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Pharmacological characterization of AM1710, a putative cannabinoid CB₂ agonist from the cannabilactone class: Antinociception without central nervous system side-effects

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Abstract

Cannabinoid CB₂ agonists produce antinociception without central nervous system (CNS) side-effects. This study was designed to characterize the pharmacological and antinociceptive profile of AM1710, a CB₂ agonist from the cannabilactone class of cannabinoids. AM1710 did not exhibit off-target activity at 63 sites evaluated. AM1710 also exhibited limited blood brain barrier penetration. AM1710 was evaluated in tests of antinociception and CNS activity. CNS side-effects were evaluated in a modified tetrad (tail flick, rectal temperature, locomotor activity and rota-rod). Pharmacological specificity was established using CB₁ (SR141716) and CB₂ (SR144528) antagonists. AM1710 (0.1–10 mg/kg i.p.) produced antinociception to thermal but not mechanical stimulation of the hindpaw. AM1710 (5 mg/kg i.p.) produced a longer duration of antinociceptive action than the aminoalkylindole CB₂ agonist (*R,S*)-AM1241 (1 mg/kg i.p.) at maximally antinociceptive doses. Antinociception produced by the low (0.1 mg/kg i.p.) dose of AM1710 was blocked selectively by the CB₂ antagonist SR144528 (6 mg/kg i.p.), whereas antinociception produced by the high dose of AM1710 (5 mg/kg i.p.) was blocked by either SR144528 (6 mg/kg i.p.) or SR141716 (6 mg/kg i.p.). AM1710 did not produce hypoactivity, hypothermia, tail flick antinociception, or motor ataxia when evaluated in the tetrad at any dose. In conclusion, AM1710, a CB₂-preferring cannabilactone, produced antinociception in the absence of CNS side-effects. Thus, any CB₁-mediated antinociceptive effects of this compound may be attributable to peripheral CB₁ activity. The observed pattern of pharmacological specificity produced by AM1710 is consistent with limited blood brain barrier penetration of this compound and absence of CNS side-effects.

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Keywords

cannabinoid; CB₂; antinociception; tetrad; pain

1. Introduction

Activation of cannabinoid CB₂ receptors produces antinociception in animal models of persistent pain (for review see Guindon and Hohmann, 2008). The CB₂ receptor represents a promising therapeutic target for the treatment of pathological pain specifically because antinociceptive efficacy is observed in the absence of unwanted central nervous system (CNS) side-effects (Hanus *et al.*, 1999; Malan *et al.*, 2001). Relative to CB₁ receptors, a paucity of CB₂ receptors is detected in the CNS of naive animals. However, CB₂ receptors are upregulated within the CNS in neuropathic pain states (Beltramo *et al.*, 2006; Wotherspoon *et al.*, 2005; Zhang *et al.*, 2003). Upregulation of CB₂ receptors may contribute to the efficacy of CB₂-specific agonists in treating neuropathic pain (for review see Guindon and Hohmann, 2008). Additional targets for drug development aimed at harnessing the analgesic potential of cannabinoid signaling systems while limiting CNS side-effects have also been described (Anand *et al.*, 2009; Schlosburg *et al.*, 2009).

A recently described class of cannabinoids, the cannabilactones, includes the CB₂-preferring agonists AM1714 and AM1710. Cannabilactones are defined and differentiated from other classes of cannabinoids by the presence of a carbonyl group in place of the 6,6-dimethyl moiety associated with the classical tricyclic structure of cannabinoids (Khanolkar *et al.*, 2007). Both AM1714 and AM1710 produce antinociception following local (i.paw) administration, suggesting that they produce antinociception, at least in part, through peripheral mechanisms (Khanolkar *et al.*, 2007). We recently demonstrated that a cannabilactone CB₂ agonist suppresses neuropathic nociception in a chemotherapy model of peripheral neuropathy through a CB₂-specific mechanism (Rahn *et al.*, 2008). However, despite the considerable therapeutic potential of these compounds, antinociceptive effects of the cannabilactones remain relatively uncharacterized. More work is necessary to characterize the *in vivo* pharmacological profile associated with cannabilactone administration and determine whether compounds of this class show limited CNS side-effects. It remains unknown whether systemic administration of cannabilactones such as AM1710 lack cardinal signs of CB₁ receptor activation (e.g. hypothermia, hypoactivity, motor ataxia) or exhibit an unfavorable CNS profile. This examination is important for validating the therapeutic potential of the cannabilactones for the treatment of pain.

The present studies were conducted to evaluate the antinociceptive properties of the cannabilactone AM1710 (Fig 1) (K_i: CB₁ vs. CB₂: 360 nM vs. 6.7 nM) (Khanolkar *et al.*, 2007), in tests of thermal (Hargreaves test) and mechanical (von Frey) sensitivity. The presence of centrally-mediated side-effects produced by AM1710 was evaluated using a modified tetrad (tail flick, rectal temperature, locomotor activity, rota-rod). Cardinal signs of cannabinoid CB₁ receptor activation include antinociception in the tail-flick test, hypothermia, hypoactivity (measured by an activity meter) and motor ataxia in the rota-rod test (Malan *et al.*, 2001; Martin *et al.*, 1991). The pharmacological profile of AM1710 was compared with the prototypical CB₂-specific agonist (*R,S*)-AM1241 (K_i: CB₁ vs. CB₂: 239.4 nM vs. 3.41 nM) (Thakur *et al.*, 2005). (*R,S*)-AM1241 (Fig. 1) is a CB₂ agonist from the aminoalkylindole class of cannabinoids that has been well-characterized in both rat and mouse (Hohmann *et al.*, 2004; Ibrahim *et al.*, 2006; Malan *et al.*, 2001; Nackley *et al.*, 2003; Rahn *et al.*, 2007). Pharmacological specificity was determined using selective antagonists for CB₁ (SR141716) and CB₂ (SR144528). Central nervous system side-effects of AM1710

were compared to the mixed cannabinoid CB₁/CB₂ agonist WIN55,212-2, as well as (*R,S*)-AM1241 tested under identical conditions.

2. Methods

2.1. Subjects

Two hundred fifteen adult male Sprague Dawley rats were used in behavioral experiments; one hundred sixteen (300–400 g; Harlan, Indianapolis, IN) animals were used in studies of antinociception and ninety-nine (250–350 g; Harlan, Indianapolis, IN) animals were used in the tetrad studies. All animals were maintained on a 12 hr light/12 hr dark cycle (7:00 – 19:00; lights on) in a temperature-controlled facility. Animals were single housed and had access to food and water *ad libitum*. Antinociceptive effects of the reference compound (*R,S*)-AM1241 (1 mg/kg i.p.; n= 8) have been reported previously by our group (Rahn *et al.*, 2010). Data from this drug condition was collected concurrently with data presented in the current report. Animal experiments were conducted in full compliance with local, national, ethical and regulatory principles and local licensing regulations of Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International's expectations for animal care and use/ethics committees.

Four male CD1 mice (initially weighing 16–18 g; Charles River Laboratories, Willmington, MA) were used for determining blood brain barrier penetration of AM1710. Mice were used for these studies because mouse CB₂ shows 90% homology with rat CB₂ (Yao and Mackie, 2009). The mice were acclimated to vivarium conditions for one week prior to experimentation. Mice were allowed access to food and water *ad libitum*. All procedures were approved by the institutional Animal Care and Use Committee in accordance with public health and safety policies and followed the guidelines for the treatment of animals of the International Association for the Study of Pain (Zimmermann, 1983).

