Published in final edited form as:

Psychopharmacology (Berl). 2016 May ; 233(10): 1901–1910. doi:10.1007/s00213-016-4237-6.

⁹-Tetrahydrocannabinol-Like Effects of Novel Synthetic Cannabinoids in Mice and Rats

Michael B. Gatch and Michael J. Forster

Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX

Abstract

Rationale—Novel cannabinoid compounds continue to be marketed as "legal" marijuana substitutes, even though little is known about their molecular and behavioral effects.

Objectives—Six of these compounds (ADBICA, ADB-PINACA, THJ-2201, RCS-4, JWH-122, JWH-210) were tested for in vitro and in vivo cannabinoid-like effects to determine their abuse liability.

Methods—Binding to and functional activity at CB1 cannabinoid receptors was tested. Locomotor activity in mice was tested to screen for behavioral activity and to identify behaviorally active dose ranges and times of peak effect. Discriminative stimulus effects of the six compounds were tested in rats trained to discriminate ⁹-tetrahydrocannabinol (⁹-THC).

Results—ADBICA, ADB-PINACA, THJ-2201, RCS-4, JWH-122, JWH-210 showed high affinity binding at the CB1 receptor at nanomolar affinities (0.59 nM to 22.5 nM) and all acted as full agonists with nanomolar potencies (0.024 to 111 nM) when compared to the CB1 receptor full agonist CP 55,940. All compounds depressed locomotor activity below 50% of vehicle responding, with depressant effects lasting 1.5 to nearly 4 h. All compounds fully substituted (<80% ⁹-THC-appropriate responding) for the discriminative stimulus effects of ⁹-THC. 3,4-Methylenedioxy-methamphetamine (MDMA) was tested as a negative control and did not substitute for ⁹-THC (11% ⁹-THC-appropriate responding).

Conclusions—All six of the compounds acted at the CB1 receptor and produced behavioral effects common to abused cannabinoid compounds, which suggest that these compounds have substantial abuse liability common to controlled synthetic cannabinoid compounds.

Keywords

cannabinoids; drug discrimination; locomotor activity; abuse liability; mouse; rat

Introduction

Use of designer drugs has been increasing, including the use of a variety of synthetic cannabinoids (Drug Enforcement Administration, 2014). Of even greater concern, these compounds have been increasingly used by youth with electronic "vaping" devices (Morean et al., 2015). In 2012, a group of 15 synthetic cannabinoids was classified as Schedule I compounds (Drug Enforcement Administration, 2013). These compounds act as agonists at CB1 cannabinoid receptors (Atwood et al., 2010; Atwood et al., 2011; Brents et al., 2012; Showalter et al., 1996). and produce behavioral effects similar to those of Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the major psychoactive compound found in marijuana (Brents et al., 2012; Gatch and Forster, 2014; 2015; Ginsburg et al., 2012; Järbe et al., 2011; Marshall et al., 2014; Wiley et al., 2013; 2014).

Although marijuana has a reputation as a fairly benign substance, the synthetic cannabinoids have been associated with serious toxicities including convulsions (Gugelmann et al., 2014), renal toxicity (Centers for Disease Control and Prevention, 2013; Buser et al., 2014), and sudden death (Behonick et al., 2014). In vitro studies have reported substantial cytotoxicity induced by several synthetic cannabinoids (inc., CP-55,940, CP-47,497, CP-47,497-C8, HU-210, JWH-018, JWH-073, JWH-122, JWH-210, AM-694, AM-2201, and MAM-2201). in a range of cell types and assays (Koller et al., 2013; Tomiyama and Funada, 2014). Taken together, these findings suggest that recreational use of the synthetic cannabinoids are more dangerous than marijuana use.

Cannabinoid recreational compounds more recently found on the street include ADBICA, ADB-PINACA, THJ-2201, RCS-4, JWH-122, and JWH-210. Structures are shown in Figure 1. ADB-PINACA and THJ-2201 have been temporarily scheduled into schedule I (DEA, 2014). Increasing use of JWH-122, JWH-210 and RCS-4 has been reported (Hermanns-Clausen et al., 2013; Lesiak et al., 2014; Tuv et al., 2014) and JWH-122 is one of the most commonly identified synthetic cannabinoids in specimens from users (Castaneto et al., 2015; Wohlfarth et al., 2015). ADB-PINACA is not as common, but there have been reports of toxicity associated with its use by recreational users. Anxiety, delirium, psychosis, aggressive behaviors and seizures were observed in 8 individuals who presented to an emergency room after smoking "Crazy Clown", which was found to contain ADB-PINACA (Schwartz et al., 2015). In Colorado, 76 patients presented to emergency rooms in a one-month period after smoking "black mamba", which also was found to contain ADB-PINACA (Monte et al., 2014). Adverse effects included altered mental status, cardiotoxicity and seizures.

There is little or no information available on the mechanisms of the behavioral effects of ADBICA, ADB-PINACA, THJ-2201, RCS-4. In contrast, the JWH-series compounds have been more thoroughly studied. Both JWH-122 and JWH-210 bind to the CB1 receptor (Cha et al., 2014; Huffman, et al., 2005). JWH-210 produced conditioned place preference at an intermediate dose and conditioned place aversion at higher doses (Cha et al., 2014). JWH-210 also produced full substitution in rats trained to discriminate Δ^9 -THC (Wiley et al., 2014). As mentioned previously, both JWH-122 and 210 produce cytotoxicity (Koller et al., 2013; Tomiyama and Funada, 2014). There is also evidence of severe adverse effects

from recreational use, including shock and myocardial damage in an individual using both JWH compounds in combination with caffeine (Nakamura et al., 2014), hyperemesis in an individual using several synthetic cannabinoids including JWH-018, JWH-073, JWH-122, AM-2201, and AM-694 (Hopkins and Gilchrist, 2013), and acute psychosis in an individual using JWH-122 in combination with the phenethylamine 1-benzofuran-6-ylpropan-2-amine (6-APB), a synthetic recreational compound with reputed psychostimulant and entactogenic effects (Chan et al., 2013).

