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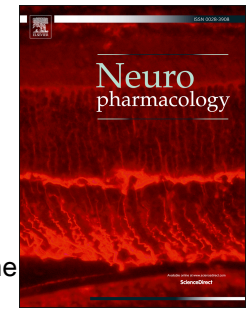

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Evaluation of first generation synthetic cannabinoids on binding at non-cannabinoid receptors and in a battery of in vivo assays in mice

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Abstract

Anecdotal reports suggest that abused synthetic cannabinoids produce cannabis-like "highs," but some of their effects may also differ from traditional cannabinoids such as Δ^9-tetrahydrocannabinol (THC). This study examined the binding affinities of first-generation indole-derived synthetic cannabinoids at cannabinoid and noncannabinoid receptors and their effects in a functional observational battery (FOB) and drug discrimination in mice. All seven compounds, except JWH-391, had favorable affinity ($\leq$ 159 nM) for both cannabinoid receptors. In contrast, binding at noncannabinoid receptors was absent or weak. In the FOB, THC and the six active compounds disrupted behaviors in CNS activation and muscle tone/equilibrium domains. Unlike THC, however, synthetic cannabinoids impaired behavior across a wider dose and domain range, producing autonomic effects and signs of CNS excitability and sensorimotor reactivity. In addition, mice acquired JWH-018 discrimination, and THC and JWH-073 produced full substitution whereas the 5-HT_{2B} antagonist mianserin did not substitute in mice trained to discriminate JWH-018 or THC. Urinary metabolite analysis showed that the compounds were extensively metabolized, with metabolites that could contribute to their in vivo effects. Together, these results show that, while first-generation synthetic cannabinoids shared some effects that were similar to those of THC, they also possessed effects that differed from traditional cannabinoids. The high nanomolar (or absent) affinities of these compounds at receptors for most major neurotransmitters suggests that these divergent effects may be related to the greater potencies and/or efficacies at CB_1 receptors; however, action(s) at noncannabinoid receptors yet to be assessed or via different signaling pathways cannot be ruled out.

Keywords: alkylindoles, functional observation battery, JWH-018, receptor binding, synthetic cannabinoids

1.0 Introduction

Over a quarter of a century ago, medicinal chemists at the pharmaceutical company Sterling-Winthrop synthesized a series of novel aminoalkylindole compounds based upon the template of a promising lead compound, pravadoline (D'Ambra et al., 1992). Although pravadoline possessed analgesic activity in animal models (Haubrich et al., 1990), toxicology studies suggested that it might be nephrotoxic (Everett et al., 1993). The mechanism for the analgesic effects of the series, while initially unknown, was later strongly suspected to be activation of the (then) newly discovered CB_1 cannabinoid receptor (D'Ambra et al., 1992). Building upon this hypothesis, academic researchers investigated the structure-activity relationships of a series of naphthoylindoles based upon the structure of one of the Sterling-Winthrop compounds, WIN 55,212-2 (Huffman et al., 1994). Subsequent studies focused on determination of the interaction of a growing number of indole- and pyrrole-derived compound series with the two identified cannabinoid receptors, CB_1 and CB_2, and characterization of their cannabimimetic in vivo pharmacological profile (Aung et al., 2000; Wiley et al., 1998). At the time, fascination with the newly discovered endocannabinoid system, and how these compounds might interact with it, resulted in less attention being paid to the possibility that these compounds might produce in vivo effects via interaction with other receptors or neurotransmitter systems.

In the early 2000s, identification of one of these synthetic cannabinoids [JWH-018: 1-pentyl-3-(1-naphthoyl)indole] in an abused product dubbed “Spice” confiscated by drug control authorities led to renewed interest in synthetic indole-derived cannabinoids (Auwarter et al., 2009; Vardakou et al., 2010). In an ironic flashback to the early days of synthetic cannabinoids, however, concentration has remained primarily on the CB_1 receptor binding and cannabimimetic in vivo activity of the structurally diverse analogs that have appeared recently rather than on full characterization of their effects at other receptor systems (Fantegrossi et al., 2014; Wiley et al., 2014a). Again, this narrow focus is understandable, given that prediction of marijuana-like

abuse liability and metabolite identification are necessary for passing drug control regulations and screening for use, respectively. Yet, anecdotal reports from human users suggest that synthetic cannabinoids may have effects that differ from those of traditional cannabinoids such as Δ^9-tetrahydrocannabinol (THC), the primary psychoactive substituent of smoked cannabis. For example, increased blood pressure (vs. decreased blood pressure seen with THC), hallucinations, psychomotor agitation, and depressed mood are various effects that have been reported in users of Spice and related synthetic cannabinoids (Schifano et al., 2009; Vardakou et al., 2010).

The objective of the present study was to evaluate selected indole-derived cannabinoids with a range of CB_1 and CB_2 binding affinities (Figure 1) in a functional observational battery (FOB) that has been used to investigate the behavioral effects of abused inhalants (Bowen et al., 1996; Tegeris and Balster, 1994) and synthetic cathinones (Marusich et al., 2014; Marusich et al., 2012) as well as to characterize potential environmental toxins on the nervous system (Moser, 2000; Moser, 2011). Separate mice were also evaluated in cannabinoid-selective drug discrimination. In addition, these compounds were screened for binding affinity and functional activation/antagonism of a wide array of non-cannabinoid receptors via the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP). To aid in development of forensic markers, preliminary analysis of urinary metabolites was also undertaken.

2.0 Materials and methods

2.1 Subjects

Adult male ICR mice (25-32g) [Harlan, Dublin, VA] and C57/Bl6J inbred mice (20-25 g) [Jackson Laboratories, Bar Harbor, ME] were housed singly in polycarbonate mouse cages. Each ICR mouse was tested with a single dose of compound in the functional observational battery (FOB). C57/Bl6J mice were used in the drug discrimination experiments. All animals were kept in a

temperature-controlled (20-22^{o}C) environment with a 12-hour light-dark cycle (lights on at 6 a.m.). ICR mice received food *ad libitum* when in their home cages. C57/Bl67 mice were maintained at 85-90% of free-feeding body weights by restricting daily ration of standard rodent chow. All mice received *ad libitum* water access when in their home cages. The in vivo studies reported in this manuscript were carried out in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and were approved by the Institutional Care and Use Committee at RTI.

2.2 Apparatus

Mice in the drug discrimination experiment were trained and tested in mouse operant chambers (Coulbourn Instruments, Whitehall, PA), housed within light- and sound-attenuating cubicles. Each chamber contained two retractable response levers, with stimulus lights over each lever, and a separate house light. A food dispenser delivered 20-mg food pellets (Bioserv Inc., Frenchtown, NJ) into a food cup (with a light) centered between the two levers. Illumination of lights, delivery of food pellets, and recording of lever presses were controlled by a computer-based system (Coulbourn Instruments, Graphic State Software, v 3.03, Whitehall, PA).

2.3 Chemicals

Δ^{9}-THC, JWH-018, JWH-073, JWH-081, JWH-210, rimonabant, and SR144528 were obtained from the National Institute on Drug Abuse (NIDA, Bethesda, MD) through the NIDA Drug Supply Program. JWH-167 and JWH-391 were synthesized by John Huffman (Clemson University, Clemson, SC). AM-2201 was purchased from Cayman Chemical (Ann Arbor, MI). For in vivo administration, all cannabinoid compounds were dissolved in a vehicle of 7.8% Polysorbate 80 N.F. (VWR, Marietta, GA) and 92.2% sterile saline USP (Butler Schein, Dublin, OH). Mianserin HCl was obtained from Sigma Aldrich (St. Louis, MO) and was dissolved in sterile saline USP. All compounds used in the FOB procedure were administered intravenously (i.v.) into the tail

vein at a volume of 10 ml/kg. Details of compound administration for the drug discrimination procedure are presented in section 2.6.

2.4 Receptor Binding and Function

Receptor binding profiles, K_i determinations, functional data (for 5-HT_{2B} receptor antagonism) and hERG data were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program (NIMH PDSP), Contract # HHSN-271-2013-00017-C. The NIMH PDSP is directed by Bryan L. Roth at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda MD, USA. Full experimental details are available on the PDSP web site http://pdsp.med.unc.edu/ (click on "Binding Assay" or "Functional Assay" on the menu bar). All results presented here are based upon secondary assessments that were conducted when compound met primary screening criteria. Values presented in the tables are the means of 3 replicates. Stably transfected cell lines expressing mainly human recombinant receptors, monoamine transporters, or ion channels were used for binding assays.

