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Finding Order in Chemical Chaos - Continuing Characterization of Synthetic Cannabinoid Receptor Agonists

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Abstract

Diversion of synthetic cannabinoids from the lab to drugs of abuse has become increasingly prevalent in recent years. Moreover, as earlier synthetic cannabinoids were banned, manufacturers introduced a new supply of novel compounds to serve as replacements. Hence, the chemical diversity of synthetic cannabinoid analogs has also rapidly increased. The present study examined 8 new synthetic cannabinoids: AM-1220, AM-2232, AM-2233, AM-679, EAM-2201, JWH-210, JHW-251, and MAM-2201. Each compound was assessed for binding affinity and functional activation of CB₁ and CB₂ receptors, and pharmacological equivalence with ⁹-tetrahydrocannabinol (THC) in THC drug discrimination. All compounds bound to and activated CB₁ and CB₂ receptors, although efficacy at the CB₂ receptor was reduced compared to that for the CB₁ receptor. Similarly, all compounds stimulated [³⁵S]GTPγS binding through the CB₁ receptor, and all compounds except AM-1220 and AM-2233 stimulated [³⁵S]GTPγS binding through the CB₂ receptor. Furthermore, these compounds, along with CP55,940, substituted for THC in THC drug discrimination. Rank order of potency in drug discrimination was correlated with CB₁ receptor binding affinity. Together, these results suggest that all test compounds share the THC-like subjective effects of marijuana. Interestingly, the most potent compounds in CB₁ binding in the present study were also the compounds that have been found recently in the U.S., MAM-2201, EAM-2201, JWH-210, AM-2233, and AM-1220. These results indicate that the evolution of the synthetic cannabinoid drug market may be focused toward compounds with increased potency.

Keywords

abuse liability; drug discrimination; receptor binding; synthetic cannabinoids; ⁹-tetrahydrocannabinol

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1.0 Introduction

Products containing synthetic cannabinoids, commonly sold as “K2” or “spice,” have proliferated the recreational drug market over the last decade. These products are purchased online as “legal” alternatives to marijuana (Baumann et al., 2014; Ford et al., 2017; White, 2017). As earlier synthetic cannabinoids were banned, manufacturers introduced a new supply of novel compounds to serve as replacements (Seely et al., 2012; Shanks et al., 2012), producing an ever-evolving drug market for synthetic cannabinoids, in which users often ingest unknown chemical substances (Knittel et al., 2016).

Because of this evolution, newer synthetic cannabinoids have been found in the recreational drug market throughout the world. Recently, AM-2232, AM-1220, AM-679, JWH-251 were detected in powder and herbal products, and in hair and serum samples throughout Europe (Gregori et al., 2013; Hermanns-Clausen et al., 2016; Hermanns-Clausen et al., 2013; Jankovics et al., 2012; Kavanagh et al., 2013; Langer et al., 2014; Salomone et al., 2014). JWH-210 was detected in urine samples from U.S. military personnel (Wohlfarth et al., 2015), and in herbal incense products in the U.S. (Logan et al., 2012; Seely et al., 2013). More recently, MAM-2201, EAM-2201, JWH-210, AM-2233, and AM-1220 have been detected in seized products in a number of U.S. regions (NDEWS, 2016a; NDEWS, 2016b; NDEWS, 2016c; NDEWS, 2016d). Additionally, MAM-2201, AM-2233, and AM-1220 were found in seized plant products in Australia (Blakey et al., 2016), and AM-2232, MAM-2201, and EAM-2201 were detected in illegal products in Japan (Uchiyama et al., 2013).

Ingesting synthetic cannabinoids can produce several adverse consequences. A combination of MAM-2201, AM-1220, and AM-2232 led to a fatality (Zaitso et al., 2015b), and a combination of 11 synthetic cannabinoids including, EAM-2201 and MAM-2201, led to diabetic ketoacidosis and death (Hess et al., 2015). JWH-210 produces immunosuppression, neurotoxicity in the nucleus accumbens, and cytotoxicity in brain, buccal, breast, and liver cells (Cha et al., 2015; Koller et al., 2013; Tomiyama & Funada, 2014; Yun et al., 2016). Furthermore, JWH-210 can cause central nervous system depression, cerebral seizures, and sympathomimetic toxicity (Hermanns-Clausen et al., 2016; Hermanns-Clausen et al., 2013). MAM-2201 suppresses neurotransmission, alters energy metabolism, inhibits CYP1A activity in liver, and produces cytotoxicity in forebrain (Ashino et al., 2014; Irie et al., 2015; Tauskela et al., 2016; Tomiyama & Funada, 2014; Zaitso et al., 2015a).

Contributing to their abuse potential, many synthetic cannabinoids produce ⁹-tetrahydrocannabinol-like (THC) effects. Multiple structural classes of psychoactive cannabinoids, including tetrahydrocannabinols, bicyclic cannabinoids (e.g., CP55,940), aminoalkylindoles (e.g., WIN55,212-2), and other indole- and pyrrole-derived cannabinoids (e.g., JWH-018), bind to and activate CB₁ receptors (Devane et al., 1988; Griffin et al., 1998). Furthermore, affinity and efficacy at the CB₁ receptor, is often correlated with potency in producing THC-like effects in vivo (Compton et al., 1993; Pertwee, 2005; Wiley et al., 1998), including locomotor suppression, antinociception, hypothermia, catalepsy, and THC-like discriminative stimulus effects (Barrett et al., 1995; Martin et al., 1991; Vann et al., 2009). Moreover, compounds that bind to and activate CB₁ receptors and produce

cannabimimetic effects in animal models frequently produce “marijuana-like” intoxication in humans (Balster & Prescott, 1992).

