose, 2 mM EDTA, 50 mM Tris-HCl, 5 mM MgCl<sub>2</sub>, pH 7.4). Homogenization using a Kontes Potter-Elvehjem glass-Teflon grinding system (Fisher Scientific, Springfield, NJ) was done to disrupt the cell membranes. Subsequent centrifugation at 3500 × g for 10

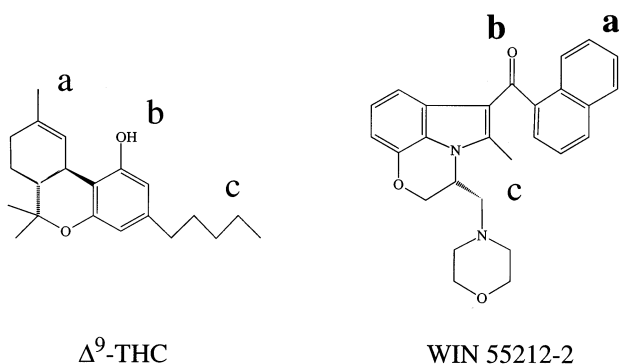


Fig. 1. Chemical structures of  $\Delta^9$ -THC and WIN 55,212-2 showing the proposed overlapping moieties involved with receptor interaction denoted A, B and C. (A) Corresponds to the cyclohexene ring and the naphthalene ring of  $\Delta^9$ -THC and WIN 55,212-2, respectively, and reflect an area of steric requirement for receptor interaction. (B) Denotes the phenolic hydroxyl ( $\Delta^9$ -THC) and carbonyl moiety (WIN 55,212-2) and represents an area thought to be involved in hydrogen bonding between the ligand and the receptor. (C) Shows the C3 alkyl side chain of  $\Delta^9$ -THC and the morpholinoethyl group of WIN 55,212-2 which are thought to be areas of hydrophobic interaction with the receptor.

min produced a supernatant which was saved and a pellet which was resuspended in centrifugation Solution 1. Homogenization and centrifugation was repeated twice and the combined supernatant fractions were centrifuged at  $48\,000 \times g$  for 1 h at  $4^{\circ}\text{C}$ . The final pellet, membrane protein (P2), was resuspended in centrifugation Solution 3 (50 mM Tris-HCl, 1 mM EDTA, 3 mM  $\text{MgCl}_2$ , pH 7.4). The protein concentration was determined by the method of Bradford (1976) using protein assay solution (Bio-Rad, Richmond, CA). The membrane preparation was divided into portions sufficient for a single binding assay and stored at  $-80^{\circ}\text{C}$ .

$\text{CB}_1$  receptor membranes were obtained directly from the isolated cortices of decapitated Sprague-Dawley rats. The tissue was homogenized in centrifugation Buffer 1 (320 mM sucrose, 2 mM EDTA, 5 mM  $\text{MgCl}_2$ ) and subsequent centrifugation at a speed of  $3650 \times g$  for 10 min. The resulting pellet was resuspended in 30 ml of Buffer 1 and the supernatant saved. Centrifugation and homogenization were repeated and the supernatant fractions combined. The combined supernatant fractions were then centrifuged at 18 000 rpm for 15 min. and the P2 pellet resuspended in Buffer Solution 2 (50 mM Tris-HCl, 2 mM EDTA, 5 mM  $\text{MgCl}_2$ , pH 7.4). Subsequent centrifugation (at  $13\,900 \times g$  and  $9600 \times g$ , respectively) and resuspension in Buffer Solution 3 (as above) produced the final P2 pellet for binding. The protein concentration was determined by the method of Bradford (1976) similar to that used with the  $\text{CB}_2$  receptor, and the tissue was frozen over dry ice in three equal aliquots and stored at  $-80^{\circ}\text{C}$ .