The purpose of the present study was identify the potential abuse liability of a set of cannabinoids temporarily scheduled by DEA (ADBICA, ADB-PINACA, THJ-2201, RCS-4) and to collect additional and/or confirmatory data for two additional compounds whose use is increasing (JWH-122 and JWH-210). Abuse liability is determined by a number of properties, including: the chemical structure of the compound is closely related to those of any known substances of abuse, the chemical has a pharmacological mechanism shared by any known substances of abuse, and the chemical produces subjective effects similar to any known substances of abuse. Chemical structures are shown in Figure 1. CB1 receptor bindings was tested to demonstrate a pharmacological mechanism shared with 9 -THC. The drug discrimination assay is a well-validated animal model of the subjective effects of behaviorally-active compounds (Young, 2009; Horton et al., 2013). The six compounds were tested in rats trained to discriminate 9 -THC. Because little or no behavioral testing has been conducted with several of these compounds, 8-h tests for locomotor stimulant effects were conducted using multiple doses to identify active time course and dose ranges of these compounds. JWH-210 has been well-characterized in these assays and served as a positive control. In addition, MDMA was tested as a negative control in the drug discrimination assay.

Methods

Subjects

Male ND4 Swiss–Webster mice were obtained from Harlan Laboratories (Indianapolis, IN) at approximately 8 weeks of age and maintained in the University of North Texas Health Science Center (UNTHSC) animal facility for two weeks prior to testing. Mice were housed 3-4 per cage on a 12:12-h light/dark cycle (lights on at 7:00 AM) and were allowed free access to food and water except during test sessions. Male Sprague-Dawley rats were obtained from Harlan Laboratories. All rats were housed individually and were maintained on a 12:12 light/dark cycle (lights on at 7:00 AM). Body weights were maintained at 320-350 g by limiting food to 15 g/day, which included the food received during operant sessions. Water was continuously available in the home cage. All housing and procedures were in accordance with Guidelines for the Care and Use of Laboratory Animals (National Research Council, 2011) and were approved by the University of North Texas Health Science Center Animal Care and Use Committee.

Locomotor Activity

Each study was conducted using 32 Digiscan locomotor activity testing chambers ($40.5 \times 40.5 \times 30.5$ cm) (Omnitech Electronics, Columbus OH) each housed within a sound-

attenuating chamber that provided dim illumination. A panel of 16 infrared beams and corresponding photodetectors were located in the horizontal direction along the sides of each activity chamber. Separate groups of 8 mice were injected with either vehicle (ethanol/Cremophor EL/0.9% saline 1:1:18) or a cannabinoid: Δ^9 -THC (2.5 – 25 mg/kg), ADBICA (0.25 – 2.5 mg/kg), ADB-PINACA (0.1 – 1 mg/kg), THJ-2201 (0.1 – 1 mg/kg), RCS-4 (1 – 10 mg/kg), JWH-122 (0.1 – 1.0 mg/kg), or JWH-210 (0.5 – 5 mg/kg), immediately prior to locomotor activity testing. Only 7 mice were tested following the 0.25 mg/kg dose of JWH-122 due to equipment failure. Each dose range included doses that were without effect to those producing at least 50% depression below vehicle control. In all studies, horizontal activity (interruption of photocell beams) was measured for 8 hours within 10-min periods, beginning at 8:00 AM (1 h after lights on).

Discrimination Procedures

Standard behavior-testing chambers (Coulbourn Instruments, Allentown, PA) were connected to IBM-PC compatible computers via Med Associates interfaces (East Fairfield, VT). The computers were programmed in Med-PC for Windows, version IV (Med Associates, East Fairfield, VT) for the operation of the chambers and collection of data.

Rats were first trained to discriminate Δ^9 -THC (3 mg/kg) from vehicle (ethanol/Cremophor EL/0.9% saline 1:1:18) using a two-lever choice methodology. Thirty minutes prior to the training sessions, rats received an injection of either saline or Δ^9 -THC and were subsequently placed in the behavior-testing chambers, where food (45 mg food pellets; Bio-Serve, Frenchtown, NJ) was available as a reinforcer for every ten responses on a designated injection-appropriate lever. Each training session lasted a maximum of 10 min, and the rats could earn up to 20 food pellets. Rats were used in tests of substitution of the experimental compounds once they had achieved 9 of 10 sessions at 85% or greater injection-appropriate responding for both the first reinforcer and total session, which occurred after approximately 60 training sessions. The training sessions occurred on separate days in a double alternating fashion (drug-drug-vehicle-vehicle-drug; etc.) until the training phase was complete, after which substitution tests were introduced into the training schedule such that at least one vehicle and one drug session occurred between each test (drug-vehicle-test-vehicle-drug-test-drug; etc.). The substitution tests occurred only if the rats had achieved 85% injection-appropriate responding on the two prior training sessions.