2.2. Drugs and Chemicals

AM1710 (3-(1',1'-dimethylheptyl)-1-hydroxy-9-methoxy-6*H*-benzo[*c*]chromene-6-one), and (*R,S*)-AM1241 ((*R,S*)-(2-iodo-5-nitrophenyl)-[1-((1-methyl-piperidin-2-yl)methyl)-1*H*-indol-3-yl]-methanone) were synthesized in the Center for Drug Discovery by two of the authors (by GT and AZ, respectively). SR141716 (5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide) and SR144528 (5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-*N*-(1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl)-1*H*-pyrazole-3-carboxamide) were provided by NIDA. WIN55,212-2 ((*R*)-(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate salt), and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich (St. Louis, MO). All drugs used in the behavioral studies were dissolved in a vehicle of 100% DMSO and delivered intraperitoneally (i.p.). This is the same vehicle that has been employed in previous work (Ibrahim *et al.*, 2005; Malan *et al.*, 2001; Nackley *et al.*, 2003; Rahn *et al.*, 2008). Cannabinoids and cannabinoid antagonists were dissolved in a volume of 1 ml/kg bodyweight with the following exception. Pharmacological specificity of AM1710 actions was determined by administering antagonists as pre-treatments 20 min prior to the agonist. In these conditions, each drug was administered in a volume of 0.5 ml/kg to ensure that all studies employed a uniform volume of DMSO.

2.3. In Vitro Screen

2.3.1. NovaScreen—The NovaScreen (Caliper Lifesciences, Hopkinton, MA) evaluated whether AM1710 exhibited off-target activity at 63 different targets including neurotransmitter-related G-protein coupled receptors, steroids, ion channels, second

messenger-related prostaglandins, growth factors/hormones, brain/gut peptides and enzymes (See supplementary file for details of all targets tested).

2.3.2. Enzyme Assays—Rat Δ TM FAAH was expressed in *E. coli* cells and purified using the procedure disclosed by Patricelli and colleagues (1998). Recombinant hexahistidine-tagged human MGL (hMGL) was expressed in *E. coli* cells and purified as reported by Zvonok and colleagues (2008a; 2008b). A high-throughput fluorometric screening assay for rFAAH inhibition using the fluorescent substrate, arachidonoyl 7-amino-4-methylcoumarin amide (AAMCA) was performed as previously reported (Ramarao *et al.*, 2005). The MGL assays followed similar procedures using the fluorescent substrate arachidonoyl, 7-hydroxy-6-methoxy-4-methylcoumarin ester (AHMMCE) (Zvonok *et al.*, 2008a). Prism software (GraphPad) was used to calculate IC₅₀ values.

2.3.3. CB₁ and CB₂ binding assay—Competitive binding assays were performed using rat brain containing CB₁ and HEK293 cells transfected with mouse CB₂ (mCB₂); membrane preparation has been previously described (Lan *et al.*, 1999). Competition binding was between the compounds to be tested and [³H]CP55940 at a final concentration of 0.76 nM (specific activity 128 Ci/mmol; NIDA) incubated at 30°C for 1 hour with the respective membrane preparation. Non-specific binding was assessed in the presence of 100 nM CP55940. The interaction was terminated by rapid filtration of the reaction suspension (Unifilter GF/B-96 Well Filter Plates; Packard Instruments) followed by five washing steps with ice-cold wash buffer (50 mM Tris-base, 5 mM MgCl₂ with 0.5% BSA); bound radioactivity was determined using a Perkin Elmer TopCount Scintillation Counter. The results were analyzed using nonlinear regression to determine the actual IC₅₀ of the ligand (Prism by GraphPad Software, Inc.) and the K_i values were calculated from the IC₅₀ (Cheng and Prusoff, 1973). All data were in triplicate with IC₅₀ and K_i values determined from at least two independent experiments.

2.4. Brain Barrier Penetration

2.4.1. Sample collection and plasma isolation—On the experimental day, AM1710 (1 mg/kg) was administered intravenously (i.v.) in a vehicle containing 3% DMSO in a 1:1:18 ratio of emulphor: ethanol: saline by injection into the lateral tail vein of mice (n = 4), weighing 25–30 g. Tissue samples were taken 15 minutes post-injection. Animals were sacrificed by cervical dislocation followed by decapitation so that trunk blood could be obtained and plasma separated by centrifugation. All samples were flash-frozen in liquid nitrogen and stored at –80°C until analysis.

2.4.2. LC-MS/MS analysis—Tissues (plasma or brain) were extracted using a modified Folch extraction (Folch *et al.*, 1957; Williams *et al.*, 2007; Wood *et al.*, 2008; Wood *et al.*, 2010) and analyzed using a Thermo-Finnigan Quantum Ultra triple quadrupole mass spectrometer with an Agilent 1100 HPLC front-end. The mobile phases used were water (A) and methanol (B) in a gradient elution starting at 95% A, transitioning in a linear gradient to 5% A and held before returning to initial conditions. Samples of 10 μ L each were injected onto a Phenomenex Gemini C18 column (2 \times 50 mm, 5 μ) with a C18 guard column. AM1710 was detected using single reaction monitoring after APCI+ ionization.

2.5. General Behavioral Experimental Methods

Methods for assessing antinociception are described previously (Rahn *et al.*, 2010). Baseline responses to mechanical stimulation of the hindpaw were evaluated at least 1 h prior to evaluation of baseline responses to thermal stimulation. In a subset of experiments (approximately 25%), the order of baseline testing was reversed (i.e. baseline responses to thermal stimulation were assessed at least 1 h prior to evaluation of baseline responses to

mechanical stimulation). This modification enabled us to confirm that hypersensitivity to thermal or mechanical stimulation was not produced by the order of testing mechanical and thermal responses (data not shown). Following completion of baseline testing, all rats were returned to their home cages for approximately 2 h prior to administration of drug or vehicle. This delay was employed to ensure that animals did not develop sensitization to repeated testing.

CNS side-effects were evaluated in two separate groups of animals that comprised the “tetrad testing”. One set of animals was used for tail flick and rectal temperature assessment. The second set of animals was used for activity meter and rota-rod testing. Baseline tail flick latencies were assessed prior to baseline assessments of rectal temperature. Following baseline measurements, animals were returned to their home cages for approximately 2 h prior to drug or vehicle administration. Training for rota-rod took place on the two days preceding the test day. Only animals that met reliability criteria for the rota-rod (i.e. ability to walk on a rotating drum for 30 sec in two separate trials) on the test day received pharmacological treatments. Subjects that failed to meet the rota-rod criteria were subsequently used in the tail flick/rectal temperature or antinociception study after an appropriate delay (i.e. several days). Animals that passed criteria for inclusion in the rota-rod study were returned to their home cages for approximately 2 h prior to drug or vehicle administration. All studies were conducted by a single experimenter who was blinded to the drug condition. Animals were randomly assigned to drug or vehicle conditions.

