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A High Efficacy Cannabinergic Ligand (AM4054) used as a Discriminative Stimulus: Generalization to other Adamantyl Analogs and Δ^9 -THC in Rats

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Abstract

In addition to endogenous lipids, the two main cloned receptors (CB₁R and CB₂R) of the endocannabinoid signaling system (ECS) can be activated (and blocked) by various exogenous ligands. A relatively novel template for CB₁R activators contains an adamantyl moiety as a key structural subunit, the first being the cannabinergic AM411. Additional chemical optimization efforts using the classical tricyclic scaffold led to AM4054. Here we explored the *in vivo* consequences of novel adamantyl analogs in rats trained to recognize the effects of the potent adamantyl cannabinergic AM4054. Rats were trained to discriminate between AM4054 (0.1 mg/kg) and vehicle. Three AM4054 analogs and Δ^9 -THC were tested for generalization (substitution) and antagonism was assessed with rimonabant. We found that all cannabinergics resulted in response generalization to the target stimulus AM4054. The order of potency was: AM4054 > AM4083 > AM4050 > AM4089 > Δ^9 -THC. The CB₁R antagonist/inverse agonist rimonabant blocked the discriminative stimulus effects of AM4054. Thus the examined structural modifications affected binding affinities but did not markedly change potencies with the exception of AM4089. *In vitro* (cAMP assay) functional data have suggested that AM4089 behaves as a partial rather than as a full agonist at CB₁R which could explain its lower potency compared to AM4054 (Thakur *et al.*, 2013). The 9 β -formyl functionality at C-9 position was identified as an important pharmacophore yielding high *in vivo* potency. Antagonism by rimonabant suggested CB₁R mediation.

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Disclosure statement

Authors declare that the study sponsors did not have any role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Keywords

adamantyl cannabinergics; antagonism; drug discrimination; rats

1. Introduction

The endocannabinoid signaling system is a major neuromodulatory network in the body. The endocannabinoid signaling system involves two relatively well established functional sites, cannabinoid receptors 1 and 2 (CB₁R and CB₂R), endogenous signaling lipids of which arachidonylethanolamine (anandamide) and arachidonoylglycerol (2-AG) are best known, as well as enzymes required for their synthesis and breakdown. A variety of agonists and antagonists for these receptors have been developed as well as ligands targeting the formation and catabolism of the endogenous signaling molecules using different chemical templates (Alger, 2013, Hanus, 2009, Makriyannis, 2014).

CB₁R is the most abundant G-protein coupled receptor (GPCR) present in the brain and is also present in several peripheral tissues. Several preclinical studies with ligands targeting this receptor have implicated a potential role in the treatment of anxiety and depression, epilepsy, pain and neurodegenerative diseases such as Parkinson's and Huntington's diseases (Pertwee, 2012). CB₁R is also responsible for the psychotropic effects of ⁹-tetrahydrocannabinol (⁹-THC), the major psychoactive constituent of marijuana. Over the last two decades, structurally diverse scaffolds with CB₁R agonist activity have been identified, developed and pharmacologically profiled. Of these, the classical cannabinoid (CC) scaffold, as represented by ⁹-THC, has been studied the most. From the different pharmacophoric groups present in CCs, the C-3 side chain at ring A (see Figure 1) has been shown to play a critical role in determining receptor sub-type affinity and selectivity (CB₁R vs. CB₂R). The northern aliphatic hydroxyl (NAH; see Figure 1) functionality at the C-9 position is another key pharmacophoric group capable of modulating affinity, potency and efficacy for CB₁R (Makriyannis, 2014).

Of particular interest for the current study was the design of a C-3 side-chain attached 1-adamantyl homolog (AM411) of the CC's tricyclic (-)-⁸-tetrahydrocannabinol (⁸-THC). ⁸-THC is a minor but more stable isomer of ⁹-THC. AM411 exhibited good affinity for CB₁R (K_i = 6.8 nM) as well as some selectivity as the K_i for CB₂R was 52.0 nM and AM411 substituted for ⁹-THC (3 mg/kg) in a rat drug discrimination bioassay (Lu *et al.*, 2005). AM411 was initially thought to be a full agonist at CB₁R (McLaughlin *et al.*, 2005), exhibiting a slow desensitizing of the receptor upon repeated administration (Luk *et al.*, 2004). Subsequent studies, however, suggested a partial rather than a full cannabinoid agonist profile (Thakur *et al.*, 2013). Also, AM411 produced profound tolerance upon repeated administration in monkeys (Desai *et al.*, 2013). Further chemical optimization efforts around the AM411 structure with a focus on the C-ring and NAH led to tetra- and hexahydrocannabinol as well as aromatic C-ring cannabinoids, all retaining the pendent 1-adamantyl chain at the C-3 position. Among these, a 9 β -hydroxymethyl analog [AM4054 (Figure 1)], which exhibited high potency, full high-efficacy with nanomolar affinity at CB₁R (Table 1), was of special interest (Thakur *et al.*, 2013). In several behavioral tests