The objective of this study was to evaluate the pharmacology of eight synthetic cannabinoids, AM-1220, AM-2232, AM-2233, AM-679, EAM-2201, JWH-210, JHW-251, and MAM-2201 (Figure 1), many of which have been identified in recently confiscated products in the U.S. (NDEWS, 2016a; NDEWS, 2016b; NDEWS, 2016c; NDEWS, 2016d). Pharmacological assays were chosen based on their ability to predict cannabinoid abuse liability (Wiley & Martin, 2009). Each compound was assessed for binding affinity and functional activation of CB₁ and CB₂ receptors, and pharmacological equivalence with THC in THC drug discrimination.

2.0 Materials and Methods

2.1 Subjects

Singly housed male C57BL/6 inbred mice (20-25g; Jackson Laboratories, Bar Harbor, ME) were used for drug discrimination. Mice were maintained at 85-90% of free-feeding body weights by restricting daily ration of standard rodent chow. Mice were housed in temperature-controlled conditions (20–24 °C) with a 12 h standard light-dark cycle (lights on at 0600), and had ad libitum access to water in their home cages. All studies were carried out in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), the ARRIVE guidelines, and in accordance with our Institutional Animal Care and Use Committee (IACUC), and other federal and state regulations.

2.2 Drugs

THC [⁹-tetrahydrocannabinol] and CP55,940 [(*-*)-cis-3-[2-Hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol] were obtained from the National Institute on Drug Abuse (NIDA, Bethesda, MD) Drug Supply Program. AM-1220 [(*R*)-(1-((1-methylpiperidin-2-yl)methyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone], AM-2232 [5-(3-(1-naphthoyl)-1H-indol-1-yl)pentanenitrile], AM-2233 [1-[(*N*-methylpiperidin-2-yl)methyl]-3-(2-iodobenzoyl)indole], AM-679 [(2-iodophenyl)(1-pentyl-1H-indol-3-yl)methanone], EAM-2201 [(4-ethyl-1-naphthalenyl)[1-(5-fluoropentyl)-1H-indol-3-yl]-methanone], JWH-201 [4-ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone], JHW-251 [2-(2-methylphenyl)-1-(1-pentyl-1H-indol-3-yl)ethenone], and MAM-2201 [(1-(5-fluoropentyl)-1H-indol-3-yl)(4-methyl-1-naphthalenyl)-methanone] were provided by the Drug Enforcement Administration (DEA). For binding studies, cannabinoids were formulated in 1 mg/mL ethanol. For in vivo tests, cannabinoids were suspended in 7.8% Polysorbate 80 (VWR, Marietta, GA), and 92.2% saline (Butler Schein, Dublin, OH), and injected at a volume of 10 ml/kg.

Guanosine 5' diphosphate, bovine serum albumin, ammonium acetate, and formic acid were purchased from Sigma Chemical (St. Louis, MO). GTP γ S was purchased from Roche Diagnostics (Branchburg, NJ). [³⁵S]GTP γ S (1150-1300 Ci/mmol) and scintillation fluid (MicroScint 20) were obtained from Perkin Elmer (Waltham, MA). HPLC grade acetonitrile

and water were purchased from Fisher Scientific (Fairlawn, NJ). Reference standards were obtained from Cayman Chemical (Ann Arbor, MI). The cannabinoid receptor binding assay utilized human CB₁ (hCB₁) and human CB₂ (hCB₂) membrane preparations purchased from Perkin Elmer, isolated from a HEK-293 expression system.

2.3 Equipment

Drug discrimination experiments were conducted in mouse operant chambers (Coulbourn Instruments, Whitehall, PA), housed within light- and sound-attenuating cubicles. Chambers contained two nose poke apertures, with stimulus lights located over each aperture, and a house light. A pellet dispenser delivered 20-mg food pellets (Bioserv Inc., Frenchtown, NJ) into an illuminated food cup that was centered between the two apertures. Experimental events and recording of data were controlled by a computer-based system (Coulbourn Instruments, Graphic State Software, v 3.03, Whitehall, PA).

2.4 Receptor Binding

Binding assays were conducted as previously described (e.g., see Zhang et al., 2010). Binding was initiated by adding 40 fmol of cell membrane proteins to assay tubes containing [³H]CP55,940 (ca. 130 Ci/mmol), and a sufficient quantity of buffer A (50 mM Tris•HCl, 1 mM EDTA, 3 mM MgCl₂, 5 mg/mL BSA, pH 7.4) to create 0.5 mL incubation volume. In the displacement assays, the concentration of [³H]CP55,940 was 0.62 nM. Nonspecific binding was determined by including 10 μM unlabeled CP55,940. Following incubation at 30°C for 1 hr, binding was terminated by vacuum filtration through GF/C glass fiber filter plates (Packard, Meriden, CT) which were pre-soaked with 0.1% polyethyleneimine (PEI) in a 96-well sampling manifold (Millipore, Bedford, MA). Reaction vessels were washed twice with 4 mL of ice cold buffer (50 mM Tris•HCl, 1 mg/mL BSA). The filter plates were air-dried and sealed on the bottom. Liquid scintillate was added to the wells and the top sealed. After incubating the plates for a minimum of 2 hrs, the radioactivity present was determined by liquid scintillation spectrometry. Assays were done in duplicate, and results represent combined data from at least three independent experiments.