Binding was initiated by the addition of membrane (50  $\mu\text{g}$  of the  $\text{CB}_2$  cell line and 35  $\mu\text{g}$  of  $\text{CB}_1$  containing cortices) to siliconized test tubes containing [ $^3\text{H}$ ] CP-55 940 in assay Buffer A (50 mM Tris-HCl, 1 mM EDTA, 3 mM  $\text{MgCl}_2$ , 5 mg/ml BSA, pH 7.4). The test tubes were siliconized by rinsing twice with a 0.1% solution of Aquasil (Pierce, Rockford, IL), inverted and air-dried for approximately 12 h in order to prevent cannabinoids from adsorbing to the sides of the tubes. Triplicates for each drug concentration, for labeled [ $^3\text{H}$ ] CP-55 940, and for unlabeled CP-55 940 were used. A sufficient amount of Buffer A was used to bring the incubation volume to 0.5 ml and the concentration of [ $^3\text{H}$ ] CP-55 940 to 1 nM. Nonspecific binding was determined by the average of triplicates containing 10  $\mu\text{M}$  of unlabeled CP-55 940. As with all the cannabinoid analogues tested, unlabeled CP-55 940 was prepared by dissolution in ethanol to obtain a 1 mg/ml stock of the compound and subsequent dilution in Buffer A to the desired concentration. Displacement studies used cannabinoid analogue concentrations ranging from 0.1 nM to 10  $\mu\text{M}$ . After binding was initiated by addition of P2 membrane (homogenized with a

Kontes Ponter-Elvehjem glass-Teflon grinding system), the reaction vessel was incubated for 1 h at  $30^{\circ}\text{C}$ . Binding was terminated by the addition of 2 ml ice cold wash Buffer B (50 mM Tris-HCl, 1 mg/ml BSA, pH 7.4) to each tube followed by vacuum filtration through pretreated filters in a 12-well sampling manifold (Millipore, Bedford, MA). The GF/C glass-fiber filters (2.4 cm., Baxter, McGaw Park, IL) were pretreated in a 0.1% solution of polyethylenimine at a pH 7.4 (Sigma Chemical Co.) for at least 3 h in order to reduce non-specific binding. Each reaction vessel was washed with an additional 2 ml of ice-cold wash Buffer B and the filters washed with 4 ml of the ice-cold wash buffer. The filters were placed in 7 ml scintillation vials (RPI Corp., Mount Prospect, IL) containing 5 ml Budget-Solve (RPI Corp.). After shaking for 1 h the quantity of radioactivity present was determined by liquid scintillation spectrometry.

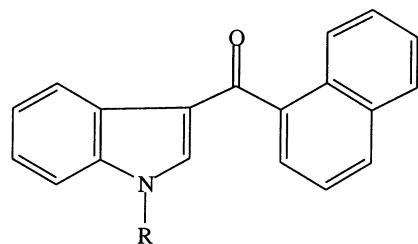
#### 2.4. Data analysis

The resulting  $\text{IC}_{50}$  and  $K_i$  values for each competition study represented the combined data of at least three experiments. Scatchard analysis was used to calculate the  $K_D$  and  $B_{\text{max}}$  values for the  $\text{CB}_2$  and  $\text{CB}_1$  receptors and analyzed using the Kell package of binding analysis programs for the Macintosh computer (Biosoft, Milltown, NJ). Using unweighted least-squared non-linear regression of log concentration-percent displacement data  $\text{IC}_{50}$  values were obtained. The  $\text{IC}_{50}$  values were then converted to  $K_i$  values using the method of Cheng and Prusoff (1973). The  $K_i$  values obtained were then used to compare the selectivity and affinity of a ligand for either receptor.

### 3. Results

The first series of indoles differ from WIN 55,212-2 by the lack of a methyl group at C-2 and the substitution of the N-methylmorpholino by a N-alkyl side chains of varying length (Table 1). Binding to both receptors was very weak with N-alkyl side chain lengths of either one or two carbon atoms. Increasing the carbon chain length to a propyl (JWH-072) had little influence on  $\text{CB}_1$  binding but affinity at  $\text{CB}_2$  increased almost 15-fold. Optimal binding at the  $\text{CB}_1$  receptor was observed with butyl, pentyl and heptyl side chains, whereas optimal  $\text{CB}_2$  binding occurred with only the pentyl and hexyl side chains. As for receptor preference, the low affinity propyl analogue (JWH-072) exhibited 6-fold selectivity for  $\text{CB}_2$  while the higher affinity pentyl analogue (JWH-018) demonstrated modest preference for  $\text{CB}_2$  (also shown in Showalter et al., 1996). Extending the N-hexyl side chain by an additional carbon to a N-heptyl chain (JWH-020), resulted in a 13-fold de-

