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Moving around the molecule: Relationship between chemical structure and in vivo activity of synthetic cannabinoids

Jenny L. Wiley^a, Julie A. Marusich^a, and John W. Huffman^b

^aRTI International, 3040 Cornwallis Road, Research Triangle Park, NC

^bDepartment of Chemistry, Clemson University, Clemson, SC

Abstract

Originally synthesized for research purposes, indole- and pyrrole-derived synthetic cannabinoids are the most common psychoactive compounds contained in abused products marketed as “spice” or “herbal incense.” While CB₁ and CB₂ receptor affinities are available for most of these research chemicals, in vivo pharmacological data are sparse. In mice, cannabinoids produce a characteristic profile of dose-dependent effects: antinociception, hypothermia, catalepsy and suppression of locomotion. In combination with receptor binding data, this tetrad battery has been useful in evaluation of the relationship between the structural features of synthetic cannabinoids and their in vivo cannabimimetic activity. Here, published tetrad studies are reviewed and additional in vivo data on synthetic cannabinoids are presented. Overall, the best predictor of likely cannabimimetic effects in the tetrad tests was good CB₁ receptor affinity. Further, retention of good CB₁ affinity and in vivo activity was observed across a wide array of structural manipulations of substituents of the prototypic aminoalkylindole molecule WIN55,212-2, including substitution of an alkyl for the morpholino group, replacement of an indole core with a pyrrole or phenylpyrrole, substitution of a phenylacetyl or tetramethylcyclopropyl group for JWH-018’s naphthoyl, and halogenation of the naphthoyl group. This flexibility of cannabinoid ligand-receptor interactions has been a particular challenge for forensic scientists who have struggled to identify and regulate each new compound as it has appeared on the drug market. One of the most pressing future research needs is determination of the extent to which the pharmacology of these synthetic cannabinoids may differ from those of classical cannabinoids.

Keywords

alkylindoles; aromatic stacking; cannabinoids; herbal marijuana; indoles; JWH-018; pyrroles; review; spice; synthetic cannabinoids

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Corresponding Author: Jenny Wiley, Ph.D., RTI International, 3040 Cornwallis Road, Research Triangle Park, North Carolina 27709-2194, Phone: (919) 541-7276, FAX: (919) 541-6499, jwiley@rti.org.

Conflict of interest statement

The authors do not have any real or perceived conflicts of interest with regard to the data presented in this manuscript.

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Introduction

The use of marijuana and other constituents of the cannabis plant (e.g., hashish) for medicinal and religious purposes can be traced back to ancient China. Yet, intensive scientific interest in marijuana awaited development of appropriate tools, the first of which arrived in 1964 when Dr. Raphael Mechoulam and colleagues isolated and identified Δ^9 -tetrahydrocannabinol (Δ^9 -THC) as the primary psychoactive constituent of the marijuana plant (Gaoni and Mechoulam, 1964). Continuing his work in the emerging field of cannabinoid science, Professor Mechoulam founded a lab that served as an incubator for several foundational discoveries that occurred later in the 1980's and 1990's. During these formative years, major research findings included discovery of the endocannabinoid system (Devane, et al., 1992; Hanus, et al., 2001; Mechoulam, et al., 1995), identification and cloning of the CB₁ and CB₂ subtypes of cannabinoid receptor (Devane, et al., 1988; Matsuda, et al., 1990; Munro, et al., 1993), and synthesis of selective antagonists for each of these receptors (Rinaldi-Carmona, et al., 1994; Rinaldi-Carmona, et al., 1998). Although cannabinoid agonists used in initial in vivo research studies were phytocannabinoids such as Δ^9 -THC and cannabidiol (Compton, et al., 1990; Martin, et al., 1981), these plant-derived cannabinoids soon shared the research arena with synthetic cannabinoids, including the novel classes of bicyclic cannabinoids (e.g., CP55,940; Little, et al., 1988) and the aminoalkylindoles (e.g., WIN55,212-2; Compton, et al., 1992), developed by scientists at Pfizer and Sterling-Winthrop, respectively, as well as the endocannabinoids (e.g., anandamide and 2-arachidonoylglycerol; Devane, et al., 1992; Mechoulam, et al., 1995).