Thirty-one rats drawn from a larger pool of Δ^9 -THC trained rats were used for testing the compounds in the present study. Five of the rats were used for testing with two of the test compounds. During test sessions, both levers were active, such that 10 consecutive responses on either lever led to reinforcement. For dose-effect experiments, data were collected until the first reinforcer was obtained, or for a maximum of 20 min. Each compound was tested in a separate group of six rats using a repeated-measures design such that each rat was tested at all doses of a given drug. Vehicle (1 ml/kg) and Δ^9 -THC (3 mg/kg) controls were tested before the start of each compound evaluation. Doses of ADBICA (0.025 – 0.25 mg/kg, 15 min pretreatment), ADB-PINACA (0.1 – 2.5 mg/kg, 60 min), THJ-2201 (0.05 – 0.5 mg/kg, 30 min), RCS-4 (0.5 – 50 mg/kg, 20 min), JWH-122 (0.1 – 1 mg/kg, 40 min), and JWH-210 (0.025 – 0.5 mg/kg, 30 min) were tested. A dose range was tested from no effect (<20% Δ^9 -

THC-appropriate responding) to full effect (80% ⁹-THC-appropriate responding or suppression of responding to less than 20% of vehicle control). Pretreatment times were based on the time of peak depression for each compound in the locomotor activity testing.

Binding

Binding to human recombinant CB1 receptors expressed in Chinese hamster ovary (CHO) cells was determined. Cell membrane homogenates were incubated with the radioligand in absence or presence of the test compound in a buffer. Nonspecific binding was determined in the presence of a specific agonist or antagonist at the target. Following incubation, the samples were filtered rapidly under vacuum through glass fiber filters presoaked in a buffer and rinsed several times with an ice-cold buffer using a 48-sample or 96-sample cell harvester. The filters were counted for radioactivity in a scintillation counter using a scintillation cocktail.

ADBICA, ADB-PINACA, RCS-4, JWH-122, and JWH-210 were screened at 100 nM (1.0E-07 M) and 10000 nM (1.0E-05 M) to determine appropriate concentration ranges for subsequent testing. They were then tested for binding at the CB1 receptor at 8 concentrations chosen between 3E-11 and 1E-06 M with first and last step of 1 log unit and middle steps of half logs and for functional activity at 8 concentrations between 1E-12 and 1E-08 M with a first step of 1 log unit and the remaining steps of half log for IC₅₀ and EC₅₀ determinations. Each experiment was conducted with duplicate wells. Two separate experiments were conducted for the binding and functional IC₅₀ and EC₅₀ studies.

Functional assays

Gi : Agonist effect—The CHO cells were suspended in HBSS buffer (Invitrogen) complemented with 20 mM HEPES (Invitrogen) (pH 7.4) then distributed in microplates and incubated at room temperature in the presence of HBSS (basal control), the reference agonist at one (stimulated control) or various concentrations (EC₅₀ determination), or the test compounds. Thereafter, the adenylyl cyclase activator NKH 477 was added to artificially increase cAMP concentration and allow agonist effect detection (decrease of cAMP levels). Following incubation, the cells were lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP antibody labeled with europium cryptate) were added. After 60 min at room temperature, the fluorescence transfer was measured at ex=337 nm and em=620 and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration was determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio).

Gi : Antagonist effect—The CHO cells were suspended in HBSS buffer (Invitrogen) complemented with 20 mM HEPES (Invitrogen) (pH 7.4) then distributed in microplates. The cells were preincubated for 5 min at room temperature in the presence of either of the following: HBSS (stimulated control), the reference antagonist at one (basal control) or various concentrations (IC₅₀ determination), or the test compounds. Thereafter, the reference agonist (CP 55940) and the adenylyl cyclase activator NKH 477 were added. For basal control measurements, reference agonist was omitted. Following incubation, the cells were lysed and the fluorescence acceptor (D2-labeled cAMP) and fluorescence donor (anti-cAMP

antibody labeled with europium cryptate) were added. After 60 min at room temperature, the fluorescence transfer was measured at $\text{ex}=337$ nm and $\text{em}=620$ and 665 nm using a microplate reader (Rubystar, BMG). The cAMP concentration was determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio).

Drugs

⁹-Tetrahydrocannabinol, ADBICA (N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1H-indole-3-carboxamide), ADB-PINACA (N-[1-(aminocarbonyl)-2,2-dimethylpropyl]-1-pentyl-1H-indazole-3-carboxamide), THJ-2201 ((1-(5-fluoropentyl)-1H-indazol-3-yl)(naphthalen-1-yl)methanone), RCS-4 ((4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone), JWH-122 ((4-methyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone), JWH-210 ((4-ethyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone) were provided by the National Institute on Drug Abuse Drug Supply Program. All drugs were dissolved in ethanol/Cremophor EL/0.9% saline (1:1:18) and were administered i.p. in a volume of 1 ml/kg.

Data Analysis

Locomotor activity data were expressed as the mean number of photocell counts in the horizontal plane (ambulation counts) during each 10-min period of testing. For each test compound, a 30-min period, beginning when maximal depression of locomotor activity first appeared as a function of dose, was used for analysis of dose-response data and calculation of ED_{50} values (dose producing 1/2 maximal depressant activity, where maximal depression = 0 counts/30 min). OriginGraph (OriginLab Corporation, Northampton, MA) was used to estimate the maximal depression induced by each cannabinoid. A two-way analysis of variance, with dose as a between-groups factor and time as a within-subjects factor, was conducted on horizontal activity counts/10 min interval.