AM4054 was found it to be a potent pharmacological tool for assessing cannabinoid receptor function. For example, AM4054 displayed characteristic tetrad effects with a potency considerably higher than that of standard agonists such as 9 -THC, WIN55,212-2 and AM356 (McLaughlin *et al.* , 2013). Like other cannabinergics, AM4054 induced diuresis and hypothermia in rodents (Chopda *et al.* , 2013, Paronis *et al.* , 2013). AM4054 has been used as a discriminative stimulus (0.01 mg/kg) in drug discrimination for monkeys (Kangas *et al.* , 2013).

In an attempt to further extend these results, in rats trained to discriminate between the potent CB₁R agonist AM4054 and vehicle, we show that AM4089 is less efficacious, *in vivo*. These results are in congruence with the *in vitro* properties of AM4089 as seen in the cAMP and β -arrestin recruitment assays (Thakur *et al.*, 2013). Additionally, two other moderate CB₁R-affinity adamantyl cannabinoids (AM4050 and AM4083), bearing dissimilar structural features at the NAH site as compared to AM411, AM4089 and AM4054, were tested so as to gain a better insight into the structure-activity relationship (SAR), *in vivo*. For the four ligands tested, we observed that the examined structural modifications at the NAH site affected binding affinities without markedly changing the potencies as observed using our validated drug discrimination protocol, with the exception of AM4089.

Additional compounds tested were 9 -THC and the CB₁R selective inverse agonist/antagonist rimonabant. The binding affinities for CB₁R for the two latter ligands are approximately 39.5 nM and 11.5 nM, respectively (Thakur *et al.* , 2005, Thakur *et al.* , 2009).

2. Methods

2.1. Animals

Twelve male Sprague-Dawley rats (Taconic Farms, Germantown, NY) were individually housed in a colony room with an average temperature of 20°C and a 12 h light/dark cycle (lights on at 7 a.m.) with free access to food and water during a one week acclimation period before implementing below described protocols (training and testing occurred during the light phase). Bedding was provided twice weekly to each cage using SoftZorb[®] (Northeastern Products Corp; Warrensburg, NY). Animals were approximately 90 days old at the time of purchase and experimentally naïve at the time of shaping the lever pressing response (see below). After the acclimation period, the animals were accustomed to being handled and injected during the subsequent week and access to food was gradually limited until the animals reached approximately 80% of their expected free-feeding weight. Animals had free access to tap water except during experimental sessions. All procedures were approved by the Animal Care and Use Committee of Northeastern University, Boston, MA, USA. The principles in “Guide for the care and use of laboratory animals, 8th ed.” (National Research Council, 2011) were followed.

2.2. Apparatus

Drug discrimination training and testing utilized eight operant conditioning chambers (ENV-001), enclosed in sound attenuating cubicles, and connected to an IBM-compatible PC via an LVB interface (SG-502), purchased from Med Associates (St. Albans, VT). Behavioral sessions were conducted and data were collected were conducted using a Med-PC software program (v. 1.16, Med Associates, St. Albans, VT). The chambers were equipped to deliver food reinforcement and had two retractable response levers. The levers were separated by a receptacle in which food (Bio-Serve® type FO299 45 mg pellets; Frenchtown, NJ) could be presented.

2.3. Procedure

The animals were shaped by successive approximation to lever press for food until they responded 10 times for each reinforcer (fixed-ratio 10 schedule of reinforcement; FR-10). The position of drug-appropriate levers was quasi randomly assigned among subjects so that it was to the right of the receptacle for half the subjects and left for the other half. Throughout the session, the aforementioned FR-10 schedule of reinforcement was in effect. When the house light was off, and the stimulus lights above the response levers were lit, completion of 10 presses on the active lever resulted in the delivery of one food pellet and a 5-sec time-out period where the food cup was illuminated by a receptacle light and the house light on. At the end of the 5-sec time-out period, the stimulus lights above the levers were re-lit, the house light turned off, and the FR-10 schedule of reinforcement contingency reinstated. Sessions ended by all lights in the box being turned off. Post session supplemental food (Harlan Rat Chow®, # 2018) took place in the afternoon / evening.