The synthetic cannabinoids, which were later hijacked for use in designer drugs labeled as "herbal incense," were originally synthesized in this milieu (Huffman, et al., 1994). JWH-018 [1-pentyl-3-(1-naphthoyl)indole], one of the first such compound identified in a forensic sample (Lindigkeit, et al., 2009), and other structurally related synthetic cannabinoids were created as part of a research program directed by Dr. John Huffman at Clemson University, Clemson, South Carolina. In effort to determine how such structurally diverse molecules as Δ^9 -THC and WIN55,212-2 could fit into the same receptor, Dr. Huffman considered the then popular 3-point attachment model of cannabinoid ligand-receptor interaction (Thomas, et al., 1991) and hypothesized that the attachment location for the morpholino group of WIN would correspond with the pentyl side chain of Δ^9 -THC (Figure 1). To test this hypothesis, he synthesized a series of indole- and pyrrole-derived cannabinoids and elicited the assistance of Dr. Billy Martin and colleagues at Virginia Commonwealth University, Richmond, Virginia to evaluate them. Dr. Martin's structure-activity relationship (SAR) analysis of synthetic cannabinoids used a two-step approach to measure binding affinity at CB₁ and CB₂ receptors and to assess the cannabinoids in a tetrad of in vivo tests in which cannabinoid agonists produce a characteristic profile of effects in mice, including suppression of motor activity, antinociception, hypothermia and catalepsy (Martin, et al., 1991). This mini-review focuses on summarizing the findings of published SAR studies which examined the in vivo activity of indole- and pyrrole-derived cannabinoids. In addition, new in vivo data are presented for structurally related indoles and pyrroles. These unpublished data were collected in the same lab and under the same experimental parameters used for the previously published data (Wiley, et al., 2012a; Wiley, et al., 2012b; Wiley, et al., 1998). Animals used in these experiments were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Virginia Commonwealth University (Richmond, Virginia) and the 'Guide For The Care And Use Of Animals' (National Research Council, 1996). Not covered in this review are manuscripts that present only binding and/or functional in vitro data. The interested reader is referred to several excellent reviews that have been written on this topic (Huffman, 1999; Huffman, 2009; Huffman and Padgett, 2005; Manera, et al., 2008). In addition, other manuscripts in this volume review separate aspects of the in vivo and behavioral

pharmacology of this class of cannabinoids, including their discriminative stimulus effects (Järbe and Gifford) and their liability for producing tolerance and dependence (Lichtman et al.).

WIN55,212-2

WIN55,212-2 represents the prototypic aminoalkylindole cannabinoid which inspired the idea for synthetic indole cannabinoids (Huffman, et al., 1994). Coincidentally, the enantiomers of WIN55,212 and Δ^9 -THC with cannabinoid activity are polar opposites: (+)-WIN55,212 (designated WIN55,212-2) and (–)- Δ^9 -THC. (+)- Δ^9 -THC and the (–)-enantiomer, (–)-WIN55,212 (designated as WIN55,212-3) are not active in the tetrad test battery in mice (Compton, et al., 1992; Martin, et al., 1991). While Δ^9 -THC binds with approximately equal affinity to both identified cannabinoid receptors (CB₁ K_i = 41 nM, CB₂ K_i = 36), WIN55,212-2 has better affinity for CB₂ (K_i = 0.28 nM) vs CB₁ (K_i = 1.89 nM) receptors (Showalter, et al., 1996). Δ^9 -THC and WIN55,212-2 also differ in their in vitro efficacy at the CB₁ receptor, with Δ^9 -THC acting as a partial agonist in functional assays such as [³⁵S]GTP γ S binding and WIN55,212-2 acting as a full agonist (Breivogel and Childers, 2000); however, both compounds show approximately equal efficacy in the mouse tetrad tests. In the present study, a full dose-effect curve determination was conducted with WIN55,212-2 in the tetrad battery (Figure 2) and serves as a comparison for other novel synthetic indole and pyrrole cannabinoids shown in Tables 1–4. Results are similar to those that have been obtained previously with WIN55,212-2 (Compton, et al., 1992), Δ^9 -THC (Martin, et al., 1991), and a variety of other psychoactive cannabinoid agonists (Compton, et al., 1993). At lower doses, WIN55,212-2 (present study; Figure 2, panel A) may increase locomotion, whereas at higher doses cannabinoid agonists, including WIN55,212-2 (present study; Compton, et al., 1992), reliably and dose-dependently suppress locomotor activity (Martin, et al., 1991). Maximum suppression typically approaches 100%. WIN55,212-2 also produced robust antinociceptive effects (100% maximum possible effect at higher doses; Figure 2, panel B) and substantial decreases in rectal temperature (–6 °C is typical at higher doses; Figure 2, panel C). WIN55,212-2 increases catalepsy in the mouse ring test, with efficacy ranging from 60 – 100% (Figure 2, panel D). Acute Δ^9 -THC produces similar biphasic locomotor effects, decreases in rectal temperature, and increases in catalepsy (Martin, et al., 1991; Sañudo-Peña, et al., 2000; Wiley, et al., 2008). ED₅₀s for the effects of WIN55,212-2 in these tests are provided in Table 1.