Drug discrimination data are expressed as the mean percentage of drug-appropriate responses occurring in each test period. Rates of responding were expressed as a function of the number of responses made divided by the time to the first reinforcer. Graphs for percent drug-appropriate responding and response rate were plotted as a function of dose of test compound (log scale). Percent drug-appropriate responding was shown only if at least 3 rats completed the first fixed ratio, whereas all rats are shown for the response rate data. Full substitution was defined as 80% drug-appropriate responding and not statistically different from the training drug. The potencies of ADBICA, ADB-PINACA, THJ-2201, RCS-4, JWH-122, and JWH-210 were calculated by fitting straight lines to the dose-response data for each compound by means of OriginGraph (OriginLab Corporation, Northampton, MA). Straight lines were fitted to the linear portion of dose-effect curves, including not more than one dose producing <20% of the maximal effect and not more than one dose producing >80% of the maximal effect. Other doses were excluded from the analyses. Response-rate data were analyzed by one-way repeated measures analysis of variance. Effects of individual doses were compared to the vehicle control value using a priori contrasts. The criterion for significance was set a priori at $p<0.05$.

Compound binding was calculated as a % inhibition of the binding of a radioactively labeled ligand specific for each target. Cellular agonist effect was calculated as a % of control response to a known reference agonist (CP 55940) for each target and cellular antagonist effect was calculated as a % inhibition of control reference agonist response for each target. The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (nH) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using Hill equation curve. This analysis was performed using software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot® 4.0 for Windows® (© 1997 by SPSS Inc.). The inhibition constants (K_i) were calculated using the Cheng Prusoff equation. A Scatchard plot was used to determine the K_D .

For the functional assays, the results were expressed as a percent of control agonist response and as a percent inhibition of control agonist response in the presence of the test compound. The EC_{50} values (concentration producing a half-maximal response) and IC_{50} values (concentration causing a half-maximal inhibition of the control agonist response) were determined by non-linear regression analysis of the concentration-response curves generated with mean replicate values using Hill equation curve fitting software as described above.

Results

Data from the in vitro studies are summarized in table 1. Each of the compounds tested showed binding to the human CB1 receptor at nanomolar affinities, ranging from 0.59 nM (ADB-PINACA) to 22.5 nM (RCS-4). All of the test compounds acted as full agonists with nanomolar potencies, ranging from 0.024 (ADB-PINACA) to 111 nM (JWH-210). Maximum effects ranged from 97.4 to 101.9% of the effect produced by the reference compound, the full agonist CP 55940. Binding affinities and potencies of functional activity were not correlated ($R^2=0.01$, $p=0.86$).

Locomotor Activity

Figure 2 shows average horizontal activity counts/10 min as a function of time and dose (top to bottom panels). No compound produced effects lasting longer than 4 h, so only the first 4 h of the test sessions are shown. Table 2 shows the ED_{50} for each compound.

Treatment with 9 -THC resulted in time- and dose-dependent depression of locomotor activity following 10 and 25 mg/kg (Fig 2). A two-way analysis of variance conducted on horizontal activity counts/10 min indicated a significant effect of Treatment $F(4,35)=4.38$, $p=.006$, 10-Minute Periods $F(47,1645)=21.54$, $p<.001$, and the interaction of Periods and Treatment $F(188,1645)=1.96$, $p<.001$. Depressant effects of 10 and 25 mg/kg occurred within 10-20 min following injection and lasted 120-210 min. The period 30-60 min was selected for analysis of dose-response data because this was the time period in which 9 -THC produced maximal effects.

ADBICA produced time- and dose-dependent depression of locomotor activity following 0.5, 1, and 2.5 mg/kg. A two-way analysis of variance conducted on horizontal activity counts/10 min failed to indicate a significant effect of dose, although significant effects were

observed for the time $F(47,1645)=26.21$, $p<.001$, and the interaction of dose and time $F(188,1645)=2.18$, $p<.001$. Depressant effects of 0.5, 1, and 2.5 mg/kg occurred within 10 min following injection and reached the low point by 20 min. The effects lasted longer as dose increased, ranging from 30 to 70 min. Treatment with ADB-PINACA resulted in time- and dose-dependent depression of locomotor activity following 0.25, 0.5, and 1 mg/kg (Fig 2). A two-way analysis of variance conducted on horizontal activity counts/10 min failed to indicate a significant effect of dose, although significant effects were observed for time interval $F(47,1974)=29.00$, $p<.001$, and the interaction of dose and time $F(235,1974)=2.45$, $p<.001$. Depressant effects of 0.25, 0.5, and 1 mg/kg occurred within 10 min following injection, peaked at 20 min, and lasted 30-60 min.

Treatment with JWH-210 resulted in time- and dose-dependent depression of locomotor activity following 1 to 10 mg/kg. A two-way analysis of variance conducted on horizontal activity counts/10 min indicated a significant effect of Treatment $F(6,49)=5.52$, $p<.001$, 10-Minute Periods $F(47,2303)=35.80$, $p<.001$, and the interaction of Periods and Treatment $F(282,2303)=2.20$, $p<.001$. Depressant effects occurred within 30 minutes following injection and lasted 190 minutes. The 10 mg/kg dose did not increase the depression or increase its duration over that produced by 1 to 5 mg/kg JWH-210, and so is not shown on the figures to enhance clarity. Tremors were observed upon handling in 6 of 8 mice, 8 h following 10 mg/kg JWH-210. THJ-2201 produced time- and dose-dependent depression of locomotor activity following 0.5 and 1 mg/kg. A two-way analysis of variance conducted on horizontal activity counts/10 min failed to indicate a significant effect of dose, although significant effects were observed for the time interval $F(47,1645)=25.49$, $p<.001$, and the interaction of dose and time interval $F(188,1645)=1.78$, $p<.001$. Depressant effects of 0.5 and 1 mg/kg occurred within 10-20 min following injection, peaked at 30 min, and lasted 40-80 min.