2.5.1. Assessment of Mechanical Withdrawal Thresholds and Thermal Paw Withdrawal Latencies—Mechanical withdrawal thresholds were assessed using a digital Electrovonfrey Anesthesiometer (IITC model Alemo 2290–4; Woodland Hills, CA) equipped with a rigid tip (0.8 mm diameter). All efforts were made by the experimenter to maintain a constant rate of stimulus application across animals. Rats were placed underneath inverted plastic cages and positioned on an elevated mesh platform. Rats were allowed 10–15 min to habituate to the chamber prior to testing. Stimulation was applied to the midplantar region of the hind paw through the floor of a mesh platform. Mechanical stimulation was terminated upon paw withdrawal; consequently, there was no upper threshold limit set for termination of a trial. Mechanical paw withdrawal thresholds are reported as the mean of duplicate determinations averaged across paws.

Paw withdrawal latencies to radiant heat were measured in duplicate for each paw using the Hargreaves test (Hargreaves *et al.*, 1988) and a commercially available plantar stimulation unit (IITC model 336; Woodland Hills, CA). Rats were placed underneath inverted plastic cages positioned on an elevated glass platform. Rats were allowed 10–15 min to habituate to the chamber prior to testing. Radiant heat was presented to the midplantar region of the hind paw through the floor of the glass platform. Stimulation was terminated upon paw withdrawal or after 40 s to prevent tissue damage. Paw withdrawal latencies are reported as the mean of duplicate determinations averaged across paws.

Baseline mechanical withdrawal thresholds and thermal paw withdrawal latencies were assessed prior to pharmacological manipulations. Mechanical paw withdrawal thresholds were re-assessed 15 min following injection of drug or vehicle. Thermal paw withdrawal latencies were measured at 30, 60 and 120 minutes post-injection to assess the time course of CB₂ agonist actions.

Antinociception to thermal (in the Hargreaves test) and mechanical (electrovonfrey) stimulation was evaluated in otherwise naive rats. Separate groups of animals received either racemic (*R,S*)-AM1241 (1 mg/kg i.p.; n = 8) [data shown in (Rahn *et al.*, 2010)], AM1710 (0.1, 0.33, 1, 5 and 10 mg/kg i.p.; n = 8 per group), or DMSO (n = 19). To determine

pharmacological specificity, SR144528 (6 mg/kg) or SR14176 (6 mg/kg i.p.) was administered 20 min prior to AM1710 (0.1 or 5 mg/kg; n = 8–9 per group). SR141716 (6 mg/kg i.p., n = 8 per group) or SR144528 (6 mg/kg i.p.; n = 8) did not alter basal nociceptive thresholds to mechanical or thermal stimulation at these doses (Rahn *et al.*, 2010). Thermal withdrawal latencies were re-determined, in duplicate for each paw, at 30, 60 and 120 min following injection.

2.5.2. Tetrad Testing

2.5.2a. Tail Flick/Rectal Temperature: A modified tetrad profile was performed to assess CNS side-effects. Tail flick latency and rectal temperature were assessed in the same animals. Tail flick (D'Amour and Smith, 1941) was assessed using a commercially available tail flick unit (IITC model 336; Woodland Hills, CA). Animals were placed in restraint tubes (IITC model 81; Woodland Hills, CA) and allowed 10 min to habituate prior to testing. Radiant heat was presented to the tail and the latency for the animal to withdraw its tail from the heat source was recorded. Stimulation was terminated when the animal withdrew its tail from the radiant heat source. A cut-off latency of 10 s was employed to prevent tissue damage. Baseline tail flick latencies are reported as the mean of six tail flick latencies. Tail flick latencies were re-determined at 30, 60, and 120 min post-injection and are reported at each time point as the mean of four tail flick latencies.

Rectal temperature was assessed using a commercially available rectal probe (Physitemp RET-2 rectal probe for rats; Clifton, NJ) and meter (Physitemp Model BAT-12R; Clifton, NJ). Following assessment of baseline tail flick latencies, rectal probes, lubricated with Vaseline®, were inserted to a depth of approximately 2.4 cm. Probes were then connected to the meter and body temperature was recorded. Baseline rectal temperature is reported as the mean of four measurements. Rectal temperatures were then determined in duplicate at 35, 65, and 125 min post-injection and are reported at each time point as the mean of duplicate determinations.

To evaluate centrally-mediated antinociception (assessed in the tail flick test) and hypothermia, separate groups of animals received either (*R,S*)-AM1241 (1 mg/kg i.p.; n = 7), AM1710 (0.1, 5 or 10 mg/kg i.p.; n = 6 per group), DMSO (n = 7), the reference cannabinoid CB₁/CB₂ agonist WIN55,212-2 (5 mg/kg i.p.; n = 7) or the CB₁ antagonist SR141716 (6 mg/kg i.p.) 20 min prior to the administration of WIN55,212-2 (5 mg/kg i.p.; n = 6).

2.5.2b. Activity Meter/Rota-rod: Locomotor activity and motor ataxia were assessed in the same animals. Distance traveled in an activity meter was assessed by placing rats individually in the center of a polycarbonate activity monitor chamber (Med Associates, St. Albans, VT) measuring 44.5 × 44 × 34 cm housed in a darkened, quiet room. A 25-watt bulb positioned one meter over the chamber provided illumination. Activity was automatically measured by computerized analysis of photobeam interrupts (Med Associates). Total distance (cm) traveled in the arena was used for data analysis. Animals were placed in the activity meter at 20 min post-injection and remained undisturbed in this chamber for 15 min. Following activity meter testing, animals were tested on the rota-rod.

Motor ataxia was assessed using a commercially available rota-rod unit (IITC model 755 RotoRod; Woodland Hills, CA). Animals were required to walk against the motion of a rotating drum increasing in speed from 4 revolutions per min (rpm) to 40 rpm, similar to that described by Fox and colleagues (2001). The descent latency (i.e. the time for an animal to fall off the rotating drum) was recorded (sec). No cut-off latency was employed in the rota-rod test to ensure that detection of subthreshold motor ataxia would not be masked by the cut-off latency employed (Taylor *et al.*, 2003). Rota-rod training took place on the two days

prior to the test day. Animals were given a minimum of three practice trials on both training days. Practice trials terminated when the animals fell off of the rotating drum. Training trials in which the animal failed to remain on the rotating drum for a minimum of 10 sec were re-run. On the test day, reliability testing was performed. Animals that could not remain on the rotating drum for 30 seconds in two separate trials failed to meet the criteria (approximately 20%) and were dropped from the experiment. Rota-rod descent latency was calculated after drug administration at 35, 65, and 125 min post-injection. Rota-rod latencies at each time point post-injection are reported as the mean of two separate rota-rod descent latencies.

To evaluate possible centrally mediated side-effects of hypoactivity and motor ataxia, animals received either the DMSO vehicle (n = 8), (*R,S*)-AM1241 (1 mg/kg i.p.; n = 6), AM1710 (0.1, 5 or 10 mg/kg i.p.; n = 8–9 per group) or WIN55,212-2 (5 mg/kg i.p.; n = 7). To assess pharmacological specificity, a separate group was pre-treated with SR141716 (6 mg/kg i.p.) 20 min prior to WIN55,212-2 (5 mg/kg i.p.; n = 7).