We used 0.1 mg/kg of AM4054 as the discriminative stimulus. The training drug was administered i.p. (2 ml/kg), 20 min before session onset. After injection(s) the animals waited in their home cages until 20 min had elapsed before being put into the experimental chambers. Rats were trained for 20-min sessions, 5 days a week (Monday through Friday). The schedule of drug- or vehicle-training sessions was non-systematic, with no more than two sessions under the same training condition occurring consecutively. Which lever was correct depended upon whether AM4054 or its vehicle had been administered before the session. Responses on the inappropriate lever were recorded but had no programmed consequences. To avoid potential inter-animal cues, the daily order of drug or vehicle sessions for animals trained in the same chamber was varied (Extance and Goudie, 1981). The criterion for the acquisition of the discrimination was the completion of the first FR on the correct, injection-appropriate lever on at least 8 out of 10 consecutive training sessions. Correct selection was defined as the number of responses for receiving the daily first reward being equal to, or less than 14, i.e., not having pressed the incorrect lever more than four times before accumulating 10 responses on the correct lever.

Testing began with different doses and drugs occurring 20 min post-administration (i.p.) after 62 discrimination training sessions with AM4054 and no drug (vehicle). Test (T) sessions were conducted on average 3 times every two weeks; on interim days, regular drug (D) or vehicle (V) training sessions of 20 min duration took place. Such scheduling assured that each test was preceded by at least one D and one V maintenance session. Typically, the

order of sessions was: D, V, T, V, D (week 1); V, T, V, D, T (week 2); V, D, T, D, V (week 3); and D, T, D, V, T (week 4). Tests were conducted only if responding during the preceding training sessions had been correct during the initial six FR-10 cycles of the session. If incorrect, animals were retrained for at least three sessions where the number of responses for receiving the daily first reward being equal to, or less than 14 before additional testing took place. In test sessions, food was delivered for 10 presses on either lever for 6 reinforcement cycles or until 20 min had elapsed, whichever occurred first. There was one session per test day. Doses and drugs were examined in a mixed order. Adamantyl ligands and 9 -THC were examined before assessing antagonism of AM4054 by rimonabant. Prior to antagonism testing, the rats were accustomed to receiving two i.p. injections (2 ml/kg each) prior to the training sessions. For each dose tested, the percentage of responding on the drug-appropriate lever was calculated from the ratio of the number of presses on the AM4054 associated lever to the total number of lever presses in a test session (excluding responding during the food time-out periods). Only data for animals receiving at least one reinforcer during the test session were considered for this measure, i.e., animals must have made a minimum of 10 presses on one of the two levers. Response rate (responses per second) across all subjects was also calculated. This measure was based on the performance of all animals, including animals not receiving reinforcement.

2.4. Statistics

Non-linear regression analyses of mean drug discrimination dose-generalization data after log-X transformation were performed using Prism software (v. 5, GraphPad Software, San Diego, CA; www.graphpad.com) to provide estimates of the independent variable when the coordinates of X intersected with $Y = 50$ and their 95% confidence limits [$ED_{50} \pm 95\%$ C.L.; regression model: log dose vs. response – variable slope ($Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$) with the top and bottom of the curves constrained to 100 and 0, respectively]. Significant differences regarding response rate were calculated by means of oneway analyses of variance, followed by Holm-Sidak post-hoc statistical test procedure using SigmaStat software (v. 11, Systat Software, Chicago, IL; www.systat.com). Differences were considered significant at the $P = 0.05$ level. Results are presented as the mean ($\pm$ SEM).