Treatment with RCS-4 resulted in time- and dose-dependent depression of locomotor activity following 5 and 10 mg/kg (Fig 2). A two-way analysis of variance conducted on horizontal activity counts/10 min failed to indicate a significant effect of dose, but indicated a significant effect of time interval $F(47,1974)=34.46$, $p<.001$, and the interaction of dose and time interval $F(235,1974)=1.76$, $p<.001$. Depressant effects of 5 and 10 mg/kg occurred within 20 min following injection and lasted 20-50 min. JWH-122 produced time- and dose-dependent depression of locomotor activity following 0.5 and 1 mg/kg. A two-way analysis of variance conducted on horizontal activity counts/10 min failed to indicate a significant effect of dose, although significant effects were observed for the time intervals $F(47,1598)=21.59$, $p<.001$, and the interaction of dose and time intervals $F(188,1598)=1.76$, $p<.001$. Depressant effects of 0.5 and 1 mg/kg occurred within 40 minutes following injection and lasted longer (90 to 150 min) as dose increased.

Drug Discrimination

Each of the synthetic cannabinoids substituted for the discriminative stimulus effects of ⁹-THC (Figure 3). RCS-4 was less potent than the other compounds, with an ED_{50} of 37.9 mg/kg, and a 95% confidence interval that did not overlap any of the other compounds (Table 2). The ED_{50} values for the other compounds ranged from 0.11 to 1.15 mg/kg, and

all had overlapping 95% confidence intervals. JWH-210 [$F(5,25)=3.47$, $p=.016$] and RCS-4 [$F(7,35)=3.54$, $p=.006$] produced modest depression of rate of responding at doses which substituted. MDMA failed to substitute for the discriminative stimulus effects of Δ^9 -THC, producing a maximum of 11% THC-appropriate responding following 1.5 mg/kg. MDMA dose-dependently depressed rate of responding [$F(3,24)=17.214$, $p<.001$], such that 6 of 9 rats failed to complete the first fixed ratio following 3 mg/kg.

Discussion

ADBICA, ADB-PINACA, THJ-2201, RCS-4, JWH-122, JWH-210, six compounds assigned to schedule 1 by the United States Drug Enforcement Agency, each produced in vitro and behavioral effects similar to Δ^9 -THC. All of the compounds tested bound with nanomolar affinity to the CB1 receptor and acted as full agonists (THJ-2201 was not tested for binding). These findings are in accord with earlier research. Both JWH-122 and JWH-210 have been previously reported to bind to the CB1 receptor (Cha et al., 2014; Huffman, et al., 2005) as have most, if not all of the synthetic cannabinoid compounds used recreationally (Showalter et al., 1996; Atwood et al., 2010, 2011; Brents et al., 2012; Gatch and Forster, 2015).

Each of the compounds produced dose-dependent depression of locomotor activity similar to that of Δ^9 -THC and other synthetic cannabinoids (Wiley et al., 1998; 2013; Gatch and Forster, 2014; 2015). All of the test compounds except RCS-4 were more potent than Δ^9 -THC, which is in keeping with earlier observations that the synthetic cannabinoids are at least as potent as Δ^9 -THC, and some are as much as 100-fold more potent (Gatch and Forster, 2014; 2015). The newer compounds (ADBICA, ADB-PINACA, THJ-2201, RCS-4) had rapid onsets and the depressant effects lasted about an hour, whereas the JWH compounds had slower onset, and were much longer acting (2.5 to 3 h).

Each of the six compounds fully substituted for the discriminative stimulus effects of Δ^9 -THC, which agrees with an earlier report that JWH-210 produced full substitution in rats trained to discriminate Δ^9 -THC (Wiley et al., 2014). RCS-4 produced an unusual profile in that the data described an inverted U between 0.5 and 25 mg/kg, with 50 mg/kg producing full substitution. The 10 and 50 mg/kg doses were repeated in separate groups of rats (data not shown) with comparable results. It is possible that RCS-4 may have other pharmacological actions at sites other than the CB1 receptor. Pretreatment times and dose ranges for the drug discrimination assay were selected based on the time of peak depression in the locomotor activity assay. When the data for RCS-4 was not considered, there was a strong correlation in locomotor depressant and discriminative stimulus potencies in the present study ($R^2=0.98$, $p=0.0001$), despite the using mice for the locomotor activity and rats for the drug discrimination. Over the seventeen compounds we have tested, the correlation between locomotor depressant and discriminative stimulus potencies is weak, but statistically significant ($R^2=0.27$, $p=0.023$). A wide range of synthetic cannabinoids also substitute for the discriminative stimulus effects of Δ^9 -THC in studies conducted in our laboratory (Gatch et al. 2014; 2015) and in others with various species, including mice (Brents et al., 2012; Marshall et al., 2014), rats (Järbe et al., 2011; Wiley et al., 2013; 2014) and monkeys (Ginsburg et al., 2012).