2.6. Statistical Analyses

Percent change in paw withdrawal latencies from baseline was calculated with the following formula: $((\text{Post-drug paw withdrawal latency} - \text{baseline}) / \text{baseline}) * 100$. Antinociception in the tail flick test was expressed as the percent of maximum possible effect (% MPE), using the formula:

$$\frac{(\text{Post} - \text{drug paw withdrawal latency} - \text{baseline})}{(\text{Cut} - \text{off value} - \text{baseline})} \times 100$$

Change in temperature (Δ °C) was calculated with the following formula: (Post-drug temperature – mean baseline temperature). Z-scores were calculated for tetrad animals tested in the activity meter and rota-rod. Three animals with Z-scores of ± 2 standard deviations from the mean in either test were excluded from analysis.

All data was analyzed using analysis of variance (ANOVA) for repeated measures, one-way ANOVA or planned comparison Student t-tests, as appropriate. SPSS 18.0 (SPSS Incorporated, Chicago, IL, USA) was used for statistical analyses. The Greenhouse-Geisser correction was applied to all repeated factors. Post hoc comparisons between control groups and other experimental groups were performed using the Dunnett test. Post-hoc comparisons between different experimental groups were also performed to assess dose-response relationships and pharmacological specificity using the Tukey test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Results of in vitro screen for target selectivity

AM1710 demonstrated 17-fold selectivity for mCB₂ ($K_i = 17 \pm 10$ nM) compared to rCB₁ ($K_i = 282 \pm 91$ nM; data are the average \pm standard deviation of five separate experiments run in triplicate). An *in vitro* screen was also used to assess the target selectivity of AM1710 for CB₂ receptors. The Novascreen failed to identify off-target activity of AM1710 at 62 different targets including neurotransmitter-related G-protein coupled receptors, steroids, ion channels, second messenger-related prostaglandins, growth factors/hormones, brain/gut peptides and enzymes (Supplementary File). In the NovaScreen, AM1710 did not inhibit [³H]CP55,940 binding to hCB₁ at 100 nM, but exhibited 50% inhibition of binding at 10,000 nM. In a fluorescence assay, AM1710, in concentrations up to 100 μ M, also failed to inhibit activity of fatty-acid amide hydrolase and monoacylglycerol lipase, enzymes implicated in endocannabinoid deactivation (data not shown).

3.2. Brain Barrier Penetration of AM1710

An *in vivo* screen was used to determine the ability of AM1710 to cross the blood brain barrier using intravenously administered doses of 1 mg/kg. The amount of AM1710 found in the unperfused brain tissue was 0.066%/g of the injected dose, while plasma contained 0.000086%/mL (Table 1). AM1710 has a low brain penetration expected, compared to other cannabimimetics screened in this class (B/P ratio range = 0.03–1.3 mL/g; unpublished results).

3.3. Behavioral Results

3.3.1. General Results—Thermal paw withdrawal latencies and mechanical paw withdrawal thresholds did not differ between right or left paws for any group. Therefore, withdrawal thresholds in all studies are presented as the mean of duplicate measurements, averaged across paws. Baseline responses (i.e. thermal paw withdrawal latencies or mechanical withdrawal thresholds) were also similar between groups prior to administration of drug or vehicle. Baseline paw withdrawal latencies did not differ between groups in any study; therefore, baselines in the log dose response plot (Fig 2) were averaged across all doses of the same drug for statistical analyses. Moreover, paw withdrawal latencies and thresholds did not differ based upon the order of thermal and mechanical testing at baseline; therefore, the two vehicle groups are combined for all studies presented.

One animal that received AM1710 (5 mg/kg i.p.) died approximately 45 min post-injection and was excluded from all analyses. The animal likely died from a misplaced injection as no other animals receiving AM1710 at this, or any other dose tested, showed similar effects or was moribund. Within the tetrad (activity meter/rota-rod), two animals from the WIN55,212-2 (5 mg/kg i.p.) group, and one animal from the AM1710 (10 mg/kg i.p.) group were excluded from all analyses based on Z-scores.

3.3.2. The Cannabimimetic AM1710 Produces Antinociception to Thermal but not Mechanical Stimulation of the Hind Paw

3.3.2a. Responses to Mechanical Stimulation: AM1710 (1 mg/kg i.p.), but not other doses of the cannabimimetic, produced modest but reliable increases in mechanical withdrawal thresholds relative to corresponding pre-injection thresholds ($P < 0.05$ planned comparison t-test; Table 1). However, this same dose did not alter post-injection thresholds relative to the vehicle condition. Moreover, antagonist pre-treatment did not alter paw withdrawal thresholds, relative to baseline (Table 2). Paw withdrawal thresholds were not altered by (*R,S*)-AM1241 (previously published; Rahn *et al.*, 2010).

3.3.2b. Responses to Thermal Stimulation in the Plantar Test: The cannabimimetic AM1710 (0.1, 1, 5 and 10 mg/kg i.p.) increased thermal paw withdrawal latencies relative to vehicle at 30 min post-drug ($F_{5,55} = 5.859$, $P < 0.001$; $P < 0.05$ for each comparison). All doses of AM1710 also increased paw withdrawal latencies relative to baseline measurements at this time point ($F_{5,78} = 17.311$, $P < 0.001$; $P < 0.05$ for each comparison; Fig 2a). Paw withdrawal latencies were maximally increased in groups receiving AM1710 (0.1, 0.33, 1, 5 and 10 mg/kg i.p.) at 30 min post-injection; percent increases ranged from 31.5 to 64.4%.

3.3.3. Comparison of Antinociceptive Effects Induced by AM1710 and (*R,S*)-AM1241—The dose of AM1710 (5 mg/kg i.p.) that produced the greatest antinociception at 30 minutes post-injection (81.5% and 64.4% increase in paw withdrawal latencies, respectively) was compared with the maximally effective dose of (*R,S*)-AM1241 (1 mg/kg i.p.) identified previously in the same test (data from Rahn *et al.*, 2010) and compared across the entire testing time course ($F_{6,72} = 4.138$, $P < 0.01$, Fig 2b). Although both drugs

produced equivalent antinociception at 30 min post-injection relative to the vehicle control ($F_{2,24} = 9.60$, $P < 0.01$, $P < 0.001$ for each comparison), the antinociceptive effects of AM1710 (5 mg/kg i.p.) outlasted those produced by (*R,S*)-AM1241 (1 mg/kg i.p.) (Fig. 2b).

3.3.4. Pharmacological Specificity—Antinociception produced by the lowest efficacious dose of the cannabimimetic AM1710 (0.1 mg/kg i.p.) was selectively blocked by the CB₂ antagonist SR144528 (6 mg/kg i.p.) ($F_{3,41} = 3.255$, $P < 0.05$; Fig 3a) but not by the CB₁ antagonist SR141716 (6 mg/kg i.p.). AM1710 (0.1 mg/kg i.p.) produced antinociception relative to the vehicle condition at 30 min ($P < 0.05$ for comparison) but not at 120 min post-injection ($P > 0.08$). By contrast, antinociceptive effects of a higher dose of AM1710 (5 mg/kg i.p.) were blocked ($F_{3,40} = 7.450$, $P < 0.001$; Fig 3b) by both SR144528 (6 mg/kg i.p.) and SR141716 (6 mg/kg i.p.) at 30 min post-injection. Antinociceptive effects of AM1710 (5 mg/kg i.p.) persisted at 120 minutes post-injection ($P < 0.05$, planned comparison t-test), suggesting that the duration of action of AM1710 (5 mg/kg i.p.) outlasted that of AM1710 (0.1 mg/kg i.p.). Paw withdrawal latencies were similar in groups receiving vehicle or pre-treatment with either antagonist at 120 minutes post-injection.