2.5 Agonist-Stimulated [³⁵S]GTPγS Binding

G-protein coupled signal transduction ([³⁵S]GTPγS) assays of test compounds were conducted in an incubation mixture consisting of a test compound (250 nM–1mM), GDP (20 μM), [³⁵S]GTPγS (100 pM), and the hCB₁ and hCB₂ membrane preparations (0.4 pM) in 0.4 mL of assay buffer [50 mM TRIS-HCl, pH 7.4, 1 mM EDTA, 100 mM NaCl, 5 mM MgCl₂, 0.5% (w/v) BSA]. Nonspecific binding was determined in 100 μM unlabeled GTPγS, and basal binding was determined in the absence of drug. Duplicate samples were incubated for 1 hr at 30°C, and the bound complex was filtered from the reaction mixture and counted in a liquid scintillation counter.

2.6 Drug Discrimination

Adult male mice (n=8) were trained to discriminate THC using previously described procedures (Vann et al., 2009). Prior to this investigation, mice were trained to respond on one of two nose poke apertures following i.p. administration of 5.6 mg/kg THC, and to

respond on the other aperture following i.p. vehicle injection (Wiley et al., 2015). Responding was reinforced on a fixed ratio 10 (FR10) schedule, under which 10 consecutive responses on the correct (injection-appropriate) aperture was reinforced with a food pellet. Responses on the incorrect aperture reset the ratio requirement on the correct aperture. Daily injections were administered on a double alternation sequence of THC and vehicle (e.g., drug, drug, vehicle, vehicle). Daily 15 min training sessions were conducted Monday-Friday until the mice consistently met three criteria: (1) the first completed FR10 was on the correct aperture, (2) 80% of the total responding occurred on the correct aperture, and (3) response rate was ≥ 0.17 responses/s. After the criteria were met, acquisition of the discrimination was established and substitution testing began.

Stimulus substitution tests were conducted up to twice a week during 15 min test sessions, with maintenance of training on intervening days. During test sessions, 10 consecutive responses on either aperture delivered reinforcement. To be tested, mice had to meet the 3 acquisition criteria on the preceding day and during the previous training session with the alternate training compound (training drug or vehicle). A dose-effect curve was determined with THC. Prior to the present study, mice were tested with other synthetic cannabinoids, as reported previously (Wiley et al., 2015). For the present study, the dose-effect curve for THC was redetermined, and dose-effect curves were obtained for JWH-210, EAM-2201, AM-1220, JHW-251, AM-2233, MAM-2201, AM-679, AM-2232, and CP55,940, in that order.

2.7 Binding Data Analysis

Specific binding was calculated as $[(\text{total binding} - \text{nonspecific binding})/(\text{total basal binding} - \text{nonspecific binding})]$. Non-specific binding was subtracted from each sample. Data were plotted and analyzed with GraphPad Prism (GraphPad Software, Inc., San Diego, CA). Displacement data were analyzed by unweighted nonlinear regression of receptor binding data. For displacement studies, curve-fitting and K_i calculation were made with GraphPad Prism, which fits the data to one- and two-site models, and compares the two fits statistically.

K_i values were estimated from IC_{50} values using the Cheng-Prusoff equation. Data for $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding experiments are reported as mean and standard error of at least three replicates. Net stimulated $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding was defined as agonist-stimulated minus basal $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding, and percent stimulation was defined as $(\text{net-stimulated}/\text{basal } [^{35}\text{S}]\text{GTP}\gamma\text{S binding}) \times 100$. Nonlinear iterative regression analyses of agonist concentration-effect curves were performed with GraphPad Prism. Significance was defined as $p < 0.05$.

2.8 Drug Discrimination Data Analysis

During the study, some mice were removed from the study due to non-drug-related health problems. Eight mice completed testing of THC, JWH-210, EAM-2201, AM-1220, and JWH-251; 7 mice completed testing of AM-2233 and MAM-2201; 6 mice completed testing of AM-679 and AM-2232; 5 mice completed testing of CP55,940. For each session, percentage of responses on the drug-associated aperture and response rate (responses/s) were

calculated. Full substitution was defined as 80% responding on the drug-associated aperture (Vann et al., 2009). ED₅₀ values were calculated on the linear part of the drug aperture selection dose-response curve for each drug using least squares linear regression analysis, followed by calculation of 95% confidence intervals (CI). Since mice that responded fewer than 10 times during a test session did not earn a reinforcer, their data were excluded from analysis of drug aperture selection, but their response rate data were included. Response-rate data were analyzed using repeated-measures ANOVA across dose. A mean substitution procedure was used to maintain equal n's across doses within drug in the case of missing data (i.e., one mouse was tested with all doses of JWH-251 except 3 mg/kg; one mouse was tested with all doses of CP55,940 except 0.3 mg/kg). Significant ANOVAs were further analyzed with Tukey post hoc tests ($\alpha = 0.05$) to specify differences between means.

Pearson product-moment coefficients were computed to assess the relationship between in vitro affinity, efficacy, and drug discrimination ED₅₀ values. Pair-wise removal of missing values was used.