Table 1  
Structure-affinity relationships of indoles



Compound	R	CB <sub>1</sub> K <sub>i</sub> (nM)	CB <sub>2</sub> K <sub>i</sub> (nM)	CB <sub>1</sub> /CB <sub>2</sub> ratio
Δ <sup>9</sup> -THC <sup>a</sup>		40.7 ± 1.7	36.4 ± 10	1.12
WIN 55,212-2 <sup>a</sup>		1.89 ± 0.09	0.28 ± 0.16	6.75
JWH-070	Methyl	> 10 000	> 10 000	ND
JWH-071	Ethyl	1340 ± 123	2940 ± 852	0.45
JWH-072	<i>N</i> -propyl	1050 ± 55.0	170 ± 54.0	6.18
JWH-073	<i>N</i> -butyl	8.90 ± 1.80	38.0 ± 24.0	0.23
JWH-018	<i>N</i> -pentyl	9.00 ± 5.00	2.94 ± 2.65	3.06
JWH-019	<i>N</i> -hexyl	9.80 ± 2.00	5.55 ± 2.00	1.77
JWH-020	<i>N</i> -heptyl	128 ± 17.0	205 ± 20.0	0.62

<sup>a</sup> Data from Showalter et al. (1996).

crease in binding affinity at the CB<sub>1</sub> receptor and a 40-fold reduction at the CB<sub>2</sub> receptor.

Based upon the above findings, a second series of *N*-alkyl substituted indole analogues was prepared with the 2-methyl moiety that is contained in WIN 55,212-2 (Table 2). Similar to the analogues described in Table 1, enhanced binding was observed with each successive increase in the chain length. The *N*-pentyl chain length (JWH-007) displayed optimum binding of 9.50 nM at CB<sub>1</sub> and 2.94 nM at CB<sub>2</sub>, affinities that were almost identical with the pentyl analogues that were devoid of the 2-methyl group. The most significant finding in this 2-methyl series was the high affinity of the *N*-propyl analogue (JWH-015) for the CB<sub>2</sub> receptor that resulted in a 24-fold receptor selectivity (Showalter et al., 1996). There is a tendency for CB<sub>2</sub> selectivity throughout the 2-methyl indole series.

Efforts to exploit this CB<sub>2</sub> selectivity of the 2-methyl indoles involved addition of a 7'-methyl in the naphthoyl moiety. The addition of this 7'-methyl did not influence the relative selectivity of JWH-015 and JWH-046 for the CB<sub>1</sub> and CB<sub>2</sub> receptors. The highest affinity for either receptor was attributed to the *N*-pentyl analogue (JWH-048). The *N*-hexyl and heptyl derivatives produced lower receptor affinities for both receptor subtypes and revealed low selectivity.

The question then arose as to whether naphthyl substitution could augment the low receptor selectivity of the indole series lacking the 2-methyl substituent that

was described in Table 1. The addition of a 4'-methoxy in the naphthoyl moiety did not enhance CB<sub>2</sub> selectivity and did relatively little to influence affinity for either receptor (JWH-077–JWH-083, Table 3). The only exception was the *N*-pentyl derivative that actually exhibited an almost 10-fold selectivity for the CB<sub>1</sub> receptor.

The last series of indoles was devised in order to determine whether combinations of *N*-alkyl and 2-alkyl substituents would augment the affinity and selectivity of the methoxy derivatives. As can be seen in Table 3 (JWH-094–JWH-100), simultaneously increasing the length of these substituents was detrimental to receptor affinity. The highest affinity was associated with the *N*-pentyl-2-methyl analogue (JWH-098) which was modestly selective for the CB<sub>2</sub> receptor.