3.3.5. Assessment of CNS Side-effects: Antinociception in the Tail Flick Test and Hypothermia—WIN55,212-2 (5 mg/kg i.p.) produced characteristic CB₁-mediated antinociception in the tail flick test that was not produced by either AM1710 or (*R,S*)-AM1241. Tail flick latencies were elevated in WIN55, 212-2-treated groups relative to vehicle, (*R,S*)-AM1241 (1 mg/kg i.p.), and all doses of AM1710 ($F_{6,38} = 10.505$, $P < 0.001$; $P < 0.05$ for each comparison; Fig 4a) at all time points post-injection (30 min: $F_{6,38} = 11.298$, $P < 0.001$; $P < 0.001$ for each comparison; 60 min: $F_{6,38} = 8.196$, $P < 0.001$; $P < 0.01$ for each comparison; 120 min: $F_{6,38} = 6.028$, $P < 0.001$; $P < 0.05$ for each comparison). The CB₁ antagonist, SR141716 (6 mg/kg i.p.), blocked the antinociceptive effects of WIN55,212-2 in the tail flick test across the entire observation interval ($P < 0.05$ for each comparison), consistent with mediation by CB₁. By contrast, AM1710 (0.1, 5, and 10 mg/kg i.p.) and (*R,S*)-AM1241 (1 mg/kg i.p.) failed to alter tail flick latencies at any post-injection time point relative to the DMSO vehicle condition ($P > 0.60$ for each comparison).

WIN55,212-2 also produced a characteristic CB₁-mediated hypothermic effect that was not produced by the cannabimimetic AM1710 or the aminoalkylindole (*R,S*)-AM1241. WIN55,212-2 (5 mg/kg i.p.) decreased rectal temperature relative to vehicle, (*R,S*)-AM1241 (1 mg/kg i.p.) and all doses of AM1710 ($F_{6,38} = 5.207$, $P < 0.01$; $P < 0.05$ for each comparison; Fig 4b) at 35 minutes post-injection ($F_{6,38} = 8.353$, $P < 0.001$; $P < 0.01$ for each comparison). A hypothermic effect of WIN55,212-2 (5 mg/kg i.p.) was still apparent, relative to vehicle, at 65 minutes ($F_{6,38} = 3.576$, $P < 0.01$, $P < 0.01$ for relevant comparison; Fig 4b) but not 125 minutes ($P > 0.13$) post-injection. The hypothermic effects of WIN55,212-2 were completely blocked by SR141716 (6 mg/kg i.p.; $P < 0.05$ for each comparison), consistent with mediation by CB₁. By contrast, AM1710 (0.1, 5, and 10 mg/kg) and (*R,S*)-AM1241 did not alter rectal temperature relative to the vehicle condition at any time point ($P > 0.32$ for each comparison).

3.3.6. Assessment of CNS Side-effects: Hypoactivity and Motor Ataxia—WIN55,212-2 produced a characteristic CB₁-mediated hypoactivity that was not produced by antinociceptive doses of (*R,S*)-AM1241 or AM1710. WIN55,212-2 (5 mg/kg i.p.) decreased distance traveled in the activity meter relative to all other groups ($F_{3,24} = 12.404$, $P < 0.001$; $P < 0.01$ for each comparison in Fig 5a; $F_{4,33} = 9.154$, $P < 0.001$; $P < 0.05$ for each comparison in Fig 5b). As expected, WIN55,212-2-induced hypoactivity was blocked by the CB₁ antagonist SR141716 (6 mg/kg i.p.; $P < 0.01$ for comparison; Fig. 5a). By contrast, (*R,S*)-AM1241 (1 mg/kg i.p.; Fig. 5a) did not alter locomotor activity relative to the

vehicle condition ($P > 0.42$). AM1710 (0.1, 5 or 10 mg/kg i.p.) did not reliably inhibit locomotor activity relative to the vehicle condition at any dose ($P > 0.11$; Fig 5b).

WIN55,212-2 produced a characteristic CB₁-mediated motor ataxia in the rota-rod test ($F_{3,24} = 5.431$, $P < 0.01$; Fig 6a). These effects were not observed with the cannabimimetic AM1710 or the aminoalkylindole (*R,S*)-AM1241 (1 mg/kg i.p.). WIN55,212-2 (5 mg/kg i.p.) impaired the ability of rats to walk on a rotating drum relative to either vehicle or (*R,S*)-AM1241 (1 mg/kg i.p.) at 35 minutes post-injection ($F_{3,24} = 9.422$, $P < 0.001$; $P < 0.01$ for each comparison; Fig 6a). As expected, WIN55,212-2-induced motor ataxia was completely blocked by SR141716 at this time point ($P < 0.001$ for relevant comparison).

WIN55,212-2 also produced motor ataxia relative to the cannabimimetic AM1710 (5 and 10 mg/kg i.p.) ($F_{4,33} = 4.790$, $P < 0.01$; $P < 0.05$ for relevant comparison; Fig 6b) at 35 min post-injection. WIN55,212-2 (5 mg/kg i.p.) did not alter rota-rod latencies relative to vehicle at subsequent time points (65 min: $P > 0.14$ and 125 min: $P > 0.36$ for Fig 6a,b), suggesting that the antinociceptive and hypothermic effects of WIN55,212-2 outlast the motor ataxic effects of the same dose.

(*R,S*)-AM1241 (1 mg/kg i.p.) and AM1710 (0.1, 5, and 10 mg/kg i.p.) did not alter rota-rod descent latencies relative to vehicle at any time point ($P > 0.46$) (Fig. 6a,b). Rota-rod latencies were lower in groups receiving WIN55,212-2 relative to groups receiving AM1710 ($P < 0.05$ for each comparison, Tukey post hoc and planned comparison t-test).

4. Discussion

The present studies demonstrate that the cannabimimetic CB₂ agonist AM1710 is highly specific for the CB₂ receptor as previously suggested by an *in vitro* screen for target selectivity (Khanolkar *et al.*, 2007). AM1710 was previously validated to be 14-fold more selective at rat compared to human CB₂ receptors (Mukherjee *et al.*, 2004). In a species comparison of binding profiles, AM1710 exhibited K_is of 28 nM and 2 nM, respectively, for inhibiting [³H]CP55,940 binding to HEK cells stably expressing human and rat CB₂ receptors respectively (Mukherjee *et al.*, 2004). Here we additionally show that AM1710 has negligible affinity for an additional 62 targets investigated in the NovaScreen 'side-effect' profile assay (Caliper Life Sciences, Hanover, MD, USA; see Supplementary Data) including TRPV1 and did not alter activity of enzymes catalyzing endocannabinoid hydrolysis (FAAH, MGL), further validating the specificity of the compound for CB₂ receptors. These observations are consistent with the results of behavioral studies documenting the absence of centrally-mediated side-effects associated with activation of CB₁ receptors. Moreover, the compound exhibited limited blood brain barrier penetration, compared to other compounds of its class.

4.1. AM1710-induced Antinociception

AM1710 produced antinociception in the plantar test in the absence of unwanted CNS side-effects. The most striking observation of our study was that doses 100-fold higher than the lowest maximally effective antinociceptive dose showed no signs of CNS activity in the tetrad (i.e. tail flick antinociception, body temperature, rotarod, locomotor activity). The lack of dose dependence observed for AM1710 suggests that this compound exhibits high potency for producing antinociception. There may also be a limit in the magnitude of antinociception that can be produced in the plantar test following CB₂ agonist administration, at least in naive animals.