Indoles

Substitutions for morpholino group

The initial series of indole-derived cannabinoids that were used to evaluate the hypothesis that the morpholino group of the aminoalkylindoles and the C3 side chain of Δ^9 -THC occupied similar spatial domains within the CB₁ receptor were a series of indole compounds in which an alkyl group was substituted for the oxazine and morpholino substituents of WIN55,212-2. One of the primary findings of this study was that a cyclic amino group at this position was unnecessary for psychoactivity. Substitution of a *n*-pentyl group resulted in a compound (JWH-018; Figure 1) with good CB₁ receptor affinity (K_i = 9 nM) and correspondingly good potency in the tetrad tests (Wiley, et al., 1998). Further, affinity and potency varied systematically with the length of the carbon chain (position R1 of naphthoylindole structure in Figure 3), with optimal activity from *n*-butyl to *n*-hexyl and absence or reduction of receptor binding at shorter or longer carbon chains. This finding is consistent with the results of a structure-activity analysis of classical and bicyclic cannabinoids, in which length and branching of the C3 carbon chain of Δ^8 -THC and CP55,940 analogs dramatically affected CB₁ affinity and in vivo potency (Compton, et al., 1993). Fluoropentyl substitution also resulted in an active compound (AM-2201) that has

been found in “herbal incense” products (Denooz, et al., 2013; Logan, et al., 2012). CB₁ receptor recognition and in vivo activity were also retained with some cyclic substitutions (cyclohexylethyl and cyclopropylmethyl), but not with others (2-phenylethyl), albeit none of these compounds were as potent as WIN55,212-2 or JWH-018 (Wiley, et al., 1998). Of the compounds tested in this early study, the most potent compounds (JWH-073, JWH-018, JWH-019, and AM-2201: *n*-butyl, *n*-pentyl, *n*-hexyl, and *n*-fluoropentyl substitutions, respectively) were detected in samples purchased in 2011 in the state of North Carolina in the United States (Cox, et al., 2012), suggesting that manufacturers of these now illicit products may have been aware of these initial findings.

Addition of substituents to indole group

The *n*-alkyl indoles described above were unmethylated at the 2-indole position (position R2 of naphthoylindole structure in Figure 3). A parallel set synthesized during the same time period contained a 2-methylindole substituent. While 2-methylation did not affect the overall pattern of results (i.e., optimal affinity and potency with *n*-butyl to *n*-hexyl chain lengths), absolute affinities and potencies the methylated *n*-butyl to *n*-hexyl analogs were 2- to 4-fold less than those of the unmethylated compounds (Wiley, et al., 1998). Further, 2-methylation appeared to result in a shift in the SAR profile such that the methylated *n*-propyl was more active and the *n*-heptyl compound was less active than the corresponding unmethylated compounds. These results are consistent with those described for the aminoalkylindole analogs of WIN55,212-2, in that the 2-methylated compound, WIN53,365, showed 2-fold less affinity for CB₁ receptors than did its unmethylated counterpart, WIN55,225 (Dutta, et al., 1997).

The effects of a series of compounds with C-5 halogenation (Br and I) of the indole group on binding and in vivo activity are presented in Table 1. Compared to compounds with *n*-alkyl groups of comparable lengths, these halogenated indoles exhibited greatly decreased affinity for the CB₁ receptor. For example, JWH-455 ($K_i = 686$ nM) showed 77-fold less CB₁ receptor affinity than did JWH-073 ($K_i = 8.9$ nM), the non-halogenated *n*-butyl analog. As with the non-halogenated series, optimal chain length was *n*-pentyl ($K_i = 145$ and 178 for the Br analog JWH-444 and the I analog JWH-452, respectively). Unlike the original non-halogenated compounds, however, none of the analogs in Table 1 showed < 100 nM CB₁ receptor affinity. Consequently, a single 30 mg/kg intravenous dose of each compound was assessed in vivo (where available supply allowed). Although one compound (JWH-453) showed poor CB₁ receptor affinity and was not active in any of the tests, all of the other tested compounds exhibited activity or partial activity in one or more test. Given the poor CB₁ receptor binding affinity of some of the compounds (e.g., JWH-446 and JWH-455), these results are somewhat surprising. One possibility is that these compounds may be metabolized to active metabolites, as has been demonstrated for other indole-derived cannabinoids (Brents et al., 2012). On the other hand, the tetrad tests are not entirely selective for cannabinoids (Wiley and Martin, 2003), suggesting that non-cannabinoid receptor actions may have played a role in the effects of these compounds at the 30 mg/kg probe dose. Assessment of the ability of rimonabant to reverse the effects would be necessary to determine whether the observed effects were CB₁ receptor mediated.