3.0 Results

3.1 Cannabinoid Receptor Binding and Agonist-Stimulated [³⁵S]GTP γ S Binding

All test compounds displaced [³H]CP55,940 at the CB₁ receptor binding site (Figure 2, panels A and B), with affinities ranging from 0.41 nM for EAM-2201 to 39.6 nM for AM-679 (Table 1). All test compounds also stimulated [³⁵S]GTP γ S turnover in hCB₁ transfected cell membranes (Figure 2, panels C and D). The high affinity positive control CP55,940 was the most potent compound at the CB₁ receptor binding site, followed by MAM-2201, AM-1220, and EAM-2201 (Table 1). However, MAM-2201, JWH-210, EAM-2201, JWH-251, and AM-2232 exhibited enhanced efficacy at CB₁ compared to CP55,940 (Table 1). The compounds with the lowest affinities for the CB₁ receptor, AM2322, JWH-251, and AM-679, also had the lowest potencies in the [³⁵S]GTP γ S assay, albeit even these compounds were able to increase [³⁵S]GTP γ S binding over basal levels.

Similar to their effects at the CB₁ receptor, all test compounds displaced [³H]CP55,940 at the CB₂ receptor binding site (Figure 3, panels A and B), with affinities that were similar to or greater than those obtained at the CB₁ receptor (Table 1). In contrast with their high affinities for the CB₂ receptor, all test compounds and CP55,940 showed reduced efficacy at CB₂ compared to CB₁ (Figure 3, panels C and D). Maximal CB₂ efficacy was observed with CP55,940, and slightly lower efficacies were observed for JWH-210 and EAM-2201. However, potencies for stimulating [³⁵S]GTP γ S turnover at the CB₂ receptor varied greatly across the compounds (Table 1). Whereas MAM-2201 was approximately 4-fold more potent than CP55,940, other compounds (including several high affinity hCB₂ ligands, such as AM-1220) failed to fully stimulate the CB₂ receptor at high concentrations, such that potency and efficacy could only be extrapolated, or were not determinable.

3.2 Drug Discrimination

Mice trained to discriminate THC from vehicle showed full, dose-dependent substitution for THC, and full substitution at the 5.6 mg/kg THC training dose (Figure 4, panels A-B). All

synthetic cannabinoids also fully substituted for THC, and all showed dose-dependent substitution except EAM-2201, which produced a bitonic function with substitution at the middle dose (Figure 4, panels A-B). Rank order of potency for substitution was EAM-2201 > CP55,940 > MAM-2201 > AM-1220 > JWH-210 > AM-2232 > AM-2233 > JWH-251 > THC > AM-679, which was similar to the rank order potency for CB₁ binding affinity (Table 1). During control tests of vehicle and 5.6 mg/kg THC, mice responded predominantly on the vehicle- and THC-associated apertures, respectively, across the course of the study (Figure 4, panels A-B).

All synthetic cannabinoids substituted for THC at a minimum of one dose that did not significantly reduce response rate. Although full substitution also occurred at 0.3 mg/kg MAM-2201, 1.0 mg/kg AM-2232, and 0.3 mg/kg CP55,940, these effects were accompanied by reductions in response rates [MAM-2201: $F(3,18)=17.06$, $p<0.05$; AM-2232: $F(3,15)=10.19$, $p<0.05$; CP55,940: $F(4,16)=28.30$, $p<0.05$] (Figure 4, panels C-D). There was also a significant effect of dose on response rate for EAM-2201 [$F(3,21)=7.48$, $p<0.05$], but no doses produced significant differences from vehicle. THC did not alter response rates compared to vehicle across the dose range tested (Figure 4, panels C-D).

Pearson product-moment coefficients were computed between each in vitro dependent variable and drug discrimination ED₅₀ values to demonstrate which in vitro measure was most highly associated with in vivo subjective effects. There was a significant positive correlation between CB₁ receptor binding affinity and drug discrimination ED₅₀ values [$r(18)=0.93$, $p < 0.05$]. No other in vitro measure was significantly correlated with drug discrimination ED₅₀ values.

4.0 Discussion

All test compounds bound to the CB₁ receptor with nanomolar affinity, and activated G-proteins, similar to CP55,940 (present study) and other psychoactive cannabinoids, including phytocannabinoids (Thomas et al., 1998), aminoalkylindoles (Compton et al., 1992a), and bicyclic cannabinoids (Compton et al., 1992b). All eight test compounds also bound to the CB₂ receptor with nanomolar affinity, although efficacy at the CB₂ receptor was reduced compared to the CB₁ receptor, which was also true for the positive control CP55,940. These results are in accordance with previous studies which found that EAM-2201, MAM-2201, JWH-210, and JWH-251 are potent and effective agonists at the CB₁ receptor (Cannaert et al., 2016; Cha et al., 2014; Cha et al., 2015; Costain et al., 2016; Gatch & Forster, 2016; Huffman et al., 2005), and MAM-2201, JWH-210, and EAM-2201 also activate the CB₂ receptor (Cannaert et al., 2016). While the present study found that JWH-251 had an affinity of 15 ± 3 nM for the hCB₂ receptor, a previous study using similar methods reported a lower CB₁ affinity of 146 ± 36 nM (Huffman et al., 2005). The reason for this discrepancy is unknown, however, the estimates for the CB₁ affinity of JWH-251 were less dissimilar between the two laboratories (16 ± 3 nM vs 29 ± 3 nM), despite having been evaluated in CB₁ receptors derived from different species (rat in previous study and human in current study).