2.5 Functional Observational Battery (FOB)

The functional observational battery (FOB), modified from a procedure commonly used by the Environmental Protection Agency to evaluate potential toxins (Moser, 2011; Moser and Boyes, 1993), provides an overall behavioral profile of a compound that allows assessment of a wide range of effects. Upon arrival in the test room, mice were weighed and were randomly assigned to receive a specific dose of cannabinoid compound or vehicle (n=6/group). Each mouse was observed individually in its home cage immediately after i.v. injection with its assigned dose of compound / vehicle. Five min later, the mouse was placed into the open field, which was a clear Plexiglas container (33.0cm x 51.0cm x 23.0cm), and was scored individually on all measures over a period of 2 min by a single trained technician who was blind to treatment

condition. The technician scored the following list of behaviors, with the scoring scale for each behavior shown in parenthesis. Home cage observations were taken on activity (ordinal), ease of handling (categorical), lacrimation (ordinal), palpebral closure (ordinal), salivation (ordinal), muscle tone (categorical), piloerection (categorical), exophthalmus (categorical), and forelimb placing (categorical). Open field observations included rearing (number of times both forelimbs completely off surface; continuous), posture (categorical), convulsions (ordinal), flattened body (ordinal), gaping (ordinal), clonus (ordinal), tonus (ordinal), gait (categorical), alertness (ordinal), activity (ordinal), tail elevation (ordinal), writhing (ordinal), circling (ordinal), bizarre behaviors (categorical), stereotypic behaviors (categorical), defecation (continuous), and urination (continuous). Responses after two types of observer interference were recorded: approach response (categorical) and startle response (categorical).

In a preliminary follow-up investigation, evaluation of cannabinoid receptor mediation of the effects of 3 mg/kg JWH-018 in the FOB was undertaken. Procedural variables were the same as those described for the primary study, except that mice were injected with two compounds. The first compound was vehicle or a cannabinoid receptor antagonist (rimonabant for CB_1 receptor or SR144528 for CB_2 receptor), which was injected intraperitoneally (i.p.). Forty min later each mouse was injected i.v. with vehicle or JWH-018. Six groups of mice (n=5-6 mice/group) were tested: vehicle/vehicle, vehicle/JWH-018 (3 mg/kg), rimonabant (3 mg/kg)/vehicle, SR144528 (3 mg/kg)/vehicle, rimonabant (3 mg/kg)/JWH-018 (3 mg/kg), and SR144528 (3 mg/kg)/JWH-018 (3 mg/kg).

2.6 Drug Discrimination

Training in the mouse discrimination procedure was similar to that described previously (Vann et al., 2009). Briefly, two groups of mice were trained in a drug discrimination procedure. Each mouse was placed in a standard operant conditioning chamber with two response levers. Mice

were trained to respond on one of the two levers following intraperitoneal (i.p.) administration of 0.3 mg/kg JWH-018 (one group) or i.p. administration of 5.6 mg/kg THC (second group) and to respond on the other lever following i.p. vehicle injection according to a fixed ratio 10 (FR10) schedule of food reinforcement, under which 10 consecutive responses on the correct (injection-appropriate) lever resulted in delivery of a food pellet. Responses on the incorrect lever reset the ratio requirement on the correct lever. Daily injections were administered on a double alternation sequence of JWH-018 or THC and vehicle (e.g., drug, drug, vehicle, vehicle). Daily 15 min training sessions were held Monday-Friday until the mice consistently met three criteria: (1) the first completed FR10 was on the correct lever, (2) ≥ 80% of the total responding occurred on the correct lever, and (3) response rate must have been ≥ 0.17 responses/s. When the criteria were met, acquisition of the discrimination was established and substitution testing began.

Stimulus substitution tests were typically conducted on Tuesdays and Fridays during 15 min test sessions, with maintenance of training continuing on intervening days. During test sessions, 10 consecutive responses on either lever delivered reinforcement. If a mouse pressed the other lever prior to completing 10 responses on a lever, the ratio requirement on the original lever was reset. To be tested in the experiment, mice must have completed the first FR10 on the correct lever, ≥ 80% of the total responding must have occurred on the correct lever, and response rate must have been ≥ 0.17 responses/s during the prior day's training session. In addition, the mouse must have met these same criteria during previous training sessions with the alternate training compound (training drug or vehicle). The JWH-018-trained mice were drug-naïve at the start of the study. In these mice, substitution dose-effect curves were determined with JWH-018, THC, and JWH-073 followed by antagonism tests with rimonabant and SR144528. In addition, the 5-HT_{2B} antagonist mianserin was tested alone and in combination with the training dose of JWH-018. In the THC-trained mice, a dose-effect curve had been determined

previously with THC and with a putative allosteric modulator of the cannabinoid CB_1 receptor (data not shown). In these mice, mianserin was tested alone and in combination with THC. All cannabinoid compounds were administered i.p. at a volume of 10 ml/kg 30 min before the start of the discrimination test session. For antagonism tests, rimonabant and SR144528 were administered 10 min prior to administration of the agonist (i.e., 40 min pre-session). Mianserin was injected i.p. at a volume of 10 ml/kg 30 min before the start of the session (10 min before injection with vehicle, THC or JWH-018).

2.7 Metabolite Analysis

Immediately following the FOB test, mice (n=6) that had received the highest dose of each compound or vehicle were placed in metabolism cages and urine was collected over a 24 h period. Urine from mice dosed with the same compound was pooled for analysis. Samples were diluted 1:3 with acetonitrile, vortexed, and centrifuged for 5 min at 10,000 rcf. The samples were filtered through 0.22 µm spin filters (Agilent Technologies, Santa Clara, CA) prior to analysis.

Samples were analyzed on a Waters Acquity ultra performance liquid chromatography (UPLC) system coupled to a Waters Synapt G2 HDMS quadrupole time-of-flight (Q-TOF) mass spectrometer (Waters, Milford, MA). The mass spectrometer was operated under resolution mode, positive electrospray ionization, source temperature of 150℃, desolvation temperature of 500℃ , desolvation gas at 1,000 L/hr, capillary vo ltage at 2.99 kV, sampling cone at 35 V, and extraction cone at 4.3 V. The mass spectrometer was externally calibrated from 50 - 1000 m/z using a sodium formate solution. Leucine enkephalin was used as a lockmass to correct for mass shifts during acquisition. Full scan data was collected in both low (4 eV) and high (15 to 40 eV ramp) collision energies nearly simultaneously for every m/z using MSE acquisition mode (Bateman et al., 2002).

Samples were separated on an Acquity BEH C18 column (1.7 µm 2.1 x 50 mm) connected to a Vanguard BEH C18 pre-column (1.7 µm x 2.1 X 5 mm) and held at 30℃. Injection volume was 5 µL. A gradient elution with a flow rate of 500 µL/min was used with mobile phase A consisting of water with 0.1% formic acid and mobile phase B consisting of acetonitrile with 0.1% formic acid. The mobile phase composition was held at 90% A for 0.5 min and linearly decreased to 5% A over 10 min, increased to 90% A in 0.1 min and held for 2.9 min for re-equilibration.

Waters MassLynx 4.1 with the aid of the Metabolynx application manager was used to analyze acquired data. Metabolynx was used to automate the screening for expected and unexpected metabolites by their protonated ion exact mass and potential fragment ions at the same retention time. Automation was supplemented with manual interrogation using mass defect filtering, precursor ion and fragment ion searching techniques. Metabolites were provisionally identified by their molecular weight, retention time, and fragment ions. No reference standards were used for confirmation.

2.8 Data Analysis

Categorical variables were assessed as normal versus abnormal. Ordinal measures were scored using an ordinal scale, with 1 = normal/no drug effect and increasing numbers corresponding to increased intensity of drug effect. Continuous variables were measured as number of incidents of the event. None of the compounds produced the following responses: lacrimation, salivation, convulsions, writhing, circling, bizarre behaviors, stereotypic behaviors, defecation and urination. For this reason, these measures were omitted from further analysis. A vehicle control group was tested for each compound. Data for these control groups were combined into a single vehicle group, to which all compound doses were compared separately. Categorical variables were analyzed with Fisher's exact tests. Ordinal variables were analyzed

with Kruskal-Wallis tests and continuous variables, with one-way ANOVA. When an ANOVA was significant ($p<0.05$), Dunnett's post-hoc tests were used to specify differences from the vehicle control. For descriptive purposes, measures included in the FOB were grouped into five domains, as illustrated in Table 2: CNS activity, CNS excitability, autonomic effects, muscle tone/equilibrium and sensorimotor reactivity (for further details, see Bowen et al., 1996).

For each drug discrimination session, percentage of responses on the drug lever and response rate (responses/s) were calculated. Since mice that responded less than 10 times during a test session did not respond on either lever a sufficient number of times to earn a reinforcer, their data were excluded from analysis of drug lever selection, but response rate data were included. Response-rate data were analyzed using separate two-way repeated-measures (dose of training drug X mianserin dose) ANOVAs for each discrimination. Significant ANOVAs were further analyzed with Tukey post hoc tests ($\alpha = 0.05$) to specify differences between means.

3.0 Results

3.1 Receptor Binding

With the exception of JWH-391 (K_i >10,000 nM), all compounds had measurable binding affinity for the CB_1 cannabinoid receptor, with a range from 0.46 to 90 nM (Table 1) and a rank order of affinities from highest to lowest: JWH-210 > AM2201 > JWH-081 > JWH-018 = JWH-073 > THC > JWH-167 > JWH-391. All compounds also had affinity for the CB_2 cannabinoid receptor, with a range from 0.49 to 1236 nM (Table 1). Similar to THC, JWH-210 had approximately equal affinities for the two cannabinoid receptors whereas JWH-018 and JWH-391 had higher affinities for CB_2 cannabinoid receptors than for CB_1 cannabinoid receptors, albeit JWH-391 did not have much affinity for either receptor. The remaining compounds (JWH-073, AM-2201, JWH-081, and JWH-167) exhibited higher affinity for CB_1 (vs. CB_2) cannabinoid receptors.