Pyrroles

Naphthoylpyrroles

Conversion of the original series of naphthoylindoles to naphthoylpyrroles resulted in compounds with greatly reduced CB₁ receptor affinities and in vivo potencies (Wiley, et al., 1998). Compounds with chain lengths of *n*-methyl to *n*-propyl (position R1 of naphthoylpyrrole structure in Figure 3) did not bind to the CB₁ receptor ($K_i > 10,000$ nM).

Whereas lengthening the chain to *n*-butyl or longer improved affinity, only the *n*-pentyl pyrrole (JWH-030) had reasonable affinity ($K_i = 87$ nM), although its potency in at least two of the in vivo tests (temperature and ring immobility) remained low (> 70 μ M/kg). Perhaps not surprisingly, these naphthoylpyrroles are not commonly detected in forensic samples, unlike the comparable naphthoylindole analogs (e.g., JWH-018, JWH-073), albeit there are exceptions: e.g., 3-naphthoyl-*n*-pentylpyrrole (JWH-030) has been detected in samples obtained over the internet (Uchiyama, et al., 2013).

Phenylpyrroles

Although the 3-point attachment model formed the original basis of the creation of indole-derived cannabinoids, exploration of their SAR led to revision of the model in favor of the hypothesis that interaction of these new cannabinoids with the CB₁ receptor might involve stacking of their aromatic naphthoyl and indole substituents (Huffman, et al., 2003; Reggio, et al., 1998). Later synthesis of a series of phenylpyrroles and evaluation of CB₁ receptor binding data was supportive of this aromatic stacking hypothesis (Huffman, et al., 2006). Table 2 presents unpublished results of in vivo tetrad tests with some of these compounds. Due to limited supplies, a full dose-effect curve could not be determined for all of the compounds. Hence, potencies could not be estimated; however, several observations may be made based upon these data. First, with the exception of JWH-309 (which could not be tested at an adequate dose due to limited supply), all of the compounds were fully or partially active in one or more of the tetrad tests. Second, these results are generally consistent with the excellent CB₁ receptor binding affinities of the majority of the compounds. JWH-363 had the worst binding affinity ($K_i=245$ nM) and showed the least activity in vivo (at 30 mg/kg i.v., inactive in two tests and minimal activity in the other two). In contrast, JWH-370 had the best binding affinity ($K_i=5.6$ nM) and was fully active in all four tests at a dose of 10 mg/kg, i.v. Third, both the nature and the position of the phenyl substituent affected in vivo activity. For example, compounds with 2- or 3-phenyl substituents (JWH-370, JWH-346, JWH-365, JWH-367, and JWH-307) were fully active in the complete tetrad whereas compounds with 4-phenyl substituents (JWH-364 and JWH-371) were inactive in at least one of the tests, despite reasonable CB₁ binding affinities. Trifluoromethylphenyl substitution (JWH-372 and JWH-363) resulted in decreased activity in one or more of the tests, as compared to compounds with other 2- or 3-phenyl substituents. Together, these results demonstrate overall concordance of in vivo activity and good CB₁ receptor binding affinities for the compounds and are supportive of the aromatic stacking hypothesis.

Naphthoyl manipulations

Addition of substituents to naphthoyl group

The steric and electronic effects of two types of substituents on the naphthoyl group have been examined in SAR with an in vivo component: electron withdrawing halogen substituents and electron donating methoxy. The effects of moderately electron withdrawing halogen substituents (Br, Cl, F, and I) at C-4 or C-8 (see chemical structure at the top of Table 3) on CB₁ and CB₂ receptor affinities and in vivo pharmacology were examined in a series of 1-alkyl-3-(1-naphthoyl)indoles (Wiley, et al., 2012b). In addition, two electroneutral structural features (the length of the N-alkyl group and the presence of a methyl at the 2-indole position) were manipulated in this series. Results observed with the two electroneutral structural manipulations were consistent with a number of previous SAR investigations of indole- and pyrrole-derived cannabinoids in which these structural manipulations produce similar patterns of alterations of CB₁ and CB₂ affinities and in vivo potencies, suggesting that the length of the alkyl substituent is as crucial for this cannabinoid structural motif (Wiley, et al., 1998) as it is for cannabinoids based on the

tetrahydrocannabinol structural motif (Compton, et al., 1993). As found in these previous studies, 1-pentyl substitution was optimal for enhancement of CB₁ receptor affinity and in vivo activity. Further, C-4 substituents showed accentuated CB₁ receptor affinity and in vivo activity compared to C-8 substituents, some of which did not bind to the receptor nor produce activity in the tetrad tests. Less variability occurred across halogen groups. The authors hypothesized that steric interference with aromatic stacking was a probable cause of the decreased activity of compounds with C-8 substituents (Wiley, et al., 2012b).