Interestingly, AM-1220 and AM-2233 showed high affinity for the CB₂ receptor, but did not stimulate [³⁵S]GTPγS binding through the CB₂ receptor. AM-1220 and AM-2233 are racemic mixtures, and their profile of affinity and efficacy suggests that the (piperidin-2-yl)methyl side chain may permit ligand binding, but favors or promotes conformations of the CB₂ receptor that do not promote G-protein activation. The racemic nature of AM-2233 also affects binding affinity, with most of AM-2233's CB₁ receptor affinity originating from the (R)-enantiomer (Deng et al., 2005). Given these findings, it is possible that AM-1220 and AM-2233 can act as CB₁ receptor agonists and CB₂ receptor antagonists, depending upon the tone of the endocannabinoid signaling system.

All test compounds substituted fully for THC in drug discrimination. Only two of the test compounds were previously examined for in vivo pharmacological effects. In accordance with the present study, JWH-210 decreased locomotor activity and balance, and produced conditioned place preference (Cha et al., 2014; Gatch & Forster, 2016), and both JWH-210 and AM-2233 substituted for THC in drug discrimination in rats (Gatch & Forster, 2016; Jarbe et al., 2011; Wiley et al., 2014). Furthermore, results of the present study are consistent with research showing that other synthetic cannabinoids, including JWH-018, JWH-073, JWH-200, JWH-203, JWH-250, AM-2201, CP 47,497-C8-homolog (Gatch & Forster, 2014), ADBICA, ADB-PINACA, THJ-2201, RCS-4, JWH-122, JWH-210 (Gatch & Forster, 2016), XLR-11, UR-144 (Wiley et al., 2013), AB-CHMINACA, AB-PINACA, and FUBIMINA (Wiley et al., 2015), also substituted for THC in drug discrimination.

The THC discrimination assay is a pharmacologically selective animal model that predicts marijuana-like subjective effects of novel compounds in humans (Balster & Prescott, 1992; Jarbe & Gifford, 2014). Its pharmacological selectivity has been empirically demonstrated in mice (McMahon et al., 2008; Vann et al., 2009), rats (Barrett et al., 1995), nonhuman primates (Wiley et al., 1995), and humans (Lile et al., 2009). Results of the present study indicate that all test compounds likely share the THC-like subjective effects that humans have reported when using other synthetic cannabinoids (Gunderson et al., 2012). Additionally, rank order potency of substitution for THC in drug discrimination was positively correlated with affinity for the CB₁ receptor in the present study. These results suggest that the discriminative stimulus effects of the test compounds are CB₁ receptor-mediated. Future studies could confirm this by examining if the CB₁ receptor antagonist rimonabant blocks these discriminative stimulus effects. The CB₁ receptor affinity of these synthetic cannabinoids correlated with their potency in the drug discrimination assay; however, this was not observed with CB₂ receptor affinity. These findings are consistent with previous observations that the discriminative stimulus effects of these synthetic cannabinoids are CB₁ receptor dependent, as verified by the CB₁R-selective inverse agonist/antagonist rimonabant eliciting rightward shifts in the dose-response curve, and pA₂ and pK_B analyses for quantitative confirmation of CB₁R mediation (Grim et al., 2016; Grim et al., 2017; Hrubá & McMahon, 2017).

The present study is the first to examine the in vivo pharmacology of AM-1220, AM-2232, AM-679, EAM-2201, JHW-251, and MAM-2201. Demonstrating rewarding or reinforcing effects of THC and other cannabinoids using common preclinical measures of abuse liability (e.g., self-administration, conditioned place preference, and intra-cranial self-stimulation) is

particularly challenging, and results have been inconsistent (Chaperon et al., 1998; Gardner et al., 1988; Lefever et al., 2014; Valjent & Maldonado, 2000; Vlachou et al., 2007). Hence, drug discrimination is particularly useful for measuring abuse liability of cannabinoids. Like those before them, these synthetic cannabinoids produced THC-like effects, suggesting high abuse liability. The potential for abuse may be particularly high for EAM-2201 and MAM-2201 because they are some of the most potent synthetic cannabinoids that have been found on the drug market to date (Cannaert et al., 2016). Furthermore, EAM-2201 and MAM-2201 have been found recently in the U.S. and Australia (Blakey et al., 2016; NDEWS, 2016a; NDEWS, 2016b; NDEWS, 2016d), both of which have not yet formally controlled these compounds, albeit controlling specific compounds is not necessarily the best solution because it would likely engender a new generation of synthetic cannabinoids on the drug market (Knittel et al., 2016).

5.0 Conclusion

In summary, all test compounds produced THC-like effects in cannabinoid receptor binding and efficacy, and in THC drug discrimination, with few departures from effects of the positive control compounds CP55,940 and THC. The primary difference between compounds was potency. All synthetic cannabinoids tested here were more potent than THC in CB₁ and CB₂ binding and drug discrimination, similar to many other synthetic cannabinoids (for a review see Jarbe & Raghav, 2017; Wiley et al., 2017), with the exception being that AM-679 was slightly less potent than THC in drug discrimination. Together, these results suggest that all test compounds would share the THC-like subjective effects of marijuana. These effects are consistent with the detection of these products in several countries (Blakey et al., 2016; Gregori et al., 2013; Hermanns-Clausen et al., 2016; Hermanns-Clausen et al., 2013; Jankovics et al., 2012; Kavanagh et al., 2013; Langer et al., 2014; Salomone et al., 2014; Uchiyama et al., 2013), including detection in recently seized products in the U.S. (NDEWS, 2016a; NDEWS, 2016b; NDEWS, 2016c; NDEWS, 2016d). Interestingly, the most potent compounds in CB₁ binding in the present study were also the compounds that have been found recently in the U.S., MAM-2201, EAM-2201, JWH-210, AM-2233, and AM-1220, suggesting that the evolution of the synthetic cannabinoid drug market may be focused toward more potent compounds.

