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Pharmacological Characterization of Novel Water-soluble Cannabinoids[1]

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Nonstandard abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol

%MPE, percent maximum possible effect

CP55940, (–)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol

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ABSTRACT

Presently, there are numerous structural classes of cannabinoid receptor agonists, all of which require solubilization for experimental purposes. One strategy for solubilizing water-insoluble tetrahydrocannabinols is conversion of the phenolic hydroxyl to a morpholinobutyryloxy substituent. The hydrochloride salts of these analogs are water-soluble and active *in vivo* when administered in saline. The present investigation demonstrated that hydrochloride salts of numerous substituted butyryloxy esters are water-soluble and highly potent. The substitutions include piperidine, piperazine, and alkyl substituted amino moieties. It was also discovered that incorporation of a nitrogenous moiety in the alkyl side chain increased the pharmacological potency of THC. For example, an analog containing a pyrazole in the side chain (O-2545) was found to have high affinity and efficacy at CB_1 and CB_2 receptors, and when dissolved in saline it was highly efficacious when administered either i.v. or i.c.v to mice. A series of carboxamido and carboxylic acid amide analogs exhibited high pharmacological potency but their hydrochloride salts were not water-soluble. On the other hand, incorporation of imidazoles into the terminus of the side chain led to water-soluble hydrochloride salts that were highly potent when administered in saline to laboratory animals. It is now possible to conduct cannabinoid research with agonists that are water-soluble and thus obviating the need of solubilizing agents.

Introduction

Marijuana has attracted considerable attention for centuries because of its psyschotropic and medicinal properties. Early scientific investigations were conducted with either smoked plant material or the plant extract. Needless to say, the synthesis of marijuana's major psychotropic constituent, Δ^9-THC, opened a new era in marijuana research (Gaoni and Mechoulam, 1964). For the first time researchers were able to conduct research in a quantitative fashion because the precise dose of Δ^9-THC could be administered.

Unfortunately, Δ^9-THC is a non-crystalline, highly lipophilic compound that requires solubilization with either a surfactant agent or adherence to a water miscible substance (albumin, Tween 80, emulphor, etc.). This high lipophilicity has placed constraints on the pharmacological evaluation of Δ^9-THC. Even when dissolved in these vehicles, Δ^9-THC has limited solubility and will precipitate if care is not exercised. This cannabinoid will adhere to solid surfaces rather than remain in solution under certain conditions. There is always the concern that the use of different vehicles in separate pharmacological studies may influence the pharmacological effects of Δ^9-THC. Undoubtedly, these challenges in drug solubilization contribute to the vagaries accompanying cannabinoid data collection. While many of the above vehicles are sufficient for systemic administration of cannabinoids, they present significant challenges for cannabinoid administration into specific sites. In a previous study examining spinal and supraspinal sites of cannabinoid action, Δ^9-THC was administered a mixture of ethanol, emulphor and saline for systemic administration and in dimethyl sulfoxide for spinal injection because neither vehicle was suitable for both routes of administration (Lichtman and Martin, 1991). *In vitro* studies present other problems in that many of the surfactants that are tolerated *in vivo* dissolve membranes thereby destroying the cell preparation. Of course, all of these potential pitfalls are

magnified with chronic drug administration or with long drug exposures *in vitro*. It is for these reasons that there have been numerous attempts to prepare water-soluble derivatives of cannabinoids.

The first successful attempt in preparing a water-soluble form of Δ^9-THC involved converting it to a morpholinobutyrl ester, the hydrochloride of which was water-soluble (Zitko et al., 1972). This compound retained cannabinoid pharmacological activity. A morpholinobutyrl ester of Δ^8-THC was also found to be equipotent to Δ^8-THC in several behavioral models (Compton and Martin, 1990). Water-soluble derivatives of Δ^8-THC were prepared in other laboratories and found to be effective in lowering intraocular pressure in rabbits (ElSohly et al., 1984). In more recent times, numerous cannabinoid analogs have been developed that are considerably more potent than Δ^9-THC (Martin et al., 1999; Khanolkar et al., 2000). One of these compounds contains a cyano group on the terminal carbon atom of the side chain in Δ^8-THC (Martin et al., 1999). Therefore, a morpholinobutryl ester of this potent cannabinoid was prepared and found to be highly active when prepared in saline and evaluated either *in vivo* or *in vitro* (Pertwee et al., 2000). It is assumed that these phenolic esters (Zitko et al., 1972; Pertwee et al., 2000) are prodrugs because a free hydroxyl group is required for pharmacological activity of Δ^9-THC at the CB_1 cannabinoid receptor (Razdan, 1986; Huffman et al., 2002). Phosphate esters of the endocannabinoids anandamide and noladin ether have also been prepared (Juntunen et al., 2003a; Juntunen et al., 2003b). These esters are rapidly hydrolyzed in biological tissues and are effective in lowering intraocular pressure in rabbits when applied in an aqueous solution.