2.5. Drugs

AM4054 (6aR,9R,10aR)-3-(Adamantan-1-yl)-9-(hydroxymethyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol, synthesized according to (Thakur *et al.*, 2013), was dissolved in ethanol, appropriate amounts withdrawn, the ethanol evaporated under a stream of nitrogen, the residue then dissolved (v/v) in a solution of dimethyl sulfoxide (DMSO), propylene glycol (PG) and Tween-80 (T-80), and stored at -20°C . Shortly before being used, the solution was diluted with normal (0.9%) saline after sonification for 20-30 min. This procedure was followed for preparing suspensions of all the other examined drugs as well. The 9 -THC (6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol), dissolved in ethanol (200 mg/ml), was provided by the National Institute on Drug Abuse (NIDA; Bethesda, MD, USA) and also stored at -20°C until used. Rimonabant, as the base (*N*-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide) was also provided by NIDA and

stored at 4°C before being dissolved in the DMSO/PG/T-80 (v/v) mixture (final suspension 2% / 4% and 4% for all the ligands) before being diluted with saline (90%). The other test probes (AM4089, AM4083, and AM4050) were synthesized at the Center for Drug Discovery, Northeastern University, Boston, MA, USA (Thakur *et al.*, 2013) and Ki values were determined as described before (Lan *et al.*, 1999, Sink *et al.*, 2008). Doses were administered 2 ml/kg i.p. Suspensions were prepared fresh daily just prior to administration.

3. Results

Figure 2 displays the substitution (top) and response rate (bottom) effects of the currently examined compounds. The order of potency for the discriminative stimulus effects was: AM4054 > AM4050 > AM4083 > AM4089 > ⁹-THC. This rank order of potency is based on the median dose-effect (ED₅₀) estimates derived from non-linear regression as shown in Table 1. The table also provides estimates on the 95% confidence limits as well as the receptor affinity values (Ki). Comparing HillSlopes, all the generalization curves are considered parallel [F (5,12) = 1.6; P > 0.05 (P = 0.0608)]. Hence, within the constraints of the regression analysis, the two partial CB₁R agonists (AM4089 and ⁹-THC) did not exhibit a generalization profile different from those of the full CB₁R agonists, of which the full CB₁R agonist AM4054 is best characterized. All but two compounds exhibited at least one dose where the rate of responding was significantly lower than the vehicle training condition [F (18, 159) = 7.095; p < 0.001]. The exceptions concerning response rate occurred with compounds AM4089 and AM4050. Limited supply of AM4089 precluded testing this compound at doses higher than 1 mg/kg; this dose yielded approximately 70% drug (0.1 mg/kg AM4054) appropriate responding. The highest test dose of AM4050 resulted in ≈ 84% drug appropriate responding. Response rates after 5.6 mg/kg ⁹-THC were exceptionally low. Out of the nine subjects tested with this ⁹-THC dose, six animals received at least one reinforcement but only two received all six possible reinforcements; one animal received five and one received two reinforcements.

Combining a fixed dose of AM4054 (0.1 mg/kg) with increasing doses of the CB₁R antagonist/inverse agonist rimonabant (dose range: 0.03 to 1.0 mg/kg) suggested that the discriminative stimulus effects of AM4054 likely were CB₁R mediated (Fig. 3). The ED₅₀ dose of rimonabant that blocked the discriminative stimulus effects of 0.1 mg/kg AM4054 was estimated to be 0.24 mg/kg (see Fig. 3, top). In two instances, involving the lowest and highest doses of rimonabant examined, the rate of responding was significantly lower than the vehicle rate preceding these antagonism tests.

4. Discussion

The current study utilized the pharmacologically selective *in vivo* assay of drug discrimination employing the full / high efficacy (defined by the cAMP and GTPγS assays) cannabinergic AM4054 (0.1 mg/kg) as the training stimulus to explore the consequences of the structural modifications outlined in Figure 1 (Thakur *et al.*, 2013). AM4054 is structurally an adamantyl cannabinoid with a 9-β-hydroxymethyl functionality within the C-ring of the classical tricyclic cannabinoid template and our previous *in vitro* studies showed that the compound exhibited high affinity, potency and efficacy at CB₁R (Thakur *et al.*,