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Abbreviations

AM-1220	(R)-(1-((1-methylpiperidin-2-yl)methyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone
AM-2232	5-(3-(1-naphthoyl)-1H-indol-1-yl)pentanenitrile
AM-2233	1-[(N-methylpiperidin-2-yl)methyl]-3-(2-iodobenzoyl)indole
AM-679	(2-iodophenyl)(1-pentyl-1H-indol-3-yl)methanone
CP55,940	(-)-cis-3-[2-Hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol
EAM-2201	(4-ethyl-1-naphthalenyl)[1-(5-fluoropentyl)-1H-indol-3-yl]-methanone
GTP	guanosine-5'-triphosphate
JWH-210	4-ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone
JHW-251	2-(2-methylphenyl)-1-(1-pentyl-1H-indol-3-yl)ethenone
MAM-2201	(1-(5-fluoropentyl)-1H-indol-3-yl)(4-methyl-1-naphthalenyl)-methanone
THC	⁹ -tetrahydrocannabinol

- The present study examined 8 new synthetic cannabinoids for pharmacological effects.
- All compounds bound to and activated CB₁ and CB₂ receptors.
- These compounds substituted for THC in THC drug discrimination.
- Potency in drug discrimination was correlated with CB₁ receptor binding affinity.
- The drug market is trending toward synthetic cannabinoids with increased potency.

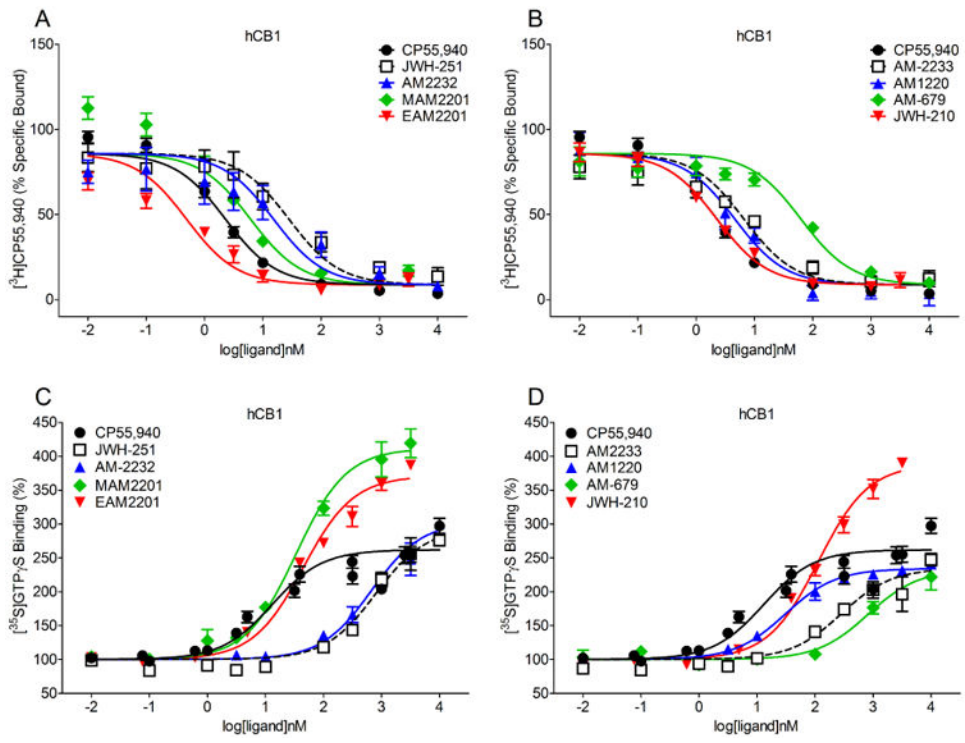


Figure 1.
Chemical structures of test compounds.