In contrast with the relatively strong cannabinoid receptor affinities for most of the synthetic cannabinoids, binding affinities at noncannabinoid receptors were notably weak or negligible (Table 1). None of the compounds showed affinity for any of the norepinephrine, histamine, opioid, sigma, $GABA_A$ or benzodiazepine receptor sub-types. While THC showed affinity for the dopamine transporter, and THC and JWH-391 had measurable affinity for the muscarinic M_1 receptor, affinities at both binding sites were weak (> 400 nM). The noncannabinoid receptor system with which most of the cannabinoids interacted was serotonin, especially 5-HT_{2B}. With the exception of JWH-210 and JWH-081, each of the compounds had measurable binding affinity to one or more serotonin receptor subtype, albeit none had high affinity (K_is ranged from 184 to 4815 nM).

3.2 In Vitro Functional Assessment

Given the notably weak binding affinities at noncannabinoid receptors observed for this set of synthetic cannabinoids, in vitro functional assessment included only two pharmacological targets, one for 5-HT_{2B} agonism and antagonism, and the other for activity at the human Ether-à-go-go-Related Gene channel (hERG). All of the test compounds failed to activate $5HT_{2B}$ receptors in the agonist assay; however, with exception of JWH-081, all indole-derived cannabinoids were efficacious inhibitors of 5-HT_{2B} receptors, with E_{max} = 100% for each compound (Table 2). Consistent with the low 5-HT_{2B} binding affinities for these indole-derived synthetic cannabinoids, their potencies (IC_{50}s) for functional inhibition of serotonin agonist were relatively weak, ranging from 17.1 μM for JWH-391 to 2870 μM for JWH-210 (Table 2).

In addition to their weak inhibition of 5-HT_{2B} receptor activation, some of the synthetic cannabinoids also inhibited hERG channels (Table 2). Full efficacy at the hERG channel was exhibited by JWH-018, with efficacies of four other compounds ranging from 59-81%. Two compounds (JWH-081 and JWH-210) lacked efficacy in the hERG channel assay. For the

compounds that showed efficacy in this assay, potencies (EC_{50}s) were weak, with the highest potency (3.35 μM) shown by AM-2201.

3.3 FOB Results

Table 3 shows results of the FOB measures for which significant effects were obtained (compared to the vehicle group), including the doses at which the effects occurred. When appropriate (i.e., ordinal or continuous data), direction of effect is also indicated. Not shown are the measures that remained unaffected by any of the compounds: convulsions, circling, bizarre behaviors, stereotyped behaviors, lacrimation, salivation, urination, defecation, and writhing.

THC's effects were concentrated primarily in the CNS activity and muscle tone/equilibrium domains (Table 3). Over the entire range of doses tested (0.3 – 10 mg/kg), THC decreased alertness, rearing and activity, both in the home cage and open field. In the muscle tone/equilibrium domain, THC impaired posture and gait and increased the frequency of lying with the abdomen on the cage floor (i.e., flattened body), with each effect occurring at 2 or more THC doses. At lower THC doses, observation revealed primarily ataxia whereas limb splay was observed at higher doses. In contrast, THC did not produce any significant autonomic effects and did not significantly affect sensorimotor reactivity. Effects on CNS excitability were sporadic, with disrupted handling reactivity and increased tail elevation occurring at a single dose only (1 and 3 mg/kg, respectively).

Table 3 also shows the effects of seven indole-derived synthetic cannabinoids in the FOB. Consistent with its lack of affinity for the CB_1 receptor, JWH-391 had minimal effects in any of the FOB domains. In contrast, the other six synthetic cannabinoids (JWH-018, JWH-073, JWH-210, AM-2201, JWH-167 and JWH-081) shared THC's profile of effects in the CNS activity and muscle tone/equilibrium domains. In common with THC, these six compounds decreased

alertness, decreased rearing, and decreased activity in home cage and open field, with each effect occurring over the range of doses tested. Similarly, each compound impaired posture and gait (including limb splay and ataxia, respectively), and increased incidence of flattened body at most doses. Abnormal muscle tone and forelimb placement were also observed following administration of most of the synthetic cannabinoids. Hence, each of the psychoactive synthetic indole-derived cannabinoids exhibited a more prominent disruption of muscle tone than did THC.

As a group, these six compounds also possessed a broader profile of impairment, one which spanned the three other domains that were largely unaffected by THC: autonomic effects, CNS excitability, and sensorimotor reactivity (Table 3), albeit THC produced a limited increase in CNS excitability that was similar in nature to that of the synthetic cannabinoids, but occurred only at single doses. With the exception of JWH-081, each of the other five psychoactive synthetic cannabinoids increased CNS excitability over a wider range of measures and/or doses, as compared to THC. JWH-018 was especially active in this domain, with significant effects on each of the 4 measures included in the domain. In the sensorimotor reactivity domain, all six psychoactive synthetic cannabinoids (but not THC) disrupted the approach and startle responses at one or more doses. For example, upon presentation of the startle stimulus, jumping and vocalization were common reactions at higher doses of the compounds. Autonomic effects (particularly gaping and eyelid closure) were also prominent among the synthetic cannabinoids, but did not occur with THC or with JWH-073.

In a follow-up evaluation of cannabinoid receptor mediation of FOB effects, 3 mg/kg JWH-018 was tested alone and in combination with 3 mg/kg rimonabant and 3 mg/kg SR144528. Table 4 shows results of these treatments on selected observations from each domain: CNS activity (alertness), CNS excitability (ease of handling), autonomic effects (palpebral closure), muscle

tone/equilibrium (forelimb placement and muscle tone), and sensorimotor reactivity (approach and startle). Normal responses for each measure were observed for all mice in the vehicle/vehicle, rimonabant/vehicle, and SR144528/vehicle groups. In contrast, mice injected with 3 mg/kg JWH-018 exhibited abnormal responses, including decreased alertness, ease of handling, and disrupted sensorimotor reactivity. Hypertonia, absence of forelimb placement response, and palpebral closure were also observed in these mice. Whereas co-administration of 3 mg/kg rimonabant completely reversed JWH-018's effects on palpebral closure, forelimb placement, muscle tone, approach and startle, the combination resulted in normalization of alertness and ease of handling responses in only 33% of the mice, suggesting incomplete antagonism. Interestingly, 3 mg/kg SR144528, a CB_2 receptor antagonist, also blocked palpebral closure in all mice and reversed attenuation of startle response in half of the mice.

3.4 Drug Discrimination

JWH-018, JWH-073 and THC fully and dose-dependently substituted for the 0.3 mg/kg JWH-018 training dose (Figure 2, panel A). Whereas JWH-073 (ED_{50} = 2.11 μmol/kg, CI = 1.71 – 2.63 μmol/kg) was about 5-fold less potent than JWH-018 (ED_{50} = 0.38 μmol/kg, CI = 0.29 – 0.50 μmol/kg), THC (ED_{50} = 4.08 μmol/kg, CI = 3.06 – 5.45 μmol/kg) was nearly 10-fold less potent. Compared to vehicle, 1 mg/kg JWH-018 and 10 mg/kg THC significantly decreased response rates [$F(5,40)=12.61$, $p<0.05$ and $F(5,40)=15.78$, $p<0.05$, respectively] whereas significant decreases were not observed with JWH-073 across the dose range tested (Figure 2, panel B).

Figure 2 (panels C and D) shows the effects of antagonism tests with the CB_1 and CB_2 receptor antagonists, rimonabant and SR144528, respectively. When co-administered with 0.3 mg/kg JWH-018, 3 mg/kg rimonabant nearly eliminated responding on the JWH-018-associated lever (Figure 2, panel C) [interaction: $F(2,12)=30.93$, $p<0.05$]. In contrast, mice injected with 3 mg/kg

SR144528 and 0.3 mg/kg JWH-018 continued to respond on this lever without any significant attenuation (compared to JWH-018 alone) [Figure 2, panel C]. Response rates were significantly decreased by rimonabant [main effect: $F(2,12)=35.06$, $p<0.05$], but not by SR144528 (Figure 2, panel D).

Figure 3 shows the effects of mianserin on percentage of responding on the drug-associated lever and response rates in mice trained to discriminate THC (panels A and B, respectively) or JWH-018 (panels C and D, respectively) from vehicle. In THC-trained mice, mice responded predominantly on the THC-associated lever following administration of the THC training dose (5.6 mg/kg) and on the vehicle-associated lever after injection with vehicle (Figure 3, left side of panel A). Mianserin doses of 0.1, 0.3, and 3 mg/kg did not alter this pattern when co-administered with either vehicle (Fig. 3, panel A, unfilled bars) or 5.6 mg/kg THC (Figure 3, panel A, filled bars); however, at 1 mg/kg mianserin plus 5.6 mg/kg THC, percentage of THC-lever responding was significantly lower than at 5.6 mg/kg THC alone [Figure 3, panel A; $F(4,32)=5.28$, $p<0.05$]. In addition, overall responding on both levers was significantly suppressed by 3 mg/kg mianserin, regardless of whether mice were also administered vehicle or THC [Figure 3, panel B; $F(4,32)=5.43$, $p<0.05$].