Of the six compounds in the present study, only JWH-210 has also been tested in the conditioned place preference assay. JWH-210, along with Δ^9 -THC, JWH-073, JWH-081 produced conditioned place preference in ICR mice at intermediate doses and either no preference or aversion at high doses (Cha et al., 2014). Other synthetic cannabinoids such as WIN 55212-2 and HU210 also produce place aversion in adult Sprague-Dawley, Wistar or Lister rats (Chaperon et al., 1998; Cheer et al., 2000; Pandolfo et al., 2009), but produced a place preference in adolescent Sprague-Dawley rats (Carvalho et al., 2014) and both adolescent and adult SHR rats, a model of attention deficit disorders (Pandolfo et al., 2009). In addition, JWH-018 produced place aversion in NIH Swiss mice unless they had been pre-exposed to Δ^9 -THC, in which case, a place preference was observed (Hyatt and Fantegrossi, 2014). These findings are of potential relevance to human recreational use as they suggest that adolescents, people with ADHD, and those with experience with marijuana may be vulnerable to the reinforcing effects of synthetic cannabinoids. Generality of these findings to other synthetic cannabinoids will be of interest.

The newer synthetic compounds (ADBICA, ADB-PINACA, THJ-2201, RCS-4), along with the JWH compounds are sold as marijuana substitutes to recreational users. As seen in Figure 1, these compounds have structures similar to synthetic cannabinoids known to be abused and which are currently scheduled. All six of the compounds bind to CB1 receptors and act as agonists as does Δ^9 -THC and other abused synthetic cannabinoids, although Δ^9 -THC is only a partial agonist at CB1 receptors. Finally, all of the compounds produced discriminative stimulus effects similar to Δ^9 -THC. Taken together, these findings suggest that the six test compounds have substantial abuse liability similar to controlled synthetic cannabinoid compounds. Tremors were observed following JWH-210. For the remaining compounds, doses which decreased locomotor activity and produced Δ^9 -THC-like discriminative stimulus effects did not produce adverse effects, and therefore may produce little acute toxicity at lower doses. However, reports of cytotoxicity (Koller et al., 2013; Tomiyama and Funada, 2014) suggest that long-term and/or use of large doses of these synthetic cannabinoids with full agonist activity may have increased risks of adverse events. In addition, due to its unusual dose-effect curve, RCS-4 may produce effects at pharmacological sites other than CB1 receptors. How this will alter its patterns of use or adverse effects profile remain to be determined.

Acknowledgments

Funding was provided by the Addiction Treatment Discovery Program of the National Institute on Drug Abuse for the behavioral data (NIH N01DA-13-8908) and for the in vitro data (N01DA-13-9881). Program staff was involved in selection of compounds and test parameters. The ATDP had no further role in study design; the collection, analysis, and interpretation of data; or the writing of the report. They have granted permission for the submission of this data for publication.

References

- Atwood BK, Huffman J, Straiker A, Mackie K. JWH018, a common constituent of 'Spice' herbal blends, is a potent and efficacious cannabinoid CB receptor agonist. *Br J Pharmacol.* 2010; 160:585–593. [PubMed: 20100276]
- Atwood BK, Lee D, Straiker A, Widlansk ITS, Mackie K. CP47,497-C8 and JWH073, commonly found in 'Spice' herbal blends, are potent and efficacious CB(1) cannabinoid receptor agonists. *Eur J Pharmacol.* 2011; 659:139–14. [PubMed: 21333643]

- Behonick G, Shanks KG, Firchau DJ, Mathur G, Lynch CF, Nashelsky M, Jaskierny DJ, Meroueh C. Four Postmortem Case Reports with Quantitative Detection of the Synthetic Cannabinoid, 5F-PB-22. *J Anal Toxicol*. 2014; 38:559–562. [PubMed: 24876364]
- Brents LK, Gallus-Zawada A, Radomska-Pandya A, Vasiljevik T, Prisinzano TE, Fantegrossi WE, Moran JH, Prather PL. Monohydroxylated metabolites of the K2 synthetic cannabinoid JWH-073 retain intermediate to high cannabinoid 1 receptor (CB1R) affinity and exhibit neutral antagonist to partial agonist activity. *Biochem Pharmacol*. 2012; 83:952–961. [PubMed: 22266354]
- Buser GL, Gerona RR, Horowitz BZ, Vian KP, Troxell ML, Hendrickson RG, Houghton DC, Rozansky D, Su SW, Leman RF. Acute kidney injury associated with smoking synthetic cannabinoid. *Clin Toxicol (Phila)*. 2014; 52:664–673. [PubMed: 25089722]
- Carvalho AF, Reyes BA, Ramalhosa F, Sousa N, Van Bockstaele EJ. Repeated administration of a synthetic cannabinoid receptor agonist differentially affects cortical and accumbal neuronal morphology in adolescent and adult rats. *Brain Struct Funct*. 2014 2014 Oct 28. [Epub ahead of print].
- Castaneto MS, Scheidweiler KB, Gandhi A, Wohlfarth A, Klette KL, Martin TM, Huestis MA. Quantitative urine confirmatory testing for synthetic cannabinoids in randomly collected urine specimens. *Drug Test Anal*. 2015; 7:483–93. [PubMed: 25231213]
- Centers for Disease Control and Prevention. Acute kidney injury associated with synthetic cannabinoid use—multiple states, 2012. *Morb Mortal Wkly Rep*. 2013; 62:93–98.
- Cha HJ, Lee KW, Song MJ, Hyeon YJ, Hwang JY, Jang CG, Ahn JI, Jeon SH, Kim HU, Kim YH, Seong WK, Kang H, Yoo HS, Jeong HS. Dependence Potential of the Synthetic Cannabinoids JWH-073, JWH-081, and JWH-210: In Vivo and In Vitro Approaches. *Biomol Ther (Seoul)*. 2014; 22:363–9. [PubMed: 25143817]
- Chan WL(1), Wood DM, Hudson S, Dargan PI. Acute psychosis associated with recreational use of benzofuran 6-(2-aminopropyl)benzofuran (6-APB) and cannabis. *J Med Toxicol*. 2013; 9:278–81. [PubMed: 23733714]
- Chaperon F, Soubrié P, Puech AJ, Thiébot MH. Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. *Psychopharmacology (Berl)*. 1998; 135:324–32. [PubMed: 9539255]
- Cheer JF, Kendall DA, Marsden CA. Cannabinoid receptors and reward in the rat: a conditioned place preference study. *Psychopharmacology (Berl)*. 2000; 151:25–30. [PubMed: 10958113]
- Drug Enforcement Administration, Department of Justice. "Establishment of Drug Codes for 26 Substances. Final rule". *Federal Register*. 2013; 78(3):664–666. [PubMed: 23289157]
- Drug Enforcement Administration, Department of Justice. National Forensic Laboratory Information System Special Report: Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS, 2010–2013. U.S. Drug Enforcement Administration; Springfield, VA: 2014.
- Gatch MB, Forster MJ. 9-Tetrahydrocannabinol-like discriminative stimulus effects of compounds commonly found in K2/Spice. *Behav Pharmacol*. 2014; 25:750–757. [PubMed: 25325289]
- Gatch MB, Forster MJ. 9-Tetrahydrocannabinol-like effects of novel synthetic cannabinoids found on the gray market. *Behav Pharmacol*. 2015; 26:460–8. [PubMed: 26061356]
- Ginsburg BC, Schulze DR, Hrubá L, McMahon LR. JWH-018 and JWH-073: 9-tetrahydrocannabinol-like discriminative stimulus effects in monkeys. *J Pharmacol Exp Ther*. 2012; 340:37–45. [PubMed: 21965552]
- Gugelmann H, Gerona R, Li C, Tsutaoka B, Olson KR, Lung D. 'Crazy Monkey' poisons man and dog: human and canine seizures due to PB-22, a novel synthetic cannabinoid. *Clin Toxicol (Phila)*. 2014; 52:635–638. [PubMed: 24905571]
- Hermanns-Clausen M, Kneisel S, Szabo B, Auwärter V. Acute toxicity due to the confirmed consumption of synthetic cannabinoids: clinical and laboratory findings. *Addiction*. 2013; 108:534–44. [PubMed: 22971158]
- Hopkins CY, Gilchrist BL. A case of cannabinoid hyperemesis syndrome caused by synthetic cannabinoids. *J Emerg Med*. 2013; 45:544–6. [PubMed: 23890687]
- Horton DB, Potter DM, Mead AN. A translational pharmacology approach to understanding the predictive value of abuse potential assessments. *Behav Pharmacol*. 2013; 24:10–36. [PubMed: 23263483]