AM1710 failed to produce antinociception to punctate mechanical stimulation relative to vehicle treatment. Withdrawal responses may occur because the mechanical stimulation is

noxious or because it represents an annoying or unpleasant touch sensation to the animal. Electrophysiological studies provide insight into the classes of primary afferents activated by mechanical stimulation of the plantar paw skin. Following stimulation of the plantar skin with calibrated von Frey filaments, mechanical thresholds for activation of A δ -nociceptors averaged 37.77 mN (i.e. approximately 3.85 g), with a range of 14–100 mN (i.e. approximately 1.4–10.2 g), whereas mechanical thresholds for activation of C-nociceptors averaged 80.24 mN (i.e. 8.19 g), with a range of 14–294 mN (i.e. approximately 1.4–29.9 g) (Leem *et al.*, 1993a). In our study, animals withdrew from the electrovonfrey at thresholds exceeding these forces (see Table 2), suggesting that electrovonfrey stimulation likely resulted in nociceptor activation. It is nonetheless important to note that methodological differences exist between the present study and the study by Leem *et al.* (1993a). The electrovonfrey used in our study offers a significant advantage over testing with traditional von Frey filaments; the area of skin stimulated with the electrovonfrey is constant regardless of the amount of force applied, eliminating the confound that is introduced when manual filaments of increasing diameters are applied to the hindpaw in the traditional method. The electrovonfrey also stimulates a larger surface area (0.8 mm for all forces applied) than most of the traditionally used von Frey filaments (which range from 0.178 – 0.813 mm in diameter for filaments applying published forces ranging from 0.407 to 75.856 g, respectively). Nonetheless, the smaller surface area of skin stimulated by mechanical versus thermal testing may also contribute to our observation of modality-specific antinociceptive effects. Differential nociceptor activation associated with mechanical versus heat stimulation may also contribute to these findings (Leem *et al.*, 1993b). The heat stimulus may activate a greater number of nociceptors than the electrovonfrey given the differences in skin surface areas stimulated by the thermal and mechanical probes. Cannabilactones have, however, been shown to suppress paclitaxel-evoked mechanical allodynia and normalize mechanical withdrawal thresholds (Rahn *et al.*, 2008) at doses that do not produce antinociception to the same stimulus modality in otherwise naive animals.

AM1710 (5 mg/kg i.p.) exhibited a similar maximal effect and a longer duration of action than the aminoalkylindole CB₂ agonist (*R,S*)-AM1241 (1 mg/kg i.p.). In previous work, both AM1714 and AM1710 produced thermal antinociception when administered locally (i.paw) (Khanolkar *et al.*, 2007). However, this is the first study to demonstrate antinociceptive effects of a cannabilactone compound following systemic administration in naive animals.

4.2. Pharmacological Specificity of AM1710-induced Antinociception

Although (*R,S*)-AM1241-induced antinociception was selectively blocked by the CB₂ antagonist SR144528, but not by the CB₁ antagonist SR141716 when tested under identical conditions (Rahn *et al.*, 2010), the *in vivo* pharmacological specificity of systemically administered AM1710 has proven more difficult to interpret. A low dose of AM1710 (0.1 mg/kg i.p.), which produced antinociception comparable to that of AM1710 (5 mg/kg i.p.) at 30 minutes (time point of maximal antinociception), showed no evidence for mediation by CB₁; antinociception produced by AM1710 (0.1 mg/kg i.p.) was insensitive to blockade by SR141716. By contrast, pre-treatment with either SR144528 or SR141716 completely blocked the antinociceptive effects of a higher dose of AM1710 (5 mg/kg i.p.). The *in vivo* pharmacology of AM1710 is more complex than would be expected from the *in vitro* binding affinities (Khanolkar *et al.*, 2007) which demonstrated that cannabilactones (AM1714 and AM1710) bind with only low affinity to CB₁ receptors. More work is necessary to determine whether differences in the bioactive transformations of the cannabilactones contribute to the *in vivo* pharmacology of these compounds. Thus, it is potentially noteworthy that AM1710 produced antinociception at 30 and 120 min post-injection, but not at 60 min post-injection. More work is necessary to identify metabolites of AM1710 and determine whether they are biologically active and brain permeable.

A CB₁ component was not observed previously in groups that received the aminoalkylindole (*R,S*)-AM1241 and the same dose of the CB₁ antagonist and tested under identical conditions (Rahn *et al.*, 2010). Locally administered AM1714 also produces thermal antinociception in the plantar test that was blocked by the CB₂-selective antagonist AM630 but not by the CB₁-selective antagonist AM251 (Khanolkar *et al.*, 2007). Thus, cannabylactone-induced antinociception is mediated, at least in part, by peripheral sites of action. No evidence for a CB₁ component in AM1710-induced antinociception was observed following administration of a lower dose of AM1710 (0.1 mg/kg i.p.), which nonetheless produced maximal antinociception (at 30 minutes) in the plantar test. Thus, increasing the dose did not further increase the antinociceptive effects of AM1710 but could presumably increase the amount of AM1710 available for metabolic transformation and/or produce a low percentage of CB₁ receptor occupancy.

Animals that received AM1710 did not exhibit cardinal signs of CB₁ receptor activation such as antinociception in the tail flick test, hypothermia, hypoactivity, or motor ataxia. Thus, any CB₁ activity produced by systemically administered AM1710 is likely to be peripheral CB₁ activity. It is important to note that the blood brain barrier penetration data indicated AM1710 does enter the brain, albeit at low levels. Consequently, AM1710 might show high antinociceptive efficacy in neuropathic pain states where, due to its blood brain barrier penetration, it could modulate upregulated CB₂ receptors in the CNS (Beltramo *et al.*, 2006; Wotherspoon *et al.*, 2005; Zhang *et al.*, 2003) without crossing the threshold for CB₁ receptor activation that produces hallmark side-effects. More work is necessary to evaluate this and other compounds from the cannabylactone class in states of induced neuropathic pain. For example, systemic cannabylactone administration suppresses chemotherapy-induced neuropathy through a CB₂-specific mechanism; the anti-allodynic effects of AM1714, observed in response to tactile stimulation, were blocked by SR144528, but not by SR141716. In fact, animals receiving SR141716 prior to administration of AM1714 showed enhanced antinociception (Rahn *et al.*, 2008). Thus, pharmacological specificity of these agonists may differ based upon whether or not these compounds are evaluated systemically or locally in the paw or under conditions (normal vs. neuropathic) in which CB₂ or CB₁ receptors may be upregulated.

Off-target effects could potentially contribute to the blockade of cannabylactone-induced antinociception produced by SR141716 in our study. However, the drug screens performed indicated that AM1710 does not significantly bind to common off-target receptors and does not inhibit activity of enzymes (i.e. FAAH, MGL) implicated in endocannabinoid deactivation. Additionally, the same dose of SR141716 (6 mg/kg) which blocked the antinociceptive effects of AM1710 (5 mg/kg i.p.) did not block antinociception produced by either a lower dose of AM1710 (0.1 mg/kg i.p.) or (*R,S*)-AM1241 (1 mg/kg i.p.). It is, however, important to note that anxiolytic effects of SR141716 (Haller *et al.*, 2002; Rodgers *et al.*, 2003; but see Thiemann *et al.*, 2009) are observed in CB₁^{-/-} knockout mice (Uriguen *et al.*, 2004), suggesting that they may be mediated through a “non-CB₁” site. SR141716 also binds TRPV1 receptors at micromolar concentrations (De Petrocellis *et al.*, 2001). However, a role for TRPV1 in cannabylactone actions is unlikely because activation of TRPV1 produces hypothermia *in vivo* (Miller *et al.*, 1982) and our studies demonstrate that AM1710 does not bind to TRPV1 or alter body temperature.