The effects of methoxy substitution on the naphthoyl group appear to support this hypothesis (Table 3; Aung, et al., 2000). Although halogen and methoxy substituents are electronic opposites (electron withdrawing vs. electron donating, respectively), C-4 substitution of either type of substituent resulted in compounds with the best CB₁ receptor affinities and in vivo activity, as compared to substitution at other positions. Unlike substituents at other positions on the naphthoyl, the rotation of C-4 substituents is less hindered and thereby, less likely to interfere with optimal aromatic stacking, which has been shown to be important for cannabinoid receptor recognition (Huffman, et al., 2003; Reggio, et al., 1998). Consistent with this idea, three of the four compounds (JWH-389 JWH-390, and JWH-391) with C-2 methoxy substituents did not bind to the CB₁ receptor. Further, none of the C-2 compounds were active in any of the tetrad tests at doses up to 30 mg/kg i.v. (Table 3). While compounds with C-6 methoxy substituents showed slightly increased activity across tests, none of these compounds were fully active in all four tests. In addition, as in previous studies, the length of the 1-alkyl and presence or absence of 2-methylation also contributed to affinity for the cannabinoid receptors and activity in the tetrad tests (Table 3). Together, these results suggest that steric effects play a stronger role in determining the nature of CB₁ receptor affinity and in vivo activity than did electronic effects.

Substitution for naphthoyl group

Because the naphthoyl group itself is involved in aromatic stacking of indole- and pyrrole-derived cannabinoids described thus far in this review, its manipulation would be expected to produce profound changes in CB₁ receptor recognition and in vivo activity and, indeed, it does. Deletion of the naphthoyl (JWH-001) or substitution of a non-cyclic group (JWH-002) eliminates both CB₁ receptor binding affinity and in vivo activity (Table 4; Huffman, et al., 1994). The number of aromatic constituents also may play a role in interaction with the CB₁ receptor and resultant pharmacological activity in the tetrad tests. For example, WIN56,098, an aminoalkylindole analog with anthracene (3 aromatic ring) substitution for the naphthalene, does not bind to the CB₁ receptor nor is it active in vivo in the tetrad tests (Compton, et al., 1992). In contrast, substitution of a two-ringed group (methyl-benzodioxole) results in a 3,4-methylenedioxyphenylacetylindole (JWH-317) that binds to CB₁ receptors with moderate affinity ($K_i=101$ nM) and is active with near maximal efficacy in suppressing locomotor activity and producing antinociception and hypothermia (Table 4). Decreasing the number of aromatic rings on the non-indole side of the carbonyl to one, as in a series of 1-pentyl-3-phenylacetylindoles (Figure 3), attenuated CB₁ receptor affinities and reduced in vivo potencies compared to 1-pentyl-3-naphthoylindole congeners (Wiley, et al., 2012a). The major structural manipulations in this series included the type of substituent (i.e., unsubstituted, methyl, methoxy, chloro, bromo, and fluoro) and the position of the substituent on the phenyl ring (i.e., 2-, 3- or 4-position). Of these manipulations, the most critical factor affecting in vivo potency was the position of the substituent. Whereas compounds with 2- and 3-phenylacetyl substituents were efficacious with good potencies, 4-substituents resulted in compounds that had poor potency or were inactive. Interestingly, this pattern also occurred for indole-derived cannabinoids with a 1-morpholinomethyl substituent instead of a 1-pentyl (Compton, et al., 1992). For example, pravadoline (WIN48,098), one of Sterling-Winthrop's original lead compounds in the aminoalkylindole

series (Haubrich, et al., 1990), has a 4-methoxyphenyl substituent in place of the naphthoyl. Pravadoline, and a similar analog without 2-methylation, do not bind to the CB₁ receptor and are not active in the tetrad tests (Compton, et al., 1992). This pattern of results suggests that steric influences are as important for aminoalkylindoles and 1-pentyl-3-phenylacetylindoles as they are for the halogenated 1-alkyl-3-(1-naphthoyl)indoles (Wiley, et al., 2012b).