Results in mice trained to discriminate 0.3 mg/kg JWH-018 from vehicle showed a similar pattern to the THC-discriminating mice at lower doses of mianserin. The training dose of JWH-018 substituted fully for itself (> 85% on the JWH-018-associated lever) whereas vehicle produced minimal responding on the JWH-018 lever (Figure 3, panel C). Substitution of the JWH-018 training dose (0.3 mg/kg) was not altered by mianserin at doses up 1 mg/kg; however, 3 mg/kg mianserin (plus vehicle) significantly increased responding on the JWH-018 lever (compared to vehicle alone) and significantly decreased responding on this lever when co-administered with 0.3 mg/kg JWH-018 (compared to 0.3 mg/kg JWH-018 alone) [Figure 3, panel

C; F(4,28)=10.73, p<0.05]. When administered with JWH-018, higher doses of mianserin (1 and 3 mg/kg) significantly suppressed overall response rate [Figure 3, panel D; F(4,28)=7.44, p<0.05].

3.5 Metabolite Identification

A summary of the metabolites found in urine from mice dosed with JWH-018, AM-2201, JWH-073, JWH-081, JWH-210, JWH-167 and JWH-391 is shown in Table 5, where I represents the phase 1 metabolites and II represents the phase II glucuronic acid conjugates. The primary metabolites excreted were monohydroxyls and monohydroxyl glucuronides, except no monohydroxyl glucuronide was observed for JWH-210. Observed fragment ions indicate that the site of monohydroxylation is on the alkyl indole structure for JWH-018 (m/z 230), AM-2201 (m/z 248), JWH-073 (m/z 344), JWH-081 (m/z 230), JWH-210 (m/z 183), JWH-167 (m/z 230), and on the 4-ethoxynaphthyl group of JWH-391 (m/z 215). AM-2201 underwent defluorination to form carboxylated JWH-018. JWH-081 and JWH-391 underwent demethylation and deethylation, respectively, to form 4-hydyoxynaphthyl and related metabolites.

4.0 Discussion

With the exception of JWH-391, all of the synthetic cannabinoids tested in the present study bind with high to moderate affinity to CB_1 and CB_2 receptors, as does THC (Table 1). Although the THC-like effects of synthetic cannabinoids have received the most attention, anecdotal reports suggest that these compounds also have a greater propensity to produce psychiatric disturbance than THC (Every-Palmer, 2011; Gunderson et al., 2012; Hermanns-Clausen et al., 2013). Mechanisms underlying these effects are largely unknown. To investigate this issue, several of the compounds identified early in the evolution of “Spice” were screened for binding affinity in a battery of receptor assays in the NIMH-funded Psychoactive Drug Screening Program. With the exception of $5\text{-}HT_{2B}$ receptors and a few other sporadic high nanomolar

binding values (Table 1), none of the compounds showed measurable affinities for any of the receptors assayed, including norepinephrine, dopamine, muscarinc acetylcholine, histamine, opioid, sigma, and $GABA_A$ receptors. Further, binding affinities for 5-HT_{2B} receptors were moderate (high nM at best), with functional tests showing that all compounds except JWH-081 were low-potency antagonists at this receptor. These results are consistent with an occasional study reporting that synthetic cannabinoids derived from different structural templates do not bind to other major noncannabinoid CNS receptors (Yao et al., 2009), and suggest that the psychological effects of the compounds are mediated through CB_1 receptor activation.

In addition to their psychological effects, synthetic cannabinoids have been reported to produce adverse cardiovascular and nephrotoxic effects (Center for Disease Control and Prevention, 2013; Hermanns-Clausen et al., 2013). While delineation of a full toxicological profile is beyond the scope of this study, some, but not all, indole-derived synthetic cannabinoids tested here inhibited hERG channels, with moderate to high efficacy, but low potency. hERG channel inhibition is associated with prolongation of QT intervals and ventricular tachyarrhythmia (Hancox et al., 2008), suggesting a possible mechanism for the cardiac disturbance sometimes observed in synthetic cannabinoid users admitted to hospital emergency departments (Hermanns-Clausen et al., 2013).

In the mouse FOB, THC's primary behavioral effects were concentrated in the CNS activation and muscle tone/equilibrium domains, with decreased alertness and posture impairment being prominent effects. This profile is consistent with previous research showing that THC and other psychoactive cannabinoids suppress locomotor activity and induce catalepsy in rodents (Martin et al., 1991) and produce ataxia in dogs (Beardsley et al., 1987) and ptosis in nonhuman primates (Beardsley et al., 1987). Anecdotal and clinical reports confirm that relaxation, drowsiness and reduced activity are also typically reported after acute marijuana use in humans

(Green et al., 2003). THC-induced autonomic effects were notably absent in the present study, which is consistent with marijuana's low acute toxicity in humans (Selden et al., 1990). Previously, the effects of THC on measures of activity and equilibrium in animal models have been shown to be blocked by rimonabant (Tseng and Craft, 2004; Varvel et al., 2005), suggesting that they are CB_1 receptor mediated. Six of the seven indole-derived synthetic cannabinoids exhibited higher CB_1 receptor affinity than THC, and not surprisingly, they also showed more prominent disruption of measures within these two FOB domains compared to other domains. This finding is consistent with the considerable observed overlap in the pharmacological effects of synthetic cannabinoids and THC in animals (Wiley et al., 1998; Wiley et al., 2014a) and the reported similarities in psychoactive effects in humans (Auwarter et al., 2009; Vardakou et al., 2010). The most likely mechanism for these effects is CB_1 receptor mediation, a hypothesis partly supported by the absence of these effects in mice that received JWH-391, a compound which did not bind to CB_1 or CB_2 receptors. In further support, CB_1 receptor antagonists have been shown to block the locomotor suppressive and cataleptic effects of synthetic cannabinoids (Wiebelhaus et al., 2012; Wiley et al., 2013).

Despite similarities of some of their effects to THC, as a group, the six psychoactive synthetic cannabinoids impaired behavior across a wider range of doses and domains, including three domains that were largely unaffected by THC: autonomic effects, CNS excitability, and sensorimotor reactivity. JWH-018 was especially active in the excitability domain, increasing excitability across all four measures. Similarly, with the exception of JWH-391, all synthetic cannabinoids, but not THC, disrupted normal sensorimotor reactivity. In humans, heightened arousal, enhanced reactivity, and avoidance are hallmark symptoms of anxiety disorders (Craske et al., 2009), suggesting that psychoactive synthetic cannabinoids may have a greater propensity to produce anxiety than does THC. Sympathetic arousal also occurs in anxiety and may produce symptoms such as tachycardia and nausea (Craske et al., 2009). In rats and mice

(i.e., species incapable of vomiting), nausea may be indicated by a gaping response (Parker and Limebeer, 2006). In the present study, several synthetic cannabinoids, but not THC, increased gaping, suggesting that their use may be associated with nausea. Case- and self-reports support occurrence of anxiety symptoms in users of synthetic cannabinoids (Brewer and Collins, 2014; Gunderson et al., 2012).

Mechanisms underlying the wider array of effects produced by synthetic cannabinoids are currently unknown. Given that their affinities for major noncannabinoid receptors tested here was low at best, the neural mechanisms underlying these distinctive behaviors may be related to their effects as cannabinoid agonists. For example, the CB_1 receptor binding affinities of synthetic cannabinoids are generally higher than THC (Manera et al., 2008), suggesting that they would have greater in vivo potency. Hence, had they been evaluated in the present study, higher doses of THC also may have produced a broader range of behaviors in the FOB. In fact, higher doses of THC have been associated with anxiety and dysphoria in anecdotal reports and a laboratory study (Hunault et al., 2014; Williamson and Evans, 2000). In addition, efficacy differences may also contribute to the findings. Whereas THC has been reported to be a partial agonist at CB_1 receptors in some in vitro functional assays (Breivogel and Childers, 2000), albeit not in others (Laaris et al., 2010), synthetic cannabinoids (to the extent that they have been tested) are full agonists (Huffman et al., 2005; Wiley et al., 2015; Wiley et al., 2013). To determine whether cannabinoid receptor activation might have contributed to the variant effects of synthetic cannabinoids in the FOB (compared to THC), JWH-018 was tested in combination with a CB_1 receptor antagonist, rimonabant, and a CB_2 receptor antagonist, SR144528. Results for this preliminary evaluation were mixed. Whereas rimonabant normalized JWH-018-disrupted effects in all mice in the domains of autonomic effects, muscle tone/equilibrium, and sensorimotor activity, it reversed JWH-018-induced decreases in alertness and ease of handling in only some of the mice. Further, SR144528 blocked palpebral closure produced by JWH-018,

suggesting that CB_2 receptors might also play a role in this effect. While caveats related to a single dose evaluation hold, these preliminary results suggest that at least some of the divergent behavioral effects of synthetic cannabinoids (compared to THC) may be related to their enhanced potencies and/or efficacies at cannabinoid receptors, albeit activity at other receptors or ion channels not measured herein is also possible.