Thus, several structural features across the different classes of cannabinomimetic indoles were shown to be important for cannabinoid receptor recognition and selectivity. CB<sub>2</sub> selectivity of the *N*-propyl carbon side chain in the series of indole and 2-methyl indole analogues was observed (JWH-072 and JWH-015) (Tables 1 and 2). Similarly, the *N*-propyl to *N*-pentyl carbon side-chain in the series of 2,7'-dimethyl indole analogues (JWH-046–JWH-048) also display selectivity for the CB<sub>2</sub> receptor (Table 2). In summary, with successive increase in the *N*-alkyl carbon side-chain length, binding affinity increases at both the CB<sub>1</sub> and CB<sub>2</sub> receptors and is optimal at 5 carbons. Beyond the sixth carbon, however, binding is weakened at both receptors.

#### 4. Discussion

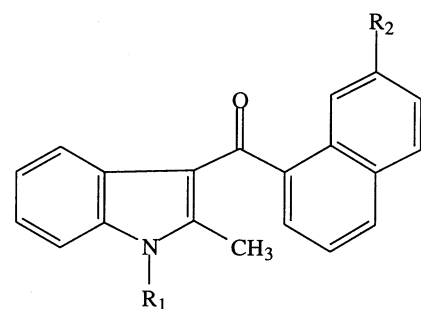
Previously, in light of evidence the cannabinoid receptor in implicating the pharmacological activity and binding affinity of WIN 55,212-2, a structural comparison to  $\Delta^9$ -THC was undertaken (Huffman et al., 1994). The best fit alignment illustrated a three-point attachment for each compound with regions of  $\Delta^9$ -THC presumed to correspond with those on the indole structure, respectively: (a) the cyclohexene and naphthalene ring; (b) the phenolic hydroxyl and carbonyl group; and (c) the carbon side chain at C3 and the morpholinoethyl group. Superimposing the two structures using this best fit alignment showed that the C3 side chain of the cannabinoid and the nitrogen substituent of the indole (equivalent to C1' on the cannabinoid) corresponded well with each other (Huffman et al., 1994). There exists a potential commonality between indoles and the classical cannabinoids. Both compounds possess a polar functionality (3-acyl indole vs. hydroxyl), a central ring system (naphthyl vs. dibenzopyran), and a lipophilic substituent (N-alkyl vs. pentyl). Based on these apparent electrostatic and steric similarities, this study was designed as an attempt to explain the effect of structurally modifying various classes of indole ana-

logues on binding at the cannabinoid receptors. In doing this, possible differences in the active sites of both receptors may be identified.

There have been various studies examining the correlation between CB<sub>1</sub> receptor affinity and potency of these novel cannabinoids to the length of the carbon chain (Huffman et al., 1994; Wiley et al., 1998), the presence of the morpholinoethyl group (Eissenstat et al., 1995; Xie et al., 1995; Wiley et al., 1998) and the naphthoyl substituent at C3 (Huffman et al., 1994). In the present study, a series of cannabimimetic indoles containing substituents at C2, and the C4' and the C7' positions have been developed in which an N-alkyl chain of varying lengths was substituted for the morpholinoethyl group.

A common trend observed across the various classes of indoles is that the manipulation of the carbon chain resulted in a hyperbolic function for binding affinities. In all five series of compounds, regardless of the presence of the substituents at C2, C4', or C7', binding affinity to both receptors steadily increased as the length of the alkyl group on the indole nitrogen was increased until maximum affinity was shown for 1-pentylindole (JWH-018), 2-methyl-1-pentylindole (JWH-007), 2,7'-dimethyl-1-pentylindole (JWH-048), 4'-

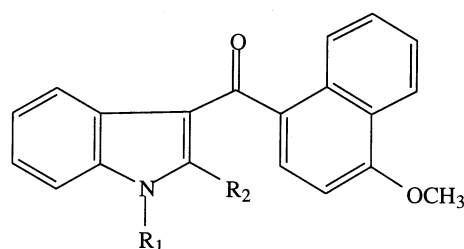
Table 2  
Structure-affinity relationships of 2-methyl and 2,7-methyl indoles