Recently, we completed assessment of two tetramethylcyclopropyl ketone indoles, UR-144 [(1-pentyl-1H-indol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone] and XLR-11 [(1-(5-fluoropentyl)-1H-indol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone]. In these compounds, a tetramethylcyclopropyl group is substituted for the naphthoyl of the parent 1-pentyl-3-naphthoylindole (Figure 3). In addition, XLR-11 has a fluoro group at the terminal end of the 1-pentyl (position R1 of tetramethylcyclopropyl ketone indole structure in Figure 3). These compounds are similar to those contained in a series of compounds synthesized by Abbott Laboratories (Frost, et al., 2010; Frost, et al., 2008), suggesting that manufacturers of “herbal incense” products have used multiple sources of information in their quest for novel psychoactive cannabinoids. While the Abbott compounds were CB₂ receptor selective, many also possessed significant affinity for the CB₁ receptor. As other compounds (e.g., JWH-018, AM-2201) have been banned, UR-144 and XLR-11 have started appearing in samples of “herbal incense” sold over the internet (Kavanagh, et al., 2013; Uchiyama, et al., 2013). Both compounds have good CB₁ and CB₂ receptor binding affinities and produced the full complement of cannabimimetic effects in the tetrad tests in mice, with potencies several-fold greater than Δ^9 -THC (Wiley et al., 2013). Given that attenuation of cannabinoid activity was observed with the 1-pentyl-3-phenylacetylindoles (JWH-205 and JWH-167, with and without 2-methylation, respectively), this result is rather surprising and suggests that the nature of the cyclic substituent (e.g., phenyl vs. tetramethylcyclopropyl), as well as their number, contribute to receptor recognition and in vivo potency. Both the phenylacetylindoles and the Abbott compounds have shown up in “herbal incense” products.

Conclusions

Originally synthesized for research purposes, indole- and pyrrole-derived synthetic cannabinoids are the most commonly identified psychoactive chemicals contained in products marketed as “herbal incense.” Although hundreds of these cannabinoids have been evaluated for their CB₁ and CB₂ receptor affinities (Aung, et al., 2000; Huffman, et al., 2006; Huffman, et al., 2003; Huffman, et al., 2005a; Huffman, et al., 2005b; Lainton, et al., 1995; Manera, et al., 2008), most have never been tested in animals before they were discovered in products confiscated from human users. The purpose of this mini-review was to summarize the findings of SAR studies in which the in vivo activity of these cannabinoids was examined. Based upon the collective data, good CB₁ affinity was the best predictor of the propensity of a given compound to produce cannabimimetic effects in the tetrad tests in mice, suggesting that these effects are mediated by CB₁ receptor activation, as has also been shown for traditional and bicyclic cannabinoids (Compton, et al., 1996; Compton, et al., 1993). Consistent with this idea, reversal of psychoactive effects of indole-derived cannabinoids has been demonstrated in nonhuman primates, rats and mice (Ginsburg, et al., 2012; Järbe, et al., 2011; Wiebelhaus, et al., 2012). Another overall observation is that good CB₁ affinity and its associated cannabimimetic in vivo activity were retained across a surprisingly wide array of structural manipulations of substituents of the prototypic WIN55,212-2 molecule, including substitution of an alkyl for the WIN55,212-2 morpholino group, replacement of an indole core with a pyrrole or phenylpyrrole, substitution of a phenylacetyl or tetramethylcyclopropyl group for JWH-018’s naphthoyl, and halogenation of the naphthoyl group. This flexibility of cannabinoid ligand-receptor interactions has severely undermined the efforts of forensic scientists who have struggled to identify and

regulate each new compound as it has appeared in marketed products (Grabenauer, et al., 2012; Vardakou, et al., 2010). Further complicating characterization of this class of abused cannabinoids is the fact that their affinities for novel cannabinoid or noncannabinoid receptors and the role that these receptors may play in mediating or modulating their pharmacological effects are largely unknown. For example, the indole structure of many of these compounds suggests that they may interact with one or more serotonin receptors. Given that the focus of extant studies has been examination of the effects of these compounds in assays specifically designed to detect cannabinoid activity, perhaps the most pressing future research need is determination of the extent to which the pharmacology of these compounds may differ from those of classical cannabinoids.

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Figure 1. Historical 3-point attachment model used to develop JWH-018. An overlay of WIN55,212-2, Δ^9 -THC and JWH-018 was hypothesized, in which each cannabinoid would attach to the CB₁ receptor at the 3 locations specified in the figure.

Figure 2.

Effects of WIN55,212-2 on spontaneous activity (panel A), antinociception (panel B), rectal temperature (panel C), and catalepsy (panel D) in adult male ICR mice. Spontaneous activity was measured as total number of photocell beam interruptions during a 10-min session and is shown as % inhibition of activity of the vehicle group. Antinociception in a tail flick assay is expressed as the percent maximum possible effect (MPE) using a 10-s maximum test latency as follows: $[(\text{test}-\text{control})/(\text{10}-\text{control})]\times 100$. Rectal temperature values are expressed as the difference between control temperature (before injection) and temperature following drug administration ($\Delta^{\circ}\text{C}$). During assessment for catalepsy, the total amount of time (in s) that the mouse remained motionless on the ring apparatus (except for breathing and whisker movement) was measured and was used as an indication of catalepsy-like behavior. This value was divided by 300 s and multiplied by 100 to obtain percent immobility, as shown in the figure. Values represent the mean ($\pm$ SEM) of 5–6 mice per group. The typical maximal effect observed for each measure is shown in the box at the upper left of each panel. ED_{50} s for data presented in these panels are provided in Table 1.