Given the indole-derived structure of this JWH compound series, a possible role of the indolamine serotonin in an abuse-related effect of synthetic cannabinoids was explored. To this end, the 5-HT_2 receptor antagonist mianserin (Peroutka and Snyder, 1981) was assessed for substitution and antagonism in mice trained in cannabinoid discrimination, an animal model of subjective effects of cannabinoids in humans (Balster and Prescott, 1992). Mice were trained to discriminate either THC or JWH-018 from vehicle. The present THC discrimination results provide systematic replication of a previous study in rats which showed that compounds with serotonin antagonism properties did not substitute for or antagonize the discriminative stimulus effects of THC (Barrett et al., 1995). The pharmacological selectivity of JWH-018 has not yet been evaluated. Although previous research showed that THC and JWH-018 substituted and cross-substituted for each other in rats (Wiley et al., 2014b), the data presented herein is the first demonstration of JWH-018 discrimination in mice. As in the THC discrimination procedure, mianserin failed to substitute for or antagonize JWH-018's discriminative stimulus effects, suggesting lack of a 5-HT_{2B} component to JWH-018 discrimination. Since noncannabinoid compounds were not tested in the previous rat study, these data serve as the first negative control for this novel discrimination. By contrast, THC and the indole-derived synthetic cannabinoid JWH-073 dose-dependently substituted for JWH-018, with rank order potencies corresponding to their affinities for the CB_1 receptor. These results suggest that the JWH-018's discriminative stimulus effects are CB_1 receptor-mediated. Further confirmation of this hypothesis is achieved through demonstration that the CB_1 receptor antagonist rimonabant

blocks these effects whereas the CB_2 antagonist SR144528 does not. The substitution and antagonism test results for JWH-018 and THC mimic those observed in rodents trained to discriminate THC from vehicle (Gatch and Forster, 2014; Järbe et al., 2006), suggesting an identical mechanism of action for THC and JWH-018.

In addition to results of the extensive binding evaluation and in vivo assessments, the present study also provides tentative identification of synthetic cannabinoid urinary metabolites in mice. All compounds studied were extensively metabolized, with only a small amount of intact parent observed in the urine for mice dosed with JWH-081, JWH-210, and JWH-167. Monohydroxylation and monohydroxylation followed by conjugation with glucuronic acid were the predominate metabolites which is in agreement with published metabolite studies (Fantegrossi et al., 2014). Other metabolites identified (e.g., dihydrodiol) were similar to published reports (Moller et al., 2011; Sobolevsky et al., 2010; Wintermeyer et al., 2010). Prior studies have demonstrated that several of these first-generation synthetic cannabinoids, including JWH-018, JWH-073, and AM-2201, have multiple hydroxylated metabolites, which in some cases, retain affinity for and activity at CB_1 receptors (Brents et al., 2012; Brents et al., 2011; Chimalakonda et al., 2012). Although not assessed here, these metabolites may contribute in an additive and/or synergistic manner to the toxicity of these compounds relative to THC.

In summary, synthetic cannabinoids and THC produced a number of similar behavioral effects in mice in domains for which CB_1 receptor mediation has been shown previously (e.g., activity and muscle equilibrium, discriminative stimulus effects). However, synthetic cannabinoids also exhibited behaviors across a wider range of domains in the FOB than did THC. While preliminary evaluation suggests that some, but not all, of these divergent effects of synthetic cannabinoids may be related to their greater potencies and/or efficacies at CB_1 or CB_2

receptors, action(s) at noncannabinoid receptors that were not assessed in the present study or via different signaling pathways cannot be ruled out. In addition, the data reported herein were collected in male mice. The degree to which synthetic cannabinoids would produce a similar toxicity profile in females has not yet been investigated and is an area for future research.

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Figure Legends

Figure 1: Chemical structures of “first generation” synthetic cannabinoids.

Figure 2: Effects of JWH-018 (filled squares), THC (unfilled squares), and JWH-073 (filled circles) on percentage of responses that occurred on the JWH-018-associated lever (panel A) and response rate (panel B). Points above VEH and JWH represent the results of control tests with vehicle and 0.3 mg/kg JWH-018 conducted before each dose-effect determination. Panels C and D show the effects of antagonist tests with 3 mg/kg rimonabant (CB_1 antagonist/inverse agonist) and 3 mg/kg SR144528 (CB_2 antagonist) in combination with vehicle (unfilled bars) and 0.3 mg/kg JWH-018 (filled bars). Each point represents the mean (± SEM) of data for 7-9 male C57/Bl6J mice. Asterisks (*) indicate significant differences ($p<0.05$) compared to respective vehicle. Pound sign (#) indicates significant difference ($p<0.05$) compared to JWH-018 alone. Dollar sign ($) indicates significant main effect of rimonabant ($p<0.05$) treatment on response rates (compared to vehicle).

Figure 3: Effects of mianserin in mice trained to discriminate 5.6 mg/kg THC (panel A) or 0.3 mg/kg JWH-018 (panel C) from vehicle on percentage of responses that occurred on the drug-associated lever (panels A and C, respectively) and response rate (panels B and D, respectively). Filled bars show the effects of mianserin plus the training dose of THC (panel A and B) or JWH-018 (panel C and D) and unfilled bars show the effects of mianserin and vehicle. Each value represents the mean (± SEM) of data for 6-9 male C57/Bl6J mice, except for the following points: percentage of drug-lever responding at 3 mg/kg mianserin and 5.6 mg/kg THC (n=3; panel A) and at 1 and 3 mg/kg mianserin plus 0.3 mg/kg JWH-018 (n=5 and 3, respectively; panel C). Asterisks (*) indicate significant differences ($p<0.05$) compared to respective combination of mianserin dose and vehicle (unfilled bar). Pound sign (#) indicates significant difference ($p<0.05$) compared to respective vehicle (mianserin dose = 0) condition.

Dollar sign ($) indicates significant main effect of mianserin dose ($p<0.05$) on response rates (compared to vehicle).

Table 1. In vitro binding affinity of THC and synthetic indole-derived cannabinoids at a panel of neuronal receptors

Receptors	THC	AM-2201	JWH-018	JWH-073	JWH-081	JWH-167	JWH-210	JWH-391
Cannabinoid*: CB_1, CB_2	CB_1: 41 CB_2: 36	CB_1: 1 CB_2: 2.6	CB_1: 9 CB_2: 2.9	CB_1: 9 CB_2: 27	CB_1: 1.2 CB_2: 12	CB_1: 90 CB_2: 159	CB_1: 0.46 CB_2: 0.49	CB_1: > 10,000 CB_2: 1236
Serotonin: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_{5A}, 5-HT_6, 5-HT_7, SERT	5-HT_{2B}: 765	5-HT_{2B}: 192 5-HT_6: 693	5-HT_{2B}: 316	5-HT_{2B}: 184	NA	5-HT_{2B}: 1249 5-HT_{2C}: 4815	NA	5-HT_{2B4}: 368 5-HT_{2C}: 1899
Dopamine: D_1, D_2, D_3, D_4, D_5, DAT	DAT: 1575	NA	NA	NA	NA	NA	NA	NA
ACh Muscarinic: M_1, M_2, M_3, M_4, M_5	M_1: 1673	NA	NA	NA	NA	NA	NA	M_1: 461
GABA: PBR	NA	PBR: 3291	PBR: 673	NA	NA	NA	NA	NA

All binding affinities are presented as K_i (in nM) and are the mean of 3 replications. If a particular receptor sub-type is not listed for the compound, binding affinity of the compound was negligible for the receptor sub-type. Binding affinities of all compounds were negligible at the following receptors (i.e., did not "pass" primary binding screen or affinity > 10,000 nM in secondary screen): norepinephrine (α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B},α_{2C}, β_1, β_2, β_3, NET); histamine (H_1, H_2, H_3, H_4); opioid (κ, μ, δ); sigma (S1, S2); $GABA_A$; benzodiazepine. * CB_1 and CB_2 binding affinities are from the following sources: THC, JWH-018, JWH-073, JWH-081, and JWH-210 (Huffman and Padgett, 2005); JWH-167 (Huffman et al., 2005); AM-2201 (Makriyannis and Deng, 2005); JWH-391 (Wiley et al., 2014a).

Table 2. In vitro functional activity of synthetic indole-derived cannabinoids at 5-HT2B receptors and hERG channels

Functional Assays	AM-2201	JWH-018	JWH-073	JWH-081	JWH-167	JWH-210	JWH-391
5-HT_{2B} Antagonism[a]	IC_{50} = 23.9 E_{max} = 100%	IC_{50} = 33.2 E_{max} = 100%	IC_{50} = 19.3 E_{max} = 100%	Not active	IC_{50} = 47.7 E_{max} = 100%	IC_{50} = 2870 E_{max} = 100%	IC_{50} = 17.1 E_{max} = 100%
hERG Channels[b]	EC_{50} = 3.35 E_{max} = 70%	EC_{50} = 236 E_{max} = 100%	EC_{50} = 9.71 E_{max} = 59%	Not active	EC_{50} = 13.8 E_{max} = 81%	Not active	EC_{50} = 4.52 E_{max} = 59%

[a] Values are presented as IC_{50} (in μM) and are the mean of 3 replicates.
[b] Values are presented as EC_{50} (in μM) and are the mean of 3 replicates.