2013). The *in vivo* (drug discrimination) rank order of potency was: AM4054 = AM4083 > AM4050 > AM4089 > Δ^9 -THC. The rank order for the compounds' ability to activate CB₁R (binding affinities) was: AM4089 > AM4054 > AM4050 > Δ^9 -THC > AM4083. Thus, the two data sets are not completely overlapping. The compound with the highest binding affinity for CB₁R was AM4089 with a K_i of 2.1 nM although its' *in vivo* potency placed it last among the adamantyl ligands. However, AM4089 was more potent *in vivo* than the phytocannabinoid Δ^9 -THC (CB₁R K_i $\approx$ 40 nM). The cAMP assay suggested that AM4089 behaves as a partial rather than a full CB₁R agonist (Thakur et al. 2013). Hence, we speculate that this partial agonist property of AM4089 may be the reason why there is a seeming discrepancy between binding affinity and *in vivo* potency of this compound, although other explanations (e.g. pharmacokinetics) are also possible. Previous studies noted that when rats are trained with a full, high efficacy CB₁R agonist (CP55,940 or AM5983), the ratio of the ED₅₀ values for the full agonists and the partial CB₁R agonist Δ^9 -THC is lower compared to a higher training dose of the full agonist (De Vry and Jentzsch, 2003, Järbe *et al.*, 2014). For example, at the training dose of 0.18 mg/kg of the full aminoalkylindole CB₁R agonist AM5983 the ratio was 3.8 which increased to 6.7 at 0.56 mg/kg AM5983. Although the training dose was not varied in the current study, it is noteworthy that the ratio of the ED₅₀ estimates for AM4054 and Δ^9 -THC was larger than 27 and full substitution by Δ^9 -THC for AM4054 was accompanied by marked decreases in the rate of responding. Substitution testing with the presumed partial cannabinergic CB₁R agonist AM4089, on the other hand, was not associated with marked changes in response rate up to the highest test dose examined, yielding 70% AM4054 appropriate responding; lack of material precluded testing of this compound at doses higher than 1 mg/kg AM4089.

The two adamantyl cannabinergic analogs AM4083 and AM4050 together with Δ^9 -THC exhibited the least favorable CB₁R binding affinities of the examined compounds. Nonetheless, the two adamantyl analogs exhibited considerable potency in the drug discrimination assay. For AM4050, our SAR studies within the tricyclic cannabinoid template concluded that 9- β -hydroxyl functionality in the C-ring imparts more affinity and potency at CB₁R compared to its 9-keto or 9- α -hydroxy congeners (Archer *et al.*, 1977, Nikas *et al.*, 2010). Our previous research also established the importance of the 9-hydroxylmethyl group in the tricyclic cannabinoid template in imparting high affinity and potency (Thakur *et al.*, 2013). So far no one-on-one drug discrimination studies have been done comparing the response of 9- β -hydroxy- and 9- β -hydroxymethyl cannabinoids. In our hand, *in vitro*, the 9- β -hydroxycannabinoid with the pendant 1-adamantyl side chain (AM4050) exhibited reduced affinity at CB₁R (K_i = 24 nM) compared to the 9-hydroxylmethyl congener AM4054 (K_i: 4.9 nM). Interestingly, AM4050 exhibited greater potency than that of AM4054 in the cAMP assay (90% inhibition at 10nM in cAMP assays compared to 42% for AM4054 at the same concentration; data not shown). In the drug discrimination assay, we noticed that AM4050 was approximately equi-potent to AM4054.

Our earlier SAR studies identified a novel pharmacophore, the 9 β -formyl moiety of AM4083, within the tricyclic cannabinoid template that imparted significant affinity to both CB₁R and CB₂R with some preference for the latter; for references see (Thakur *et al.*, 2013). We wanted to explore if this compound behaves as a cannabinoid agonist also *in vivo* and hence it was included in the experiments. AM4083 fully substituted for AM4054 and

exhibited high potency ($ED_{50} = 0.07$ mg/kg). The data suggests that this novel moiety or its bioisosteric groups can be further explored to develop potent cannabinergic ligands.

The blockade by rimonabant of the discriminative stimulus effects of AM4054 is in keeping with the notion that CB_1R is the primary, initial pathway for the discrimination; see also (Kangas *et al.*, 2013). Although speculative, the two significant decreases in the response rate in all likelihood are due to the intrinsic activity by AM4054 at the lowest test dose of rimonabant (0.03 mg/kg; see Fig. 3, bottom panel) and additional intrinsic activity by rimonabant itself at the highest dose examined (1 mg/kg). As noted elsewhere, CB_1R induced alteration of operant response rates seems to involve also non-cannabinoid mechanisms (Rodriguez and McMahon, 2014).