Compound	R	R <sub>2</sub>	CB <sub>1</sub> K <sub>i</sub> (nM)	CB <sub>2</sub> K <sub>i</sub> (nM)	CB <sub>1</sub> /CB <sub>2</sub> ratio
$\Delta^9$ -THC <sup>a</sup>			40.7 ± 1.7	36.4 ± 10	1.12
WIN 55,212-2 <sup>a</sup>			1.89 ± 0.09	0.28 ± 0.16	6.75
JWH-042	Methyl	H	> 10 000	5050 ± 192	> 1.98
JWH-043	Ethyl	H	1180 ± 44.0	964 ± 242	1.23
JWH-015	<i>N</i> -propyl	H	336 ± 36.0	13.8 ± 4.60	24.3
JWH-016	<i>N</i> -butyl	H	22.0 ± 1.50	4.29 ± 1.63	5.13
JWH-007	<i>N</i> -pentyl	H	9.50 ± 4.50	2.94 ± 2.60	3.23
JWH-004	<i>N</i> -hexyl	H	48.0 ± 13.0	4.02 ± 1.46	11.9
JWH-009	<i>N</i> -heptyl	H	311 ± 106	141 ± 14.5	2.21
JWH-046	<i>N</i> -propyl	CH <sub>3</sub>	343 ± 38.0	16.3 ± 4.90	21
JWH-047	<i>N</i> -butyl	CH <sub>3</sub>	58.7 ± 3.00	3.47 ± 1.80	17
JWH-048	<i>N</i> -pentyl	CH <sub>3</sub>	10.7 ± 1.00	0.49 ± 0.13	21.8
JWH-049	<i>N</i> -hexyl	CH <sub>3</sub>	55.1 ± 17.0	32.3 ± 2.40	1.7
JWH-050	<i>N</i> -heptyl	CH <sub>3</sub>	342 ± 6.00	526 ± 133	0.65

<sup>a</sup> Data from Showalter et al. (1996).

Table 3  
Structure-affinity relationships of 4-methoxy and 2-alkyl-4-methoxy indoles



Compound	R <sub>1</sub>	R <sub>2</sub>	CB <sub>1</sub> K <sub>i</sub> (nM)	CB <sub>2</sub> K <sub>i</sub> (nM)	CB <sub>1</sub> /CB <sub>2</sub> ratio
Δ <sup>9</sup> -THC <sup>a</sup>			40.7 ± 1.7	36.4 ± 10	1.12
WIN 55,212-2 <sup>a</sup>			1.89 ± 0.09	0.28 ± 0.16	6.75
JWH-077	Methyl	H	> 10 000	> 10 000	ND
JWH-078	Ethyl	H	817 ± 60.0	633 ± 116	1.29
JWH-079	<i>N</i> -propyl	H	63.0 ± 3.00	32.0 ± 6.00	1.97
JWH-080	<i>N</i> -butyl	H	5.60 ± 1.00	2.21 ± 1.30	2.53
JWH-081	<i>N</i> -pentyl	H	1.20 ± 0.03	12.4 ± 2.23	0.1
JWH-082	<i>N</i> -hexyl	H	5.30 ± 0.80	6.40 ± 0.94	0.83
JWH-083	<i>N</i> -heptyl	H	106 ± 12.0	102 ± 50.0	1.04
JWH-094	<i>N</i> -propyl	Methyl	476 ± 67.0	97.3 ± 2.70	4.89
JWH-093	<i>N</i> -propyl	<i>N</i> -butyl	40.7 ± 2.80	59.1 ± 10.5	0.69
JWH-096	<i>N</i> -butyl	Methyl	33.7 ± 2.90	13.3 ± 5.60	2.53
JWH-095	<i>N</i> -butyl	<i>n</i> -pentyl	140 ± 4.30	312 ± 83.0	0.45
JWH-098	<i>N</i> -pentyl	Methyl	4.50 ± 0.10	1.88 ± 0.30	2.39
JWH-097	<i>N</i> -pentyl	<i>N</i> -hexyl	455 ± 28.0	121 ± 15.0	3.76
JWH-099	<i>N</i> -hexyl	Methyl	35.3 ± 9.00	17.8 ± 2.87	1.98
JWH-100	<i>N</i> -heptyl	Methyl	381 ± 102	155 ± 74.3	2.46

<sup>a</sup> Data from Showalter et al. (1996).