Table 3. Effects of THC and indole-derived synthetic cannabinoids in a FOB procedure*

Domain **Doses tested:**	**THC** **(0.3-10)**	**AM-2201** **(0.03-1)**	**JWH-018** **(0.1-3)**	**JWH-073** **(0.1-3)**	**JWH-081** **(0.03-1)**	**JWH-167** **(1-30)**	**JWH-210** **(0.1-3)**	**JWH-391** **(0.3-10)**
CNS Activity								
Alertness	↓ (0.3-10)	↓ (0.03-1)	↓ (0.1-3)	↓ (0.3-3)	↓ (0.3-1)	↓ (3-30)	↓ (0.1-3)	----
Rearing	↓ (0.3-10)	↓ (0.03-1)	↓ (0.1-3)	↓ (0.3-3)	↓ (0.3-1)	↓ (1-30)	↓ (0.1-3)	----
Home cage activity	↓ (0.3-10)	↓ (0.03-1)	↓ (0.1-3)	↓ (0.3-3)	↓ (0.1-1)	↓ (3-30)	↓ (0.1-3)	↓ (10)
Open field activity	↓ (0.3-10)	↓ (0.03-1)	↓ (0.1-3)	↓ (0.3-3)	↓ (0.3-1)	↓ (3-30)	↓ (0.1-3)	----
CNS Excitability [a]								
Handling reactivity	(1)	(0.1-1)	(0.3-3)	(1-3)	(1)	(3-30)	(0.3-3)	----
Clonus	----	----	↑ (0.3)	----	----	----	----	----
Tonus	----	↑ (0.3-1)	↑ (1)	----	----	↑ (10)	↑ (0.3 & 3)	----
Tail elevation	↑ (3)	----	↑ (1)	↑ (1)	----	----	----	----
Autonomic Effects [b]								
Gaping	----	↑ (1)	↑ (0.3-3)	----	↑ (1)	↑ (3 & 30)	----	----
Palpebral closure	----	↑ (0.3-1)	↑ (1-3)	----	↑ (0.1 & 1)	↑ (3-30)	↑ (1-3)	↑ (10)
Piloerection	----	----	----	----	----	(10-30)	(0.3 & 3)	----
Exophthalmus	----	----	----	----	----	(1)	(0.3)	----

Muscle tone / Equilibrium								
Muscle tone	----	(0.1-1)	(0.1-3)	(1-3)	(0.3)	(3-30)	(0.3-3)	----
Posture	(3-10)	(0.03-1)	(0.1-3)	(1-3)	(0.3-1)	(1-30)	(0.1-3)	----
Forelimb placing	----	----	(3)	(0.3)	----	(10-30)	(0.3 & 3)	----
Flattened body	↑ (0.3-10)	↑ (0.03-1)	↑ (0.1-3)	↑ (1-3)	↑ (0.1-1)	↑ (3-30)	↑ (0.1-3)	----
Gait	(0.3, 1 & 10)	(0.01-1)	(0.1-3)	(0.3-3)	(0.3-1)	(3-30)	(0.1-3)	----
Sensorimotor Reactivity								
Approach response	----	(0.1-1)	(0.3-3)	(1-3)	(1)	(3-30)	(0.1 & 0.3)	----
Startle response	----	(0.3 & 1)	(3)	(3)	(1)	(3-30)	(0.3-3)	----

[*] Doses at which significant differences (compared to vehicle) were observed are shown in parentheses in each cell. Arrows denote direction of effect compared to vehicle. N = 6 male mice / dose.

[a] CNS Excitability measures with no effect: convulsions, circling, bizarre behaviors, stereotyped behaviors.

[b] Autonomic measures with no effect: lacrimation, salivation, urination, defecation, writhing.

Table 4. Evaluation of antagonism of selected JWH-018 effects in a FOB procedure

Condition [b]	**Selected Measures** [a]					
	Alertness	Ease of Handling	Palpebral Closure	Forelimb Placement	Approach Response	Startle Response
Veh / Veh	100	100	100	100	100	100
Veh / JWH	0	0	20	0	0	0
Rim / JWH	33	33	100	100	100	100
SR2 / JWH	16	0	100	0	0	50

[a] Values represent % of mice showing a normal response for the measure (n=5 for Veh/JWH group and 6 for other groups).
[b] Results for the following conditions are shown: Vehicle / Vehicle (Veh/Veh), Vehicle / 3 mg/kg JWH-018 (Veh/JWH), Rimonabant 3 mg/kg / 3 mg/kg JWH-018 (Rim/JWH), and SR144528 3 mg/kg / 3 mg/kg JWH-018 (SR2/JWH). Mice were also tested with Rimonabant 3 mg/kg / Vehicle and SR144528 3 mg/kg / Vehicle; however, results for these two groups were the same as for the Veh/Veh group (data not shown).

Table 5. Phase I and phase II metabolites excreted in urine identified for indole-derived synthetic cannabinoids

Metabolites	JWH-018		AM-2201		JWH-073		JWH-081		JWH-167		JWH-210		JWH-391	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Parent							X		X		X			
Monohydroxylation	X	X	X	X	X	X	X	X	X	X	X		X	X
Dihydroxylation	X	X			X	X	X		X	X	X	X		
Carboxylation or dihydroxylation + desaturation	X	X			X	X			X	X	X			
Hydroxylation+carboxylation									X	X				
Dihydrodiol formation	X	X	X		X				X	X			X	X
N-dealkylation													X	
Monohydroxylation and dihydrodiol	X	X			X				X	X				
Monohydroxylation and desaturation									X				X	
Defluorination + carboxylation			X											
Demethylation							X	X						
Demethylation+hydroxylation							X	X						
Demethylation+hydroxylation+desaturation							X	X						
Deethylation													X	X
Deethylation+hydroxylation													X	X
Deethylation+dihydrodiol													X	X

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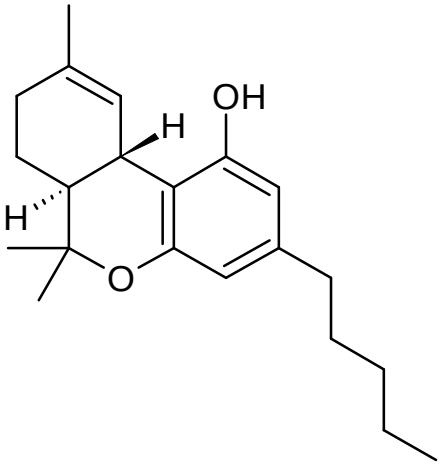

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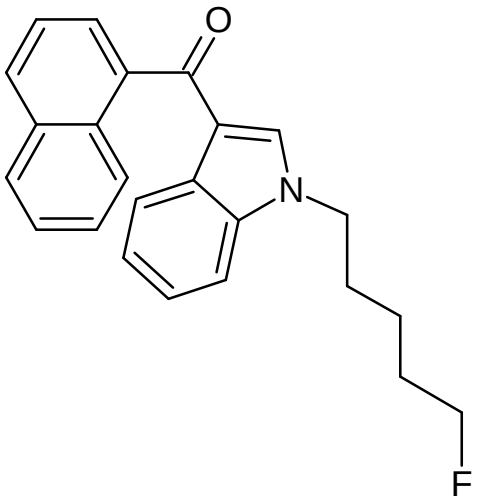

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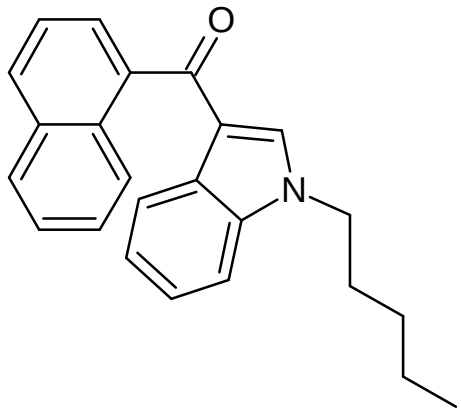

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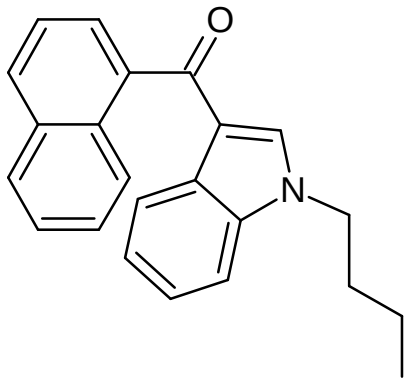

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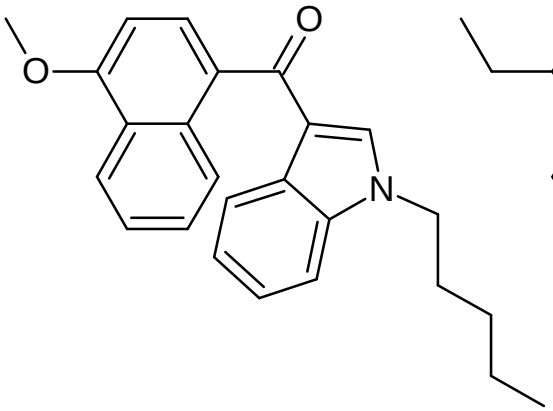