There have also been numerous attempts to prepare analogs with reduced lipophilicity. Early receptor binding studies conducted with radiolabeled Δ^8-THC were of limited success because of the extensive non-specific binding by this highly lipophilic agent (Harris et al., 1978).

In an effort to reduce non-specific binding, Nye et al. (Nye et al., 1988) prepared a radiolabeled trimethylammonium analog of Δ^8-THC. This charged analog allowed them to label a specific binding site in brain, although it remains to be established that this site is a true cannabinoid receptor. A nitrogen mustard analog of Δ^9-THC was found to be behaviorally active when administered centrally but not when administered peripherally, presumably because of its reduced lipophilicity (Little et al., 1987). In addition to the Δ^9-THC analogs cited above, numerous aminoalkylindole and biarylpyrazole cannabinoid analogs have been prepared that have reduced lipophilicity (Gatley et al., 1998; Kumar et al., 2004; Deng et al., 2005; Willis et al., 2005). Many of these compounds were developed as possible positron emission tomography radiotracers. Some of these antagonists were prepared in saline and administered to animals, but only at tracer concentrations. It is unlikely that they can be administered in saline at sufficient levels to achieve a pharmacological response.

The present investigation was undertaken in order to further explore possible alterations in the Δ^8-THC structure that would render it water-soluble. An additional objective was to develop a series of water-soluble analogs that were not prodrugs.

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Materials and Methods

Materials

Male ICR mice (Harlan Laboratories, Indianapolis, IN) weighing between 24 to 30 g were used in all experiments. Mice were maintained on a 14:10-hr light/dark cycle with food and water available *ad lib*. All test groups consisted of 6 to 12 mice. Δ^9-THC was obtained from NIDA and dissolved in a vehicle consisting of ethanol, emulphor and saline in a ratio of 1:1:18. Analogs were dissolved either in the vehicle or saline depending upon their water solubility. All chemicals for receptor binding studies were purchased from Sigma (St. Louis, MO) except the following: [^{35}S]GTPγS (1250 Ci/mmol) was purchased from New England Nuclear Group (Boston, MA), GTPγS from Boehringer Mannheim (New York, NY), Dulbeco's modified Eagle's medium (DMEM) from GIBCO BRL (Grand Island, NY), Whatman GF/B glass fiber filters from Fischer Scientific (Pittsburg, PA), fetal calf serum (FCS) and fetal bovine serum (FBS) from HyClone Laboratories (Logan, UT) and Budget-Solve scintillation fluid from RPI Corp. (Mount Prospect, Ill).

Membrane Preparations

HEK-293 cells stably expressing the human CB_1 receptor were cultured in DMEM with 10% FBS and Chinese Hamster Ovary (CHO) cells stably expressing the human CB_2 receptor were cultured in DMEM with 10% FCS. Cells were harvested by replacement of the media with cold phosphate-buffered saline containing 1 mM EDTA followed by centrifugation at 1000 x g for 5 min at 4°C. The pellet was resuspended in 50 mM Tris-HCl containing 320 mM sucrose, 2 mM EDTA and 5 mM MgCl2 (pH 7.4) (centrifugation buffer), then centrifuged at 1000 x g for 10 min at 4°C and the resulting supernatant was saved. This process was repeated twice. The

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supernatant fractions were combined and centrifuged at 40,000 x g for 30 min at 4°C. The resulting P2 pellet was resuspended in assay buffer (50 mM Tris-HCl (pH 7.4), 3 mM $MgCl_2$, 0.2 mM EGTA, and 100 mM NaCl) and protein was measured. Membranes were stored at -80°C until use.

Receptor Binding

Membrane homogenates (50 µg) were incubated with 0.5 nM [^{3}H]CP55,940 in the presence of varying concentrations (1 nM-10 µM) of test compounds in 0.5 ml of buffer containing bovine serum albumin (5 mg/ml). Non-specific binding was measured in the presence of 1 µM CP55,940. The assay was incubated at 30°C for 1 hr and terminated by addition of ice cold 50 mM Tris-HCl containing bovine serum albumin (1 mg/ml) (pH 7.4) followed by filtration under vacuum through Whatman GF/B glass fiber filters with 3 washes with cold Tris buffer. Bound radioactivity was determined by liquid scintillation spectrophotometry at 50% efficiency after extraction by shaking samples for 30-60 min with Budget-Solve scintillation fluid. Data are reported as the mean ± SEM of three experiments, each performed in triplicate. K_i values were calculated from displacement data using Equilibrium Binding Data Analysis (BIOSOFT, Milltown, NJ).