In summary, using the pharmacologically selective drug discrimination bioassay, four adamantyl cannabinoids were examined and compared to the classical cannabinergic Δ^9 -THC. As noted before (Dixon *et al.*, 2010, Le Goanvic and Tius, 2006, Lu *et al.*, 2005, Thakur *et al.*, 2013), the bulky C-3 side chain adamantyl substituent can be accommodated/recognized by both CB_1R and CB_2R , exhibiting various binding affinities for the two receptors *in vitro*. Functionally, the current *in vivo* bioassay is geared towards CB_1R and used the potent adamantyl ligand AM4054 as the reference point. CB_1R rather than CB_2R mediation is supported by previous data summarized in e.g. (Järbe, 2011) as well as the dose-related blockade of the discriminative stimulus effects of AM4054 by rimonabant in the current study. CB_1R binding affinity did not completely correspond to potency, e.g. AM4083 had the least affinity for the receptor yet the ligand was nearly as potent as AM4054. Thus, the Δ^9 -formyl functionality at the C-9 position was identified as an important pharmacophore yielding high *in vivo* potency in the drug discrimination bioassay ($ED_{50} = 0.07$ mg/kg). Further characterization of AM4083 in functional assays measuring downstream signaling is warranted. The relatively low potency of the high-affinity ligand AM4089 might reflect its *in vitro* partial rather than full CB_1R agonist functionality as based on the cAMP assay (Thakur *et al.*, 2013). In contrast to the partial agonist Δ^9 -THC, response rate was not affected over the dose range examined. Thus, AM4089 may be a potential additional tool for probing the functional significance of full versus partial agonism in the functions of the endocannabinoid signaling system.

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Highlights

Adamantyl (AM4054) cannabinoid drug discrimination in rats

Substitution with other adamantyl cannabinoids and the marijuana constituent THC

Cannabinoid receptor 1 mediation because of rimonabant (antagonist) blockade

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Figure 1.
Chemical structures of adamantyl cannabinoids as well as Δ^9 -THC examined in drug discrimination

Figure 2.

Generalization test data for five cannabinoids (top) and the corresponding response rate data (bottom) for rats trained to discriminate between vehicle and 0.1 mg/kg AM4054. Each data point represents the average ($\pm$ SEM) of 7 to 12 (AM4054), 6 to 10 (AM4089), 6 to 8 (AM4083), 8 to 12 (AM4050), and 9 to 10 (Δ^9 -THC) observations and examined on separate test days, respectively. Vehicle response rate (mean $\pm$ SEM) was 1.87 ± 0.12 responses per second (horizontal lines), based on the initial 6 reinforcement cycles of the non-drug maintenance sessions immediately preceding the above tests. Asterisk indicates significant

difference from the vehicle rate at $P = 0.05$ (Holm-Sidak post-hoc multiple comparison procedure involving a control mean).

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Figure 3.

Antagonism test data for AM4054 (top) and the corresponding response rate data (bottom) for rats trained to discriminate between vehicle and 0.1 mg/kg AM4054. Tests were conducted 20 min after concurrent i.p. administrations of rimonabant (dose range: 0.03 to 1 mg/kg) and 0.1 mg/kg AM4054. Each data point represents the average (± SEM) of 7 to 8 observations and examined on separate test days. Vehicle response rate (mean ± SEM) was 1.99 ± 0.27 responses per second (horizontal lines), based on the initial 6 reinforcement cycles of the non-drug maintenance sessions immediately preceding the above tests. *Asterisk*

indicates significant difference from the vehicle rate at $P = 0.05$ (Holm-Sidak post-hoc multiple comparison procedure involving a control mean).

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Table 1

Median dose-effect estimates (ED₅₀) and corresponding 95% confidence limits ($\pm$ 95% C.L.) as well as binding affinities (K_i; nM) for the cannabinergic drug discrimination data

Test drug	ED ₅₀ ($\pm$ 95% C.L.) mg/kg	K _i CB ₁ R	K _i CB ₂ R
AM4054	0.04837 (0.0415 - 0.0565)	4.9	12.1
AM4083	0.07707 (0.0757 - 0.0785)	52.9	5.5
AM4050	0.05464 (0.0196 - 0.1521)	23.9	40.5
AM4089	0.6316 (0.4964 - 0.8036)	2.1	46.7
⁹ -THC	1.324 (0.5309 - 3.300)	39.5 ^a	40.0 ^a

^a ⁹-THC K_i values from (Thakur *et al.*, 2005)