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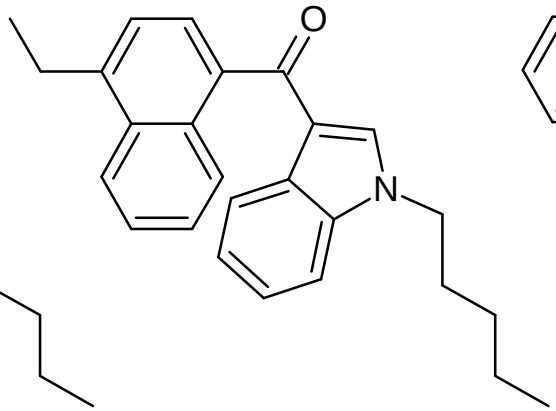

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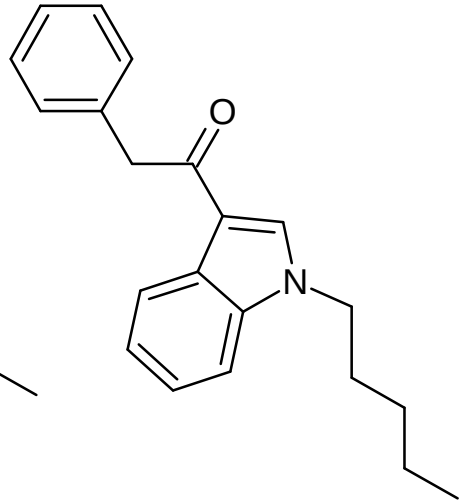

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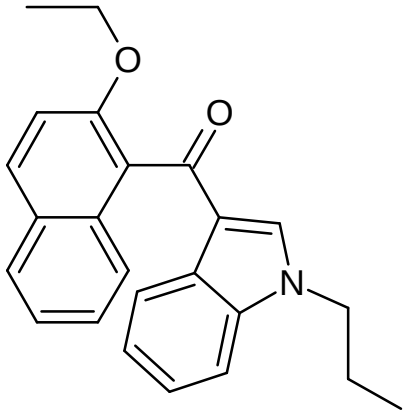

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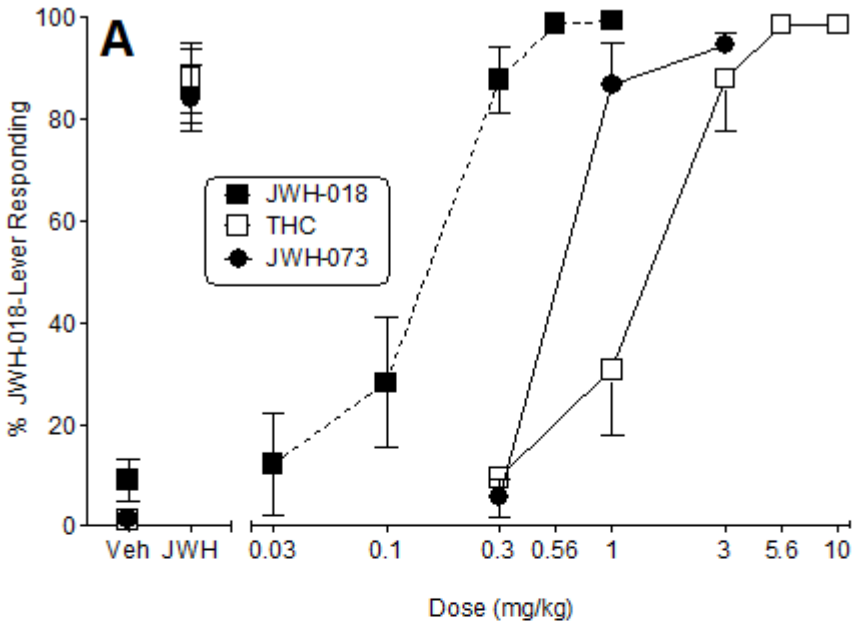
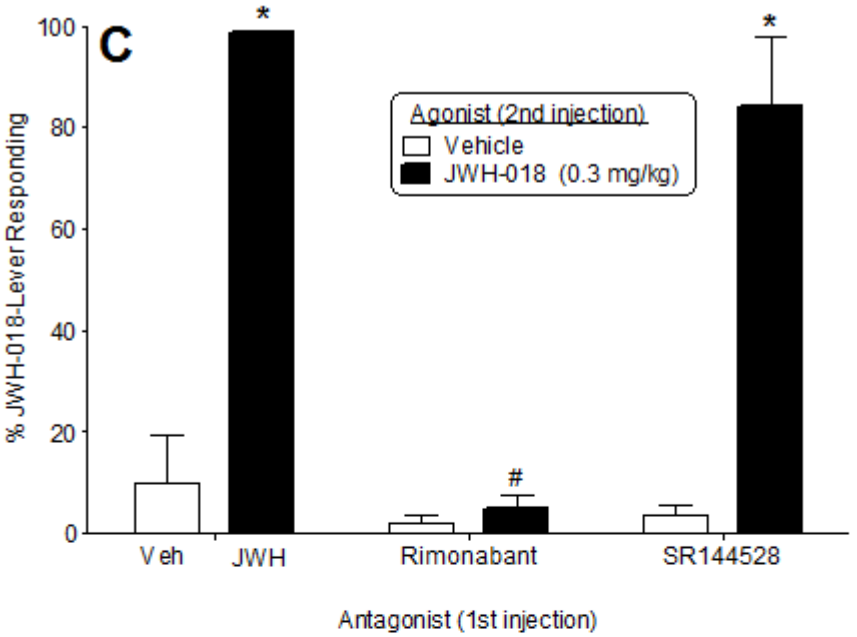
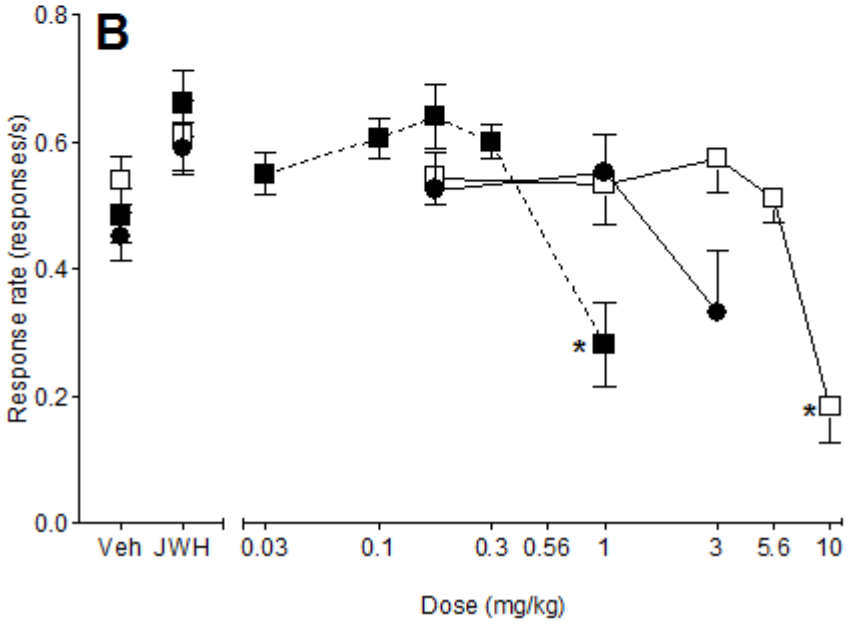
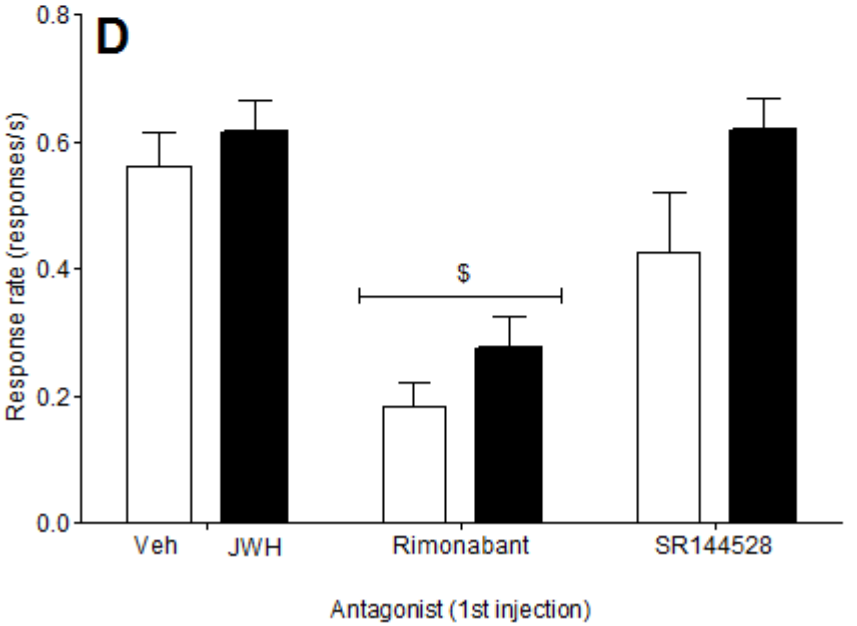
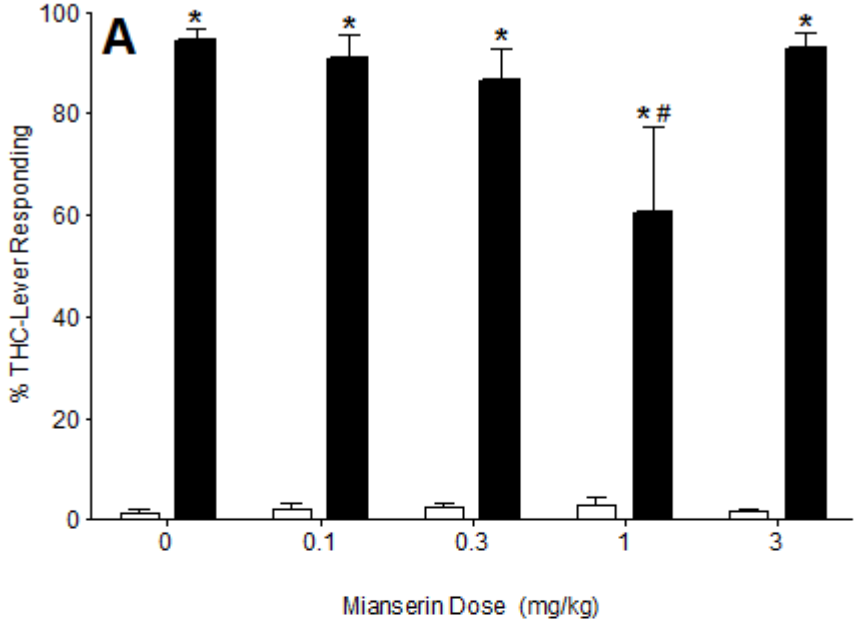
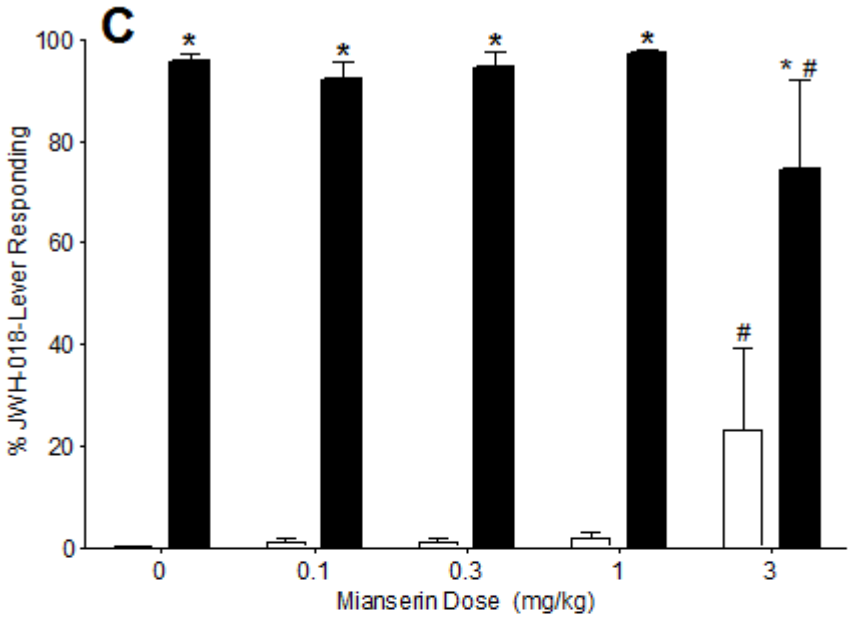