- Huffman JW1, Zengin G, Wu MJ, Lu J, Hynd G, Bushell K, Thompson AL, Bushell S, Tartal C, Hurst DP, Reggio PH, Selley DE, Cassidy MP, Wiley JL, Martin BR. Structure-activity relationships for 1-alkyl-3-(1-naphthoyl)indoles at the cannabinoid CB(1) and CB(2) receptors: steric and electronic effects of naphthoyl substituents. New highly selective CB(2) receptor agonists. *Bioorg Med Chem*. 2005; 13:89–112. [PubMed: 15582455]
- Hyatt WS, Fantegrossi WE. 9-THC exposure attenuates aversive effects and reveals appetitive effects of K2/'Spice' constituent JWH-018 in mice. *Behav Pharmacol*. 2014; 25:253–7. [PubMed: 24625557]
- Järbe TU, Deng H, Vadivel SK, Makriyannis A. Cannabinergic aminoalkylindoles, including AM678=JWH018 found in 'Spice', examined using drug (9)-tetrahydrocannabinol) discrimination for rats. *Behav Pharmacol*. 2011; 22:498–507. [PubMed: 21836461]
- Koller VJ, Zlabinger GJ, Auwärter V, Fuchs S, Knasmueller S. Toxicological profiles of selected synthetic cannabinoids showing high binding affinities to the cannabinoid receptor subtype CB1. *Arch Toxicol*. 2013; 87:1287–97. Erratum in *Arch Toxicol* 2013 Jul;87(7):1299. [PubMed: 23494106]
- Lesiak AD, Musah RA, Domin MA, Shepard JR. DART-MS as a preliminary screening method for "herbal incense": chemical analysis of synthetic cannabinoids. *J Forensic Sci*. 2014; 59:337–43. [PubMed: 24313746]
- Marshall R, Kearney-Ramos T, Brents LK, Hyatt WS, Tai S, Prather PL, Fantegrossi WE. In vivo effects of synthetic cannabinoids JWH-018 and JWH-073 and phytocannabinoid (9)-THC in mice: Inhalation versus intraperitoneal injection. *Pharmacol Biochem Behav*. 2014; 124:40–47. [PubMed: 24857780]
- Monte AA, Bronstein AC, Cao DJ, Heard KJ, Hoppe JA, Hoyte CO, Iwanicki JL, Lavonas EJ. An outbreak of exposure to a novel synthetic cannabinoid. *N Engl J Med*. 2014; 370:389–90. [PubMed: 24450915]
- Morean ME, Kong G, Camenga DR, Cavallo DA, Krishnan-Sarin S. High School Students' Use of Electronic Cigarettes to Vaporize Cannabis. *Pediatrics*. 2015; 136:611–6. [PubMed: 26347431]
- Nakamura Y, Nakano M, Nakamura M, Miyazaki D, Okamori S, Akuzawa H, Yuasa M. [Case of law-evading herbs poisoning that induced shock and myocardial damage]. *Chudoku Kenkyu*. 2014; 27:333–8. [PubMed: 25771668]
- National Research Council. Guide for the Care and Use of Laboratory Animals. The National Academies Press; Washington, D.C.; 2011.
- Pandolfo P, Vendruscolo LF, Sordi R, Takahashi RN. Cannabinoid-induced conditioned place preference in the spontaneously hypertensive rat-an animal model of attention deficit hyperactivity disorder. *Psychopharmacology (Berl)*. 2009; 205:319–26. [PubMed: 19407992]
- Schwartz MD, Trecki J, Edison LA, Steck AR, Arnold JK, Gerona RR. A common source outbreak of severe delirium associated with exposure to the novel synthetic cannabinoid ADB-PINACA. *J Emerg Med*. 2015; 48:573–80. [PubMed: 25726258]
- Showalter VM, Compton DR, Martin BR, Abood ME. Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *J Pharmacol Exp Ther*. 1996; 278:989–999. [PubMed: 8819477]
- Tomiyama K, Funada M. Cytotoxicity of synthetic cannabinoids on primary neuronal cells of the forebrain: the involvement of cannabinoid CB1 receptors and apoptotic cell death. *Toxicol Appl Pharmacol*. 2014; 274:17–23. [PubMed: 24211273]
- Tuv SS, Krabseth H, Karinen R, Olsen KM, Øiestad EL, Vindenes V. Prevalence of synthetic cannabinoids in blood samples from Norwegian drivers suspected of impaired driving during a seven weeks period. *Accid Anal Prev*. 2014; 62:26–31. [PubMed: 24129318]
- Wiley JL, Lefever TW, Cortes RA, Marusich JA. Cross-substitution of (9)-tetrahydrocannabinol and JWH-018 in drug discrimination in rats. *Pharmacol Biochem Behav*. 2014; 124:123–128. [PubMed: 24887450]
- Wiley JL, Marusich JA, Lefever TW, Grabenauer M, Moore KN, Thomas BF. Cannabinoids in disguise: 9-Tetrahydrocannabinol-like effects of tetramethylcyclopropyl ketone indoles. *Neuropharmacology*. 2013; 75:145–154. [PubMed: 23916483]