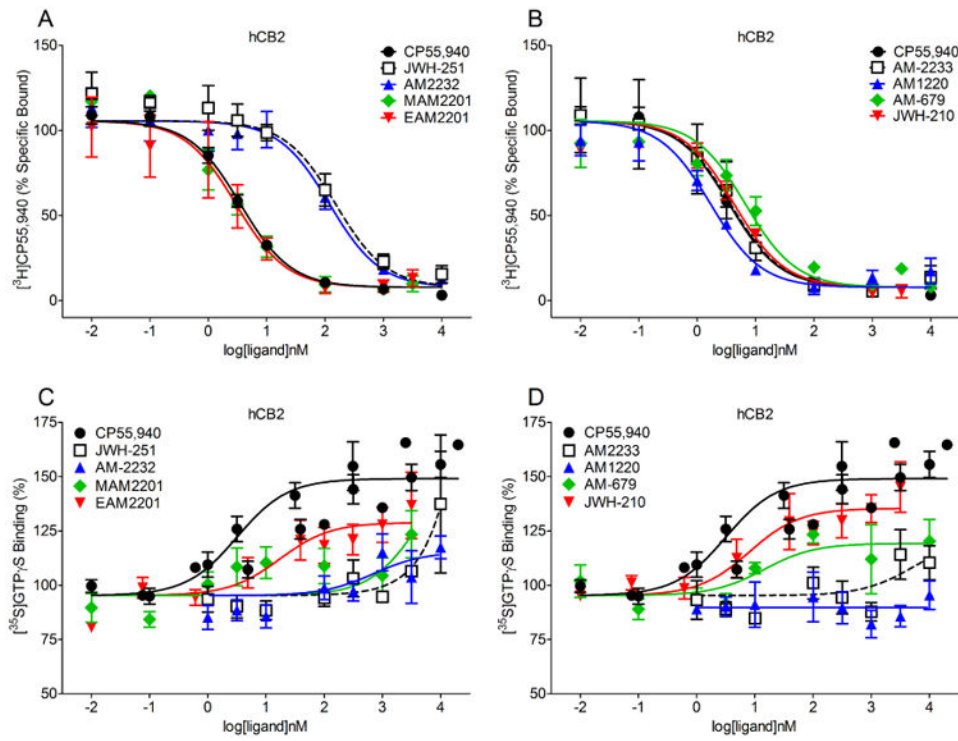


Figure 2. Displacement of [³H]CP55,940 (panels A-B) and stimulation of [³⁵S]GTP_γS turnover (panels C-D) at the hCB₁ receptor (± SEM).

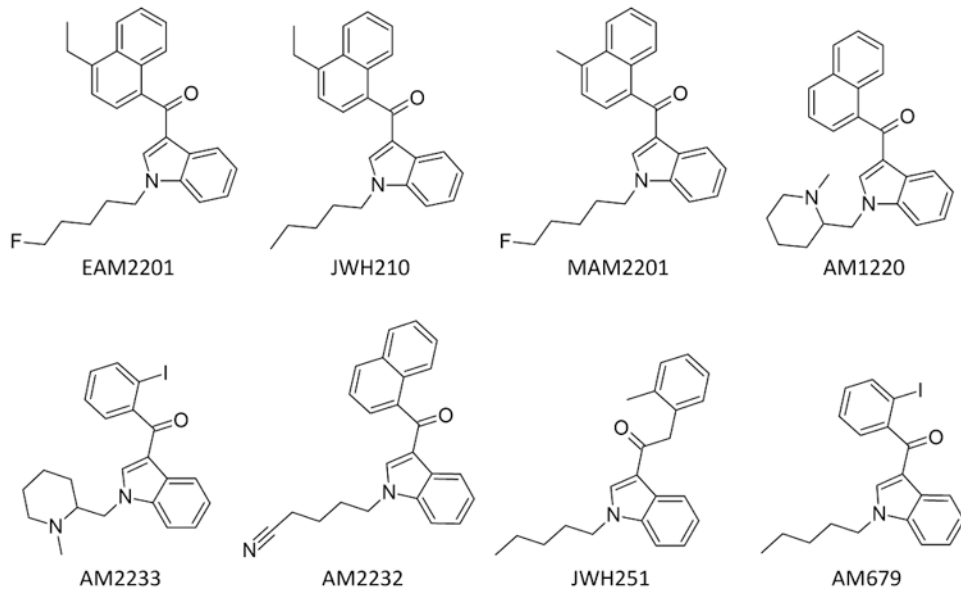


Figure 3. Displacement of [³H]CP55,940 (panels A-B) and stimulation of [³⁵S]GTPγS turnover (panels C-D) at the hCB₂ receptor (± SEM).