[^{35}S]GTPγS Binding Assays

Concentration-effect curves were generated by incubating membranes (10 µg) in assay buffer + BSA (1 mg/ml) with various concentrations of test compounds in the presence of 20 µM GDP and 0.1 nM [^{35}S]GTPγS in a 1 ml total volume. [^{35}S]GTPγS binding stimulated by 2 µM CP55,940 was used as an internal standard in each assay. Basal binding was assessed in the

absence of agonist, and nonspecific binding was measured in the presence of 10 μM GTPγS. The reaction was incubated for 90 min at 30°C and terminated by filtration under vacuum through Whatman GF/B glass fiber filters with 3 washes with cold (4°C) Tris buffer (50 mM Tris-HCl, pH 7.4). Bound radioactivity was determined by liquid scintillation spectrophotometry at 95% efficiency after extraction overnight in ScintiSafe Econo 1 scintillation fluid. Data are reported as mean ± SEM of three experiments, each performed in triplicate. Nonlinear regression analysis was conducted by iterative fitting using JMP (SAS for Macintosh). Net-stimulated binding is defined as [^{35}S]GTPγS binding in the presence of drug minus basal and percent stimulation is expressed as (net stimulated [^{35}S]GTPγS binding/basal) x 100%.

Behavioral Evaluations

All animals were allowed to acclimate to the observation room overnight. Behavioral effects were assessed in the tetrad model to measure potency in producing antinociception, catalepsy, hypothermia, and hypomobility. The baselines for tail-flick latency (2-4 sec) and rectal temperature were determined prior to i.v. injections. Baseline rectal temperatures were measured using a telethermometer and a thermometer probe inserted to 25 mm (Yellow Springs Instrument Co., Yellow Springs, OH). The mice treated i.v. with an analog were placed in individual photocell activity chambers 5 min later. Spontaneous activity was monitored for 10 min in a Digiscan Animal Activity Monitor (Omnitech Electronice, Inc., Columbus, OH) as measured by the number of interruptions of 16 photocell beams per chamber. The total number of beam interruptions during the 10-min period was determined and presented as total counts. The mice were then assessed at 20 min following the i.v. injection for antinociception using the tail-flick reaction time to a heat stimulus. A 10-sec maximum latency was used in order to avoid

tail injury. The results are presented as % MPE and are calculated as follows: %MPE = [(test latency-control latency)/(10 sec – control latency)] x 100.

Rectal temperature was measured 30 min after the i.v. injection. The change in rectal temperature (Δ°C) following analog administration was calculated for each animal. Relative immobility (catalepsy) was measured 40 min after the i.v. injection by the ring-immobility test. Mice were placed on a ring 5.5 cm in diameter attached to a stand at a height of 16 cm. The amount of time the mice spent motionless on the ring during the 5-minute procedure was measured, with the criteria of immobility being defined as the absence of all voluntary movements, including whisker movement, but excluding respiration. The percent immobility was calculated as: % immobility = [time immobile (sec)]/[length of session (sec)] x 100. Mice that fell from the ring or actively jumped were allowed five attempts. After the fifth escape these mice were removed from the ring and not included in the calculations. Data were collected from 6-12 mice for each condition tested.

A similar protocol was used to determine the effects of analogs following either i.t. or i.c.v. injection. The method of Hylden and Wilcox (Hylden and Wilcox, 1980) was used to inject 5 µl of solution i.t. between L5 and L6 of the spinal cord with a 30-gauge needle. I.c.v. injections were performed as described earlier (Pedigo et al., 1975). Mice were anesthetized with 2.5% isoflurane, and a transverse incision was made in the scalp. A free-hand 5 µl injection of drug was made into the lateral ventricle. Mice were tested as described above in the activity champers 5 to 15 min after the injection. Antinociception, body temperature, and catalepsy were quantitated 20, 20 and 40 min, respectively, after the injection.

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All studies were carried out in accordance with the Declaration of Helsinki and Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

Data analysis

Based on data obtained from numerous previous *in vivo* studies with cannabinoids, maximal cannabinoid effects in each *in vivo* pharmacological procedure were estimated as follows: 90% inhibition of spontaneous activity, 100% MPE in the tail flick procedure, and -6 ^{o}C change in rectal temperature. Means and standard error (S.E.) were calculated for %MPE, number of photocell disruptions, % ring immobility and Δ°C. Analysis of variance (ANOVA) was used to determine significant differences between control and treatment groups followed by Dunnett's t-test post-hoc analysis. Statistical analysis was performed using StatView, version 5.0 (SAS Institute, Cary, NC). Significance was defined as a "$p < 0.05$. ED_{50}'s were defined as the dose at which half maximal effect occurred. For drugs that produced one or more cannabinoid effect, ED_{50}'s were calculated separately using least-squares linear regression on the linear part of the dose-effect curve for each measure in the mouse tetrad, plotted against $\log_{10}$ transformation of the dose. For the purposes of potency comparison, potencies are expressed as μmol/kg.