4.3. Central Nervous System Side-effect Profile of AM1710

This is the first study to assess CNS side-effects in the tetrad produced by the cannabylactone AM1710. Previously, AM1714 (3.3 mg/kg i.p.) was tested in the rota-rod where it showed no activity relative to baseline measurements (Khanolkar *et al.*, 2007). No centrally-mediated side-effects were observed in animals that received AM1710 (0.1, 5 or 10 mg/kg i.p.) or (*R,S*)-AM1241 (1 mg/kg i.p.). Similar results have been reported for (*R,S*)-AM1241

in a tetrad which did not include tail flick (Malan *et al.*, 2001). However, higher doses of (*R,S*)-AM1241 (10 mg/kg i.p.) produce modest increases in tail flick latencies in mice (Ibrahim *et al.*, 2006), whereas similar doses of AM1710 in rats (10 mg/kg i.p.) did not alter tail-flick latency or produce any sign of CNS side-effects in the tetrad.

4.4. Conclusions

Our results demonstrate that the cannabylactone AM1710, like the aminoalkylindole (*R,S*)-AM1241, produces antinociception in the plantar test without producing cardinal signs of CB₁ receptor activation. The plantar test may be more sensitive than the tail flick test to detection of CB₂-mediated and peripherally-mediated antinociceptive effects (see Guindon and Hohmann, 2008 for review). Our studies suggest that cannabylactones such as AM1710 produce cannabinoid receptor-mediated antinociception at doses that do not produce CNS side-effects typical of CB₁ receptor activation. These observations suggest that the cannabylactone compound, AM1710, is representative of a promising class of novel cannabinoid analgesics which lack unwanted CNS side-effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

i.p. intraperitoneal

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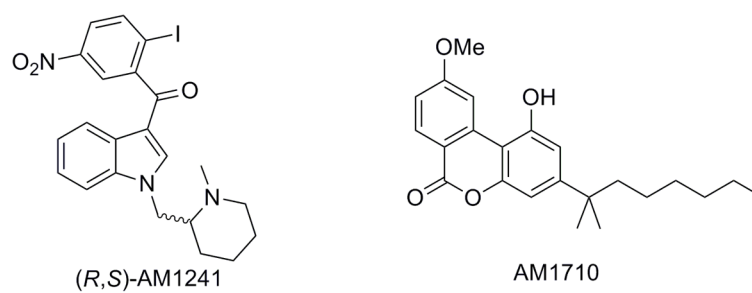


Figure 1.
Chemical structures of the aminoalkylindole (R,S)-AM1241 and the cannabiolactone AM1710.

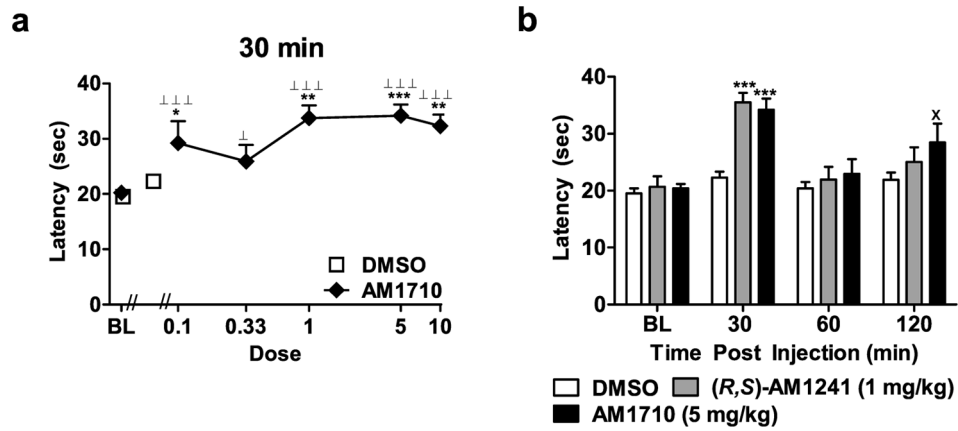


Figure 2.

(a) Log dose response for AM1710-induced antinociception in the plantar test. (b) Time course of antinociceptive effects observed following administration of AM1710 (5 mg/kg i.p.) in comparison with (R,S)-AM1241 (1 mg/kg i.p.) (n = 8 previously published in Rahn et al., 2010). Withdrawal latencies to thermal stimulation in the plantar test are shown. BL denotes baseline paw withdrawal latencies observed prior to agonist or vehicle injection. Doses are in mg/kg. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. DMSO control condition, $\perp P < 0.05$, $\perp\perp\perp P < 0.001$ vs. baseline (ANOVA; Dunnett and Tukey post hoc tests), $\times < 0.05$ vs. DMSO control condition (Student t-test). N = 8–19 per group.

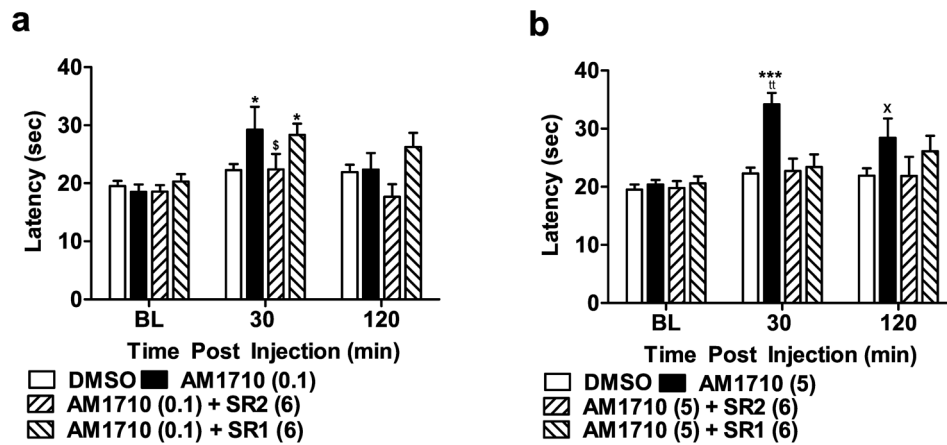


Figure 3. Pharmacological specificity of antinociceptive effects of AM1710 in the plantar test. (a) Antinociceptive effects of AM1710 (0.1 mg/kg i.p.) were blocked by the CB₂ antagonist (SR2; 6 mg/kg i.p.), but not the CB₁ antagonist (SR1; 6 mg/kg i.p.) (b) Antinociceptive effects of AM1710 (5 mg/kg i.p.) were blocked by either SR141716 (6 mg/kg i.p.) or SR144528 (6 mg/kg i.p.). * $P < 0.05$, *** $P < 0.001$ vs. DMSO control, ^t $P < 0.01$ vs. all drug groups, (ANOVA; Dunnett and Tukey post hoc tests). ^s $P < 0.05$ vs. AM1710 (0.1) + SR1 (6), ^x $P < 0.05$ vs. DMSO control (Student t-test). N = 8–19 per group.