Figure 3.

Templates for major structural classes of synthetic cannabinoids discussed in this review: naphthoylindoles, naphthoylpyrroles, 1-pentyl-3-phenylacetylindoles and tetramethylcyclopropyl ketone indoles. Additional structural templates are shown in the tables.

Cannabinoid receptor binding and *in vivo* effects of indoles with structural variations in the side chain and indole substituent

Table 1

Compound	R	R'	Affinities (nM)			In Vivo Tests*			
			CB ₁	CB ₂	SA	%MPE	RT	RI	
WIN55,212-2**	morpholino	H	1.9 ± 1 ^a	0.28 ± 0.16 ^a	87% (10)	100% (10)	-5.0 (10)	87% (10)	
					1.3	0.3	0.6	2.2	
JWH-446	propyl	Br	3322 ± 430	2011 ± 239	83% (30)	76% (30)	-3.6 (30)	64% (30)	
JWH-443	butyl	Br	364 ± 14	332 ± 84	ND	ND	ND	ND	
JWH-444	pentyl	Br	145 ± 8	207 ± 5	79% (30)	100% (30)	-5.0 (30)	76% (30)	
JWH-445	hexyl	Br	197 ± 14	499 ± 117	ND	ND	ND	ND	
JWH-453	propyl	I	1967 ± 478	778 ± 50	inactive (30)	inactive (30)	inactive (30)	inactive (30)	
JWH-455	butyl	I	686 ± 48	722 ± 157	79% (30)	68% (30)	-3.3 (30)	inactive (30)	
JWH-452	pentyl	I	178 ± 6	193 ± 19	74% (30)	62% (30)	-5.4 (30)	45% (30)	
JWH-454	hexyl	I	152 ± 10	364 ± 15	66% (30)	inactive (30)	-2.5 (30)	ND	

* SA = spontaneous activity, %MPE = % maximum possible antinociceptive effect, RT = rectal temperature, RI = ring immobility. ND = not determined. Single dose tests are indicated by magnitude of effect and dose tested (mg/kg, in parentheses). "Inactive" refers to doses that produced less than 30% of the maximal cannabimimetic effect for the measure (see Figure 2).

** Values for *in vivo* tests for WIN55,212-2 were calculated based on data shown in Figure 2 and are presented for comparison and for illustration of the system used for data presented in all tables. Numbers in top row exemplify testing of a single 10 mg/kg dose; values on the bottom row show ED₅₀s for dose-effect data presented in Figure 2.

^aShowalter, et al., 1996

Cannabinoid receptor binding and *in vivo* effects of 2-aryl-4-(1-naphthoyl)-N-pentyl-pyrroles

Table 2

Compound	R	Affinities (nM) ^a			In Vivo Tests [*]			
		CB ₁	CB ₂	SA	%MPE	RT	RI	
JWH-309	1-naphthyl	41 ± 3	49 ± 7	inactive (3)	inactive (3)	inactive (3)	ND	
JWH-366	3-pyridyl	191 ± 12	24 ± 1	91% (30)	86% (30)	-4.25 (30)	44% (30)	
JWH-370	2-methylphenyl	5.6 ± 0.4	4.0 ± 0.5	93% (10)	100% (10)	-6.2 (10)	94% (10)	
JWH-346	3-methylphenyl	67 ± 6	39 ± 2	1.5	1.3	2.6	ND	
JWH-365	2-ethylphenyl	17 ± 1	3.4 ± 0.2	86% (10)	95% (10)	-5.1 (10)	48% (10)	
JWH-364	4-ethylphenyl	34 ± 3	29 ± 1	inactive (30)	76% (30)	-5.1 (10)	48% (10)	
JWH-371	4-butylphenyl	42 ± 1	64 ± 2	76% (30)	74% (30)	-2.7 (30)	inactive (30)	
JWH-367	3-methoxyphenyl	53 ± 2	23 ± 1	83% (30)	100% (30)	-5.0 (30)	58% (30)	
JWH-307	2-fluorophenyl	7.7 ± 1.8	3.3 ± 0.2	0.42	0.53	0.96	ND	