methoxy-1-pentylindole (JWH-081) and 2-methyl-4'-methoxy-1-pentylindole (JWH-098). In pharmacological assays evaluating antinociception, hypomotility, hypothermia and ring mobility, along with CB<sub>1</sub> binding studies conducted by Wiley et al. (1998), *N*-alkyl chains of four to six carbons were shown to produce optimal in vitro and in vivo activity. However, binding to both receptors is weakened with a heptyl substituent on the nitrogen. Indoles with short chain lengths (methyl or ethyl) either displayed an absence of binding to both receptors as with 1-methylindole (JWH-070), 1-methyl-4'-methoxy indole (JWH-077) and 1,2-dimethyl-indole (JWH-042), or very weak binding in the case of the *N*-ethyl analogues (JWH-043, JWH-071 and JWH-078). Wiley et al. (1998) also found that these short side chains resulted in inactive compounds in vivo. Maximal displacement of [<sup>3</sup>H] CP-55 940 occurred with carbon chain lengths ranging from butyl through hexyl across all the classes of indoles. The significance of these findings illustrate that, as with classical and bicyclic cannabinoids and anandamide (Compton et al., 1993; Ryan et al., 1997; Seltzman et al., 1997), the length of the indole alkyl chain is important in the prediction of in vivo and in vitro potency.

The addition of a methyl substituent at the C2 position in all the cannabimimetic indoles described in Tables 1–3 resulted in a slight decrease in CB<sub>1</sub> receptor affinity relative to those analogues lacking a substituent at C2. The C7' ethyl substituent present in JWH-046–JWH-049 had relatively little effect upon CB<sub>1</sub> receptor affinity. In all series receptor affinity is enhanced at both receptors with increasing length of the *N*-alkyl chain until a carbon chain length of five is reached. This effect is more pronounced at CB<sub>2</sub> as evidenced by a 24-fold selectivity for CB<sub>2</sub> for the 2-methyl-1-propylindole (JWH-015) as opposed to the *N*-propylindole (JWH-072) which has only six-fold selectivity for CB<sub>2</sub>. Furthermore, the 2,7'-dimethyl-propyl through pentyl indoles which contain an additional methyl substituent at the C7' position each has an approximately 20-fold selectivity for CB<sub>2</sub>. Binding at CB<sub>2</sub> appears to be favored by the presence of nonpolar substituents on different portions of the nucleus. In particular, the presence of a methyl group at C2 enhances CB<sub>2</sub> affinity. This may be explained by the fact that indoles, such as WIN 55,212-2 are thought to bind at a unique site on the CB<sub>1</sub> receptor that is not shared by other cannabinoids (Song and Bonner, 1996). It is not known if

WIN 55,212-2 and other cannabimimetic indoles bind to the CB<sub>2</sub> receptor at the same site.

To investigate the effect of steric hindrance and electronic effects on binding affinity to both the central and peripheral cannabinoid receptors, the 4'-methoxyindoles and 2-alkyl-4-methoxyindoles (Table 3) were examined. In this series of compounds, the carbon chain lengths at two regions, N1 and C2, were manipulated. The simultaneous increase in the chain lengths (a maximum of five carbons at N1 and 6 carbons at C2) is accompanied by successive decreases in binding affinities at both receptors. Eissenstat et al. (1995) also demonstrated that increasing steric bulk at the C2 position in indole cannabinoids greatly decreased affinity for receptors. In the 4'-methoxy series, compounds with either a methyl group or no substituent at C2 showed enhanced affinities for the CB<sub>1</sub> receptor relative to the analogues which are unsubstituted on the naphthalene system. The same trends prevail regarding the length of the N1 alkyl chain which were observed in the indole and the 2-methylindole series.

In summary, this report enhances our understanding of both the similarities and the differences between the binding of indole-based cannabinoid receptor ligands and the classical cannabinoids, such as  $\Delta^9$ -THC. The subtle differences in the binding of these compounds to CB<sub>1</sub> and CB<sub>2</sub> receptors allow further refinement of the pharmacophore models for each of these receptors and will help lead to a basis upon which the synthesis of additional selective ligands may be derived.

## Acknowledgements

This research was supported by NIDA grants DA 03590 and DA 03672 (to B.R.M.), and DA 05274 (to M.E.A.) and DA-03590 (to J.W.H.). The authors would also like to thank Michelle Phillips, Dr Julia A.H. Lainton and Dong Dai for the preparation of compounds described previously.

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