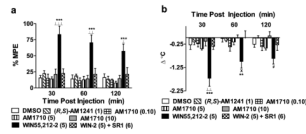


Figure 4.

(a) Effects of cannabimimetic and aminoalkylindole cannabinoid agonists on tail flick antinociception and hypothermia. WIN55,212-2 (WIN-2; 5 mg/kg i.p.) produced CB₁-mediated antinociception in the tail flick test. This effect was blocked by the CB₁ antagonist SR141716 (SR1; 6 mg/kg i.p.). Neither (*R,S*)-AM1241 (1 mg/kg i.p.) nor AM1710 (0.1, 5, and 10 mg/kg i.p.) produced antinociception in the tail flick test. (b) WIN55,212-2 (5 mg/kg i.p.) decreased rectal temperature relative to baseline through a CB₁ mechanism; this effect was blocked by SR141716 (SR1; 6 mg/kg i.p.). Neither (*R,S*)-AM1241 (1 mg/kg i.p.) nor AM1710 (0.1, 5, and 10 mg/kg i.p.) altered rectal temperature. ** $P < 0.01$, *** $P < 0.001$ vs. DMSO control condition, $\perp P < 0.05$, $\perp\perp P < 0.01$, $\perp\perp\perp P < 0.001$ vs. all drug conditions, $\times P < 0.05$ vs. AM1710 (10 mg/kg i.p.), WIN-2 + SR1, and (*R,S*)-AM1241 (1 mg/kg i.p.), $\# P < 0.05$ vs. AM1710 (10 mg/kg i.p.) (ANOVA; Dunnett and Tukey post hoc tests). $N = 6-7$ per group.

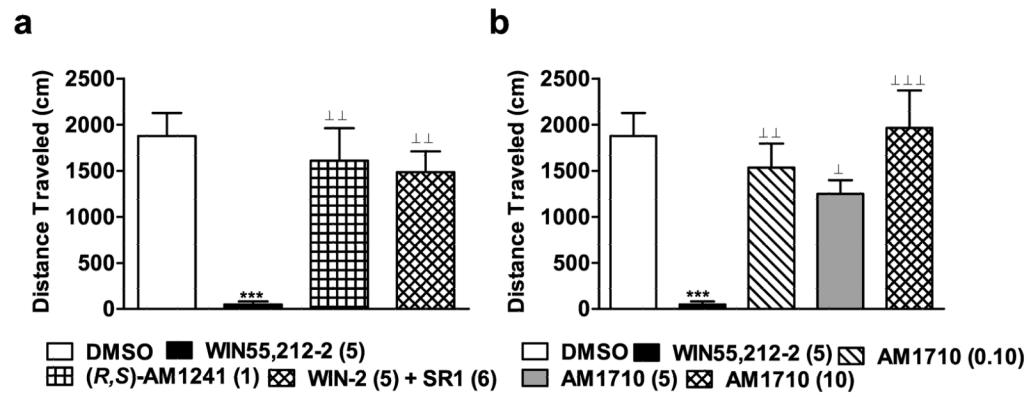


Figure 5.

(a) Effects of cannabimimetic and aminoalkylindole cannabinoid agonists on locomotor activity. WIN55,212-2 (WIN-2; 5 mg/kg i.p.) reduced total distance traveled (cm) through a CB₁ mechanism. This effect was blocked by the CB₁ antagonist SR141716 (SR1; 6 mg/kg i.p.). (R,S)-AM1241 (1 mg/kg i.p.) did not alter locomotor activity. (b) AM1710 (0.1, 5, and 10 mg/kg i.p.) did not alter locomotor activity. *** $P < 0.001$ vs. DMSO control condition, ± $P < 0.05$, ±± $P < 0.01$, ±±± $P < 0.001$ vs. WIN55,212 (5 mg/kg i.p.), (ANOVA; Dunnett and Tukey post hoc tests). $N = 6-8$ per group.

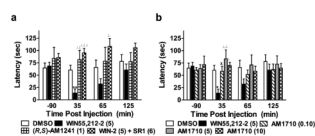


Figure 6.

(a) Effects of cannabimimetic and aminoalkylindole cannabinoid agonists on motor ataxia. WIN55,212-2 (WIN-2; 5 mg/kg i.p.) produced CB₁-mediated motor ataxia, manifested as a decrease in descent latency (sec) in the rota-rod test. This effect was blocked by SR141716 (SR1; 6 mg/kg i.p.). Neither (R,S)-AM1241 (1 mg/kg i.p.) nor (b) AM1710 (0.1, 5, and 10 mg/kg i.p.) altered rota-rod latency relative to the vehicle condition. * $P < 0.05$, ** $P < 0.01$ vs. DMSO control condition, $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$ vs. WIN55,212 (5 mg/kg i.p.) (ANOVA; Dunnett and Tukey post hoc tests). $\times P < 0.05$ vs. WIN55,212-2 (5 mg/kg i.p.) (Student t-test). N = 6–8 per group.

Table 1

Brain barrier penetration of AM1710 (1 mg/kg i.v.)

Plasma concentration	75.25 ± 12.29 ng/mL
Brain concentration	17.38 ± 2.63 ng/g
Brain-to-plasma ratio	0.23 mL/g

Data are mean ± standard deviation. Plasma and brain samples were removed 15 min post-injection, flash-frozen in liquid nitrogen and stored at -80°C until processing and analysis by LC-MS/MS.

Table 2

Paw withdrawal thresholds (g) to punctuate mechanical stimulation in animals that received the cannabidiol AM1710

Group	Pre-injection	Post-Injection
DMSO	67.8 ± 3.6	71.0 ± 4.8
AM1710 (0.1 mg/kg)	79.1 ± 4.2	74.0 ± 5.9
AM1710 (0.33 mg/kg)	66.1 ± 3.1	71.4 ± 4.6
AM1710 (1 mg/kg)	63.9 ± 3.6	73.3 ± 2.3 ⁺
AM1710 (5 mg/kg)	70.2 ± 3.7	64.7 ± 5.3
AM1710 (10 mg/kg)	63.9 ± 3.3	68.8 ± 5.5
AM1710 (0.1 mg/kg)	79.1 ± 4.2	74.0 ± 5.9
AM1710 (0.1) + SR2 (6)	73.7 ± 5.4	71.6 ± 4.7
AM1710 (0.1) + SR1 (6)	68.0 ± 4.2	69.3 ± 4.7
AM1710 (5 mg/kg)	70.2 ± 3.7	64.7 ± 5.3
AM1710 (5) + SR 2 (6)	81.4 ± 3.7	72.3 ± 3.1
AM1710 (5) + SR1 (6)	70.5 ± 5.7	64.1 ± 8.5
SR141716 (6 mg/kg)	63.3 ± 5.5	75.6 ± 5.7
SR144528 (6 mg/kg)	70.9 ± 5.1	62.8 ± 5.8

Data are mean ± s.e.mean. Doses are in mg/kg. SR1 = SR141716; SR2 = SR144528. Statistical comparisons were performed on groups separated by line divisions.

⁺ $P < 0.05$ vs. same group pre-injection threshold (Student t-test).