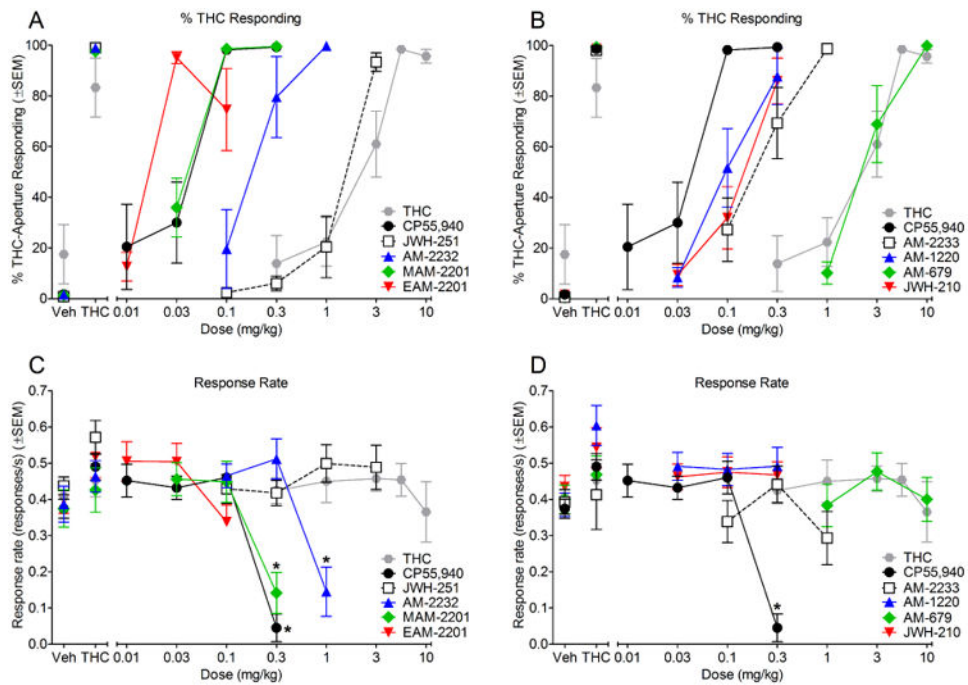


Figure 4. Effects of cannabinoids on percentage of responses on the THC-associated aperture (panels A-B) and response rate (panels C-D). Points above Veh and THC show data for tests of vehicle and 5.6 mg/kg THC, respectively, conducted before each dose-effect determination. All doses of THC and JWH-251 (n=7-8); all doses of JWH-210, EAM-2201, and AM-1220 (n=8); all doses of AM-2233 (n=6-7); all doses of MAM-2201 (n=5-7); all doses of AM-679 (n=6); 0.1-0.3 mg/kg AM-2232 (n=6), 1.0 mg/kg AM-2232 (n=3 for % THC responding and n=6 for response rate); 0.01-0.1 mg/kg CP55,940 (n=5), 0.3 mg/kg CP55,940 (n=1 for % THC responding and n=4 for response rate). Asterisks (*) indicate significant differences (p<0.05) compared to vehicle.

Binding affinity, potency and efficacy for stimulation of [³⁵S]GTPγS turnover at hCB₁ and hCB₂ receptors, and potencies for substitution in THC discrimination.

Table 1

Compound	CB ₁ K _i ^a		[³⁵ S]GTPγS Turnover ^b		CB ₂ K _i ^a		[³⁵ S]GTPγS Turnover ^b		ED ₅₀ (μmol/kg) ^e
	EC ₅₀ ^c	E _{max} ^d	EC ₅₀ ^c	E _{max} ^d	EC ₅₀ ^c	E _{max} ^d	EC ₅₀ ^c	E _{max} ^d	
CP55,940 (376.6)	1.15 (0.13) 21	163.3 (8.0) 13	163.3 (8.0) 13	163.3 (8.0) 13	0.55 (0.05) 16	0.55 (0.05) 16	9.07 (4.94) 15	55.1 (4.1) 15	0.07 (0.04-0.14)
THC (314)	40.7 ^f (1.70)	77.5 ^g (30.4)	77.5 ^g (30.4)	236 ^g (12)	36.4 ^f (10.0)	36.4 ^f (10.0)	12.3 ^g (6.8)	139 ^g (13)	4.96 (3.41-7.21)
EAM-2201 (387.5)	0.41 (0.04) 5	46.8 (5.0) 4	46.8 (5.0) 4	270.3 (5.1) 4	0.38 (0.05) 3	0.38 (0.05) 3	91.9 (65.1) 4	40.2 (11.1) 4	0.04 (0.04-0.05)
JWH-210 (369.5)	1.43 (0.39) 4	116.1 (8.2) 4	116.1 (8.2) 4	287.4 (8.6) 4	0.94 (0.19) 3	0.94 (0.19) 3	51.0 (15.5) 4	43.6 (6.5) 4	0.33 (0.23-0.46)
MAM-2201 (373.5)	1.86 (0.29) 4	36.1 (4.7) 3	36.1 (4.7) 3	310.2 (20.9) 3	0.59 (0.12) 3	0.59 (0.12) 3	1.93 (0.86) 4	23.2 (8.2) 4	0.11 (0.08-0.13)
AM-1220 (382.5)	2.40 (0.30) 4	44.4 (12.4) 3	44.4 (12.4) 3	131.5 (2.9) 3	0.26 (0.04) 4	Not determinable	Not determinable	Not determinable	0.26 (0.18-0.38)
AM-2233 (458.3)	6.63 (0.78) 5	213.3 (19.8) 3	213.3 (19.8) 3	143.6 (14.6) 3	0.48 (0.06) 3	0.48 (0.06) 3	715.1 (663.9) 2	21.0 (3.4) 2	0.42 (0.27-0.64)
AM-2232 (352.4)	14.5 (7.1) 4	833.2 (57.6) 3	833.2 (57.6) 3	203.5 (8.1) 3	11.2 (1.2) 3	11.2 (1.2) 3	406.4 (323.2) 2	31.3 (10.4) 2	0.54 (0.32-0.92)
JWH-251 (319.4)	16.2 (3.2) 4	918.4 (165.2) 3	918.4 (165.2) 3	227.6 (31.6) 3	15.1 (3.3) 3	15.1 (3.3) 3	1518.1 (1450.9) 2	18.3 3.1 2	4.07 (3.11-5.31)
AM-679 (417.3)	39.6 (7.3) 4	880.6 (170.0) 3	880.6 (170.0) 3	131.7 (19.5) 3	2.10 (0.66) 3	2.10 (0.66) 3	19.0 (9.2) 4	31.1 (8.6) 4	5.80 (4.30-7.84)

^aValues represent K_is (± SEM) in nM for [³H]CP55,940 displacement at specified receptor.

^bFor each measure, n is shown below the SEM.

^cValues represent EC₅₀s (± SEM) in nM for [³⁵S]GTPγS binding at specified receptor.

^dValues represent % maximal increase (± SEM) for [³⁵S]GTPγS binding over basal at specified receptor.

^eED₅₀s (± 95% confidence limits) are expressed in μmol/kg. Molecular weights are provided in parentheses below the compound names.

^f(Showalter et al., 1996)

^g(Rosati et al., 2014)