A
% JWH-018-Lever Responding
JWH-018
THC
JWH-073
Veh
JWH
0.03
0.1
0.3
0.56
1
3
5.6
10
Dose (mg/kg)

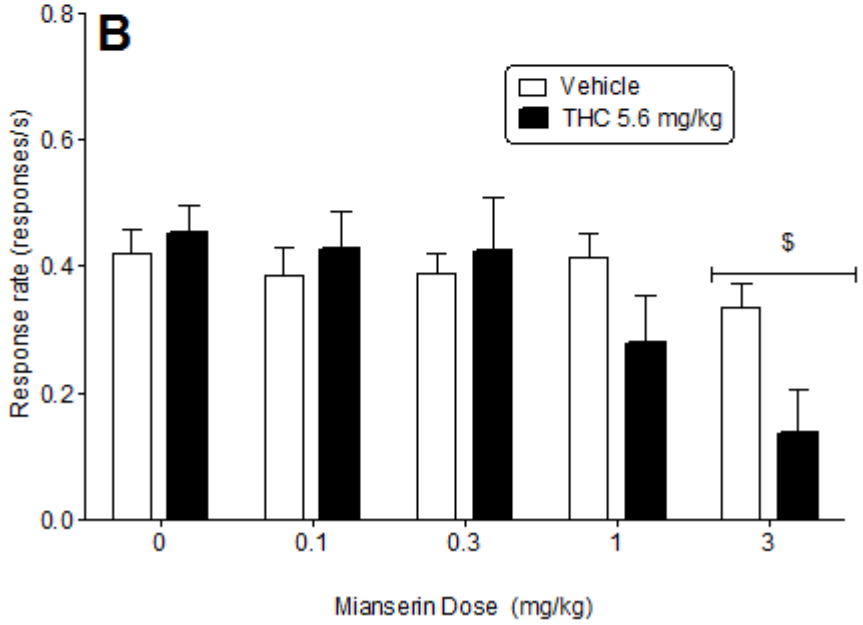

C
% JWH-018-Lever Responding
Agonist (2nd injection)
Vehicle
JWH-018 (0.3 mg/kg)
*
#
Veh
JWH
Rimonabant
SR144528
Antagonist (1st injection)

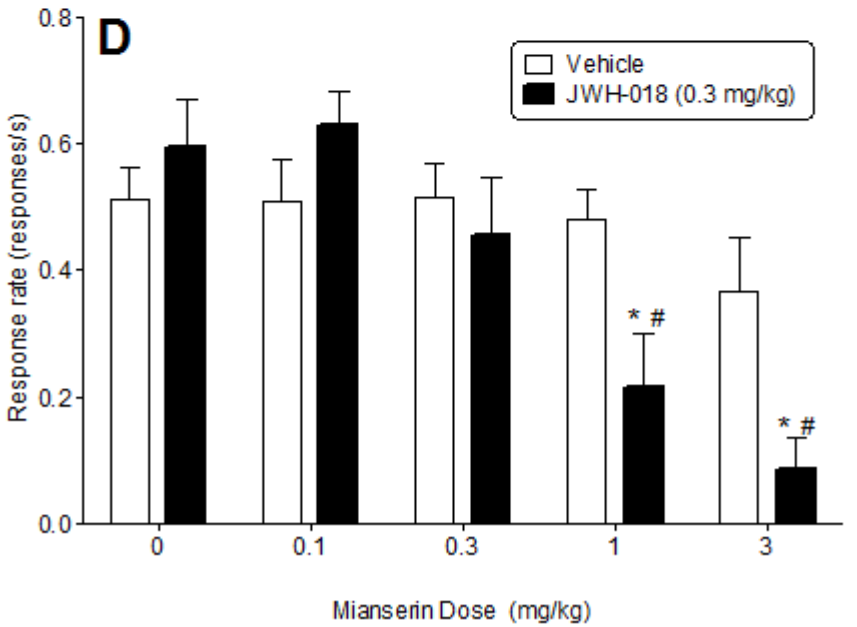

B
Response rate (responses/s)
Veh
JWH
0.03
0.1
0.3
0.56
1
3
5.6
10
Dose (mg/kg)


D
Response rate (responses/s)
$
Veh
JWH
Rimonabant
SR144528
Antagonist (1st injection)


A
% THC-Lever Responding
100
80
60
40
20
0
*
*
*
*#
*
0
0.1
0.3
1
3
Mianserin Dose (mg/kg)


C
% JWH-018-Lever Responding
100
80
60
40
20
0
*
*
*
*
*#
#
0
0.1
0.3
1
3
Mianserin Dose (mg/kg)


B
Response rate (responses/s)
0.8
0.6
0.4
0.2
0.0
Vehicle
THC 5.6 mg/kg
$
0
0.1
0.3
1
3
Mianserin Dose (mg/kg)


D
Response rate (responses/s)
0.8
0.6
0.4
0.2
0.0
Vehicle
JWH-018 (0.3 mg/kg)
*#
*#
0
0.1
0.3
1
3
Mianserin Dose (mg/kg)

Highlights

- Synthetic cannabinoids (SCBs) share some, but not all, behavioral effects with THC.
- SCBs show moderate to poor binding affinity at noncannabinoid receptors.
- JWH-018 and THC, but not mianserin, share discriminative stimulus effects in mice.
- SCBs are extensively metabolized, with identified urinary metabolites.