- Wohlfarth A, Scheidweiler KB, Castaneto M, Gandhi AS, Desrosiers NA, Klette KL, Martin TM, Huestis MA. Urinary prevalence, metabolite detection rates, temporal patterns and evaluation of suitable LC-MS/MS targets to document synthetic cannabinoid intake in US military urine specimens. *Chem Lab Med*. 2015; 53:423–34.
- Young, R. Drug Discrimination. In: Buccafuso, Jerry J., editor. *Methods of Behavior Analysis in Neuroscience*. 2nd. CRC Press, Taylor & Francis Group LLC; Boca Raton: 2009.

Figure 1.
Chemical structures of the six cannabinoid test compounds and Δ^9 -THC.

Figure 2. Time course of locomotor activity

Mean horizontal activity (Ambulation counts) as a function of time (10 min bins) and dose for each test compound (left to right). Only data from the first four hours are shown. Data are from independent groups of 8 mice per dose, except the 0.25 mg/kg dose of JWH-122 ($n=7$). * indicates depressant effects ($p < 0.05$) against vehicle control.

Figure 3. Substitution for the discriminative stimulus effects of Δ^9 -THC: Dose-Effect

Top panels show percentage of total responses made on the drug-appropriate lever. Bottom panels show rate of responding in responses per second (r/s). All of the cannabinoids fully substituted for the discriminative stimulus effects of Δ^9 -THC (>80% drug-appropriate responding). Cannabinoids, n=6 except where shown. MDMA, n=9, except where shown. Ctrl indicates vehicle and Δ^9 -THC control values. * indicates response rate different from vehicle control ($p < 0.05$).

Table 1

Binding affinity and functional activity of test compounds at human CB1 cannabinoid receptors expressed in Chinese hamster ovary (CHO) cells. Dose effect curves were tested in duplicate and the values shown are means of the two determinations, except JWH-210, which was tested in triplicate. ND=not determined. Maximum Effect (%) column shows the peak effect of each test compound relative to the comparison compound, the full agonist CP 55940.

Drug	hCB1 Binding K _i (nM)	Functional Activity EC ₅₀ (nM)	Maximum Effect (%)
ADBICA	1.3	0.38	97.4
ADB-PINACA	0.59	0.024	99.6
THJ-2201	ND	0.64	96.6
RCS-4	22.5	20	100.6
JWH-122	3.35	2.8	101.9
JWH-210	1.01	111	98.2
CP 55940	0.67	0.30	--

Table 2

ED₅₀ values (mg/kg) for discriminative stimulus effects of cannabinoids in THC-trained rats and locomotor depressant effects in mice. Data are depicted as mean with 95% confidence interval.

Drug	Drug Discrimination	Locomotor Activity
THC	1.15 (0.21 to 6.27)	12.99 (2.51 to 67.21)
ADBICA	0.11 (0.001 to 2.75)	1.07 (0.04 to 32)
ADB-PINACA	0.58 (0.05 to 6.33)	0.31 (0.07 to 1.5)
THJ-2201	0.15 (0.01 to 4.08)	0.53 (0.1 to 2.82)
RCS-4	37.9 (21.61 to 66.48)	5.54 (0.77 to 39.89)
JWH-122	0.31 (0.07 to 1.35)	0.61 (0.19 to 1.94)
JWH-210	0.11 (0.001 to 3.79)	0.78 (0.34 to 1.77)