Compound	R	Affinities (nM) ^a			In Vivo Tests [*]		
		CB ₁	CB ₂	SA	%MPE	RT	RI
JWH-308	4-fluorophenyl	41 ± 1	33 ± 2	2.4	60% (3)	-2.6 (3)	ND
JWH-372	2-trifluorophenyl	77 ± 2	8.2 ± 0.2	63% (30)	100% (30)	-4.6 (30)	27% (30)
JWH-363	3-trifluorophenyl	245 ± 5	71 ± 1	48% (30)	inactive (30)	-2.0 (30)	inactive (30)

^{*} SA = spontaneous activity, %MPE = % maximum possible antinociceptive effect, RT = rectal temperature, RI = ring immobility. ND = not determined. Single dose tests are indicated by magnitude of effect and dose tested (mg/kg, in parentheses). "Inactive" refers to doses that produced less than 30% of the maximal cannabimimetic effect for the measure (see Figure 2). Whenever supplies allowed determination of a dose-effect curve, ED₅₀s (mg/kg) are provided for active compounds.

^aHuffman, et al., 2006

Table 3

Cannabinoid receptor binding and *in vivo* effects of methoxy naphthoyl indoles

Compound	Substituents		Affinities (nM)			In Vivo Tests*			
	R	R'	X**	CB ₁	CB ₂	SA	%MPE	RT	RI
JWH-391	butyl	H	2-OCH ₃	> 10,000	1236 ± 103	inactive (30)	67% (30)	inactive (30)	inactive (30)
JWH-390	butyl	CH ₃	2-OCH ₃	> 10,000	> 10,000	stim (30)	inactive (30)	inactive (30)	inactive (30)
JWH-388	pentyl	H	2-OCH ₃	2398 ± 228	147 ± 8	inactive (30)	inactive (30)	inactive (30)	inactive (30)
JWH-389	pentyl	CH ₃	2-OCH ₃	> 10,000	> 10,000	inactive (30)	inactive (30)	-2.1 (30)	inactive (30)
JWH-080	butyl	H	4-OCH ₃	5.6 ± 1 ^a	2.2 ± 1.3 ^a	2.7	1.5	0.88	ND
JWH-081	pentyl	H	4-OCH ₃	1.2 ± 0.03 ^a	12.5 ± 2.23 ^a	0.06	0.084	0.06	ND
JWH-082	hexyl	H	4-OCH ₃	5.3 ± 0.80 ^a	6.4 ± 0.94 ^a	1.7	0.62	1.1	ND
JWH-083	heptyl	H	4-OCH ₃	106 ± 12 ^a	102 ± 50 ^a	stim	24	28	ND
JWH-411	butyl	H	6-OCH ₃	2036 ± 203	561 ± 54	inactive (30)	inactive (30)	inactive (30)	inactive (30)
JWH-410	butyl	CH ₃	6-OCH ₃	2682 ± 397	322 ± 46	64% (30)	75% (30)	-2.7 (30)	33% (30)
JWH-408	pentyl	H	6-OCH ₃	190 ± 12	91 ± 3	80% (30)	96% (30)	-4.3 (30)	62% (30)
JWH-409	pentyl	CH ₃	6-OCH ₃	746 ± 77	232 ± 20	83% (30)	42% (30)	-3.9 (30)	38% (30)

* SA = spontaneous activity, %MPE = % maximum possible antinociceptive effect, RT = rectal temperature, RI = ring immobility, ND = not determined. Stim = stimulation of locomotor activity of at least 10% above vehicle levels (i.e., -10% inhibition of activity). Single dose tests are indicated by magnitude of effect and dose tested (mg/kg, in parentheses). "Inactive" refers to doses that produced less than 30% of the maximal cannabimimetic effect for the measure (see Figure 2). Whenever supplies allowed determination of a dose-effect curve, ED50s (mg/kg) are provided for active compounds.

** X indicates substituent and its position on the naphthoyl group.

^a Aung, et al., 2000

Table 4

Cannabinoid receptor binding and *in vivo* effects of miscellaneous indoles

Compound	Structure	Affinities (nM)		<i>In Vivo</i> Tests*		
		CB ₁	CB ₂	SA	%MPE	RT
JWH-001		> 10,000	ND	inactive (100)	inactive (100)	inactive (100)
JWH-002		> 10,000	ND	inactive (100)	inactive (100)	ND
JWH-317		101 ± 1	257 ± 3	89% (30)	100% (30)	-5.5 (30)
						ND

* SA = spontaneous activity, %MPE = % maximum possible antinociceptive effect, RT = rectal temperature, RI = ring immobility. ND = not determined. Single dose tests are indicated by magnitude of effect and dose tested (mg/kg, in parentheses). "Inactive" refers to doses that produced less than 30% of the maximal cannabimimetic effect for the measure (see Figure 2).