Synthesis of compounds

All compounds were synthesized from various intermediates **2a-c** prepared using our published procedure (Singer et al., 1998), starting with the commercially available 5-cyano-dimethoxyresorcinol **1** (Scheme 1). Compounds listed in Table 1 were synthesized from the acid **2c** using standard procedures for the preparation of amides. The reverse amides (O-2589, O-2590, O-2619 and O-2620) were synthesized from **2a** by conversion to the amine via the azide,

followed by condensation with the appropriate acids using either the acid chloride or the carbodiimide (EDCI/DMAP) procedures. All the N-alkylated compounds listed in Table 2 were synthesized from **2a** by protection of the phenol as the TBDMS derivative, which was treated with the appropriate amine in the presence of NaH/DMF, to give the target compounds. The C-alkylated imidazole derivative (O-2737) was synthesized from **2c** by conversion of the acid group to the aldehyde, followed by condensation with glyoxal/NH_3 to form the 2-imidazole derivative (Dhanak et al., 2001). The phenolic esters listed in Table 3 were synthesized from **2b** using our published procedure (Razdan et al., 1976). The various acids used in their preparation were prepared according to literature procedures (Blicke et al., 1941; Cruickshank and Sheehan, 1961; Razdan et al., 1976). The quarternary compounds were synthesized by the treatment of the amines with CH_3I in ether. The compound listed in Table 4 was synthesized from the amide O-2372 (see Table 1) and diisopropylaminobutyric acid.HCl using the EDCI/DMAP procedure and the free base thus obtained was converted to its hydrochloride. All compounds showed appropriate ^{1}H NMR profiles (Jeol Eclipse 300 MHZ; Jeol USA, Inc., Peabody, MA) and were characterized on the basis of their ^{1}H NMR profiles, TLC, and elemental analyses.

cLogP calculations

cLogP (logarithm of the partition coefficient between n-octanol and water) calculations were performed using ChemDraw Ultra, CambridgeSoft Corp., Cambridge, MA.

Plasma stability

Assays were performed at 37°C using a substrate concentration of 3 µM in plasma (Wistar rat, Harlan Sera-Lab Ltd, Loughborough, UK; Human, Biochemed Pharmacologicals Inc, Winchester, VA) or 0.5M Tris buffer, pH 7.4. Samples (50 µl) were taken at 0, 10, 30, 60

and 150 minutes and were added to 150 µl acetonitrile containing internal standard (500 ng/ml desipramine). Following centrifugation at 4°C, supernatant was transferred and was made 50% (v/v) aqueous with the addition of water. Analysis was performed by LC-MS/MS (Micromass Ultima triple quadropole mass spectrophotometer with MUX interface).

Microsomal stability

Human hepatic microsomes were commercially obtained (Gentest, USA). ICR mouse liver microsomes were prepared in-house from male animals fasted overnight. Freshly thawed microsomes were diluted to yield a final P450 concentration of 250 pmol/ml. The final concentration of test compound in the incubation was 1 µM (with 1.25% acetonitrile (v/v), 0.25% DMSO (v/v)). The microsomal mixture was pre-incubated for 15 min in a shaking incubator at 37°C. NADPH (1mM final concentration) was then added to initiate the reaction. Samples (50 µl) were taken at 0, 10, 30 and 60 min, and were terminated by adding to 150µl acetonitrile containing internal standard (500 ng/ml desipramine). Water (100 µl) was added to the samples to give 50:50 aqueous/methyl cyanide. Samples were centrifuged at 4°C with analysis by LC-MS/MS as described for plasma stability.

P450 inhibition

Assays were performed using human cDNA-expressed enzymes (Supersomes™; BD Gentest, USA) with probe substrates that were metabolized to strongly fluorescent compounds. Each P450-specific assay was performed using a substrate concentration at the apparent K_m for that enzyme (as determined by BD Gentest). The following substrates and incubation times were used: CYP1A2, 7-ethoxy-3-cyanocoumarin (CEC) (5 µM), 15 min; CYP2C9, 7-methoxy-4-

trifluoromethylcoumarin (75 μM), 45 min; CYP2C19, 7-ethoxy-3-cyanocoumarin (25 μM), 30 min; CYP2D6, 3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin (1.5 μM), 30 min; CYP3A4, 7-benzyloxyquinoline (40 μM), 30 min. Incubations were performed according to the BD Gentest protocol using eight different final concentrations of test compound, in three-fold dilutions, from 75 to 0.03μM (stock solution 3.75mM in 50:50 acetonitrile/H_2O). For each set of serial dilutions, two incubations in the absence of test compound (100% metabolite formation) and two with quenching solution added prior to P450 addition (background fluorescence) were also performed. All incubations were performed at a final concentration of 1% acetonitrile together with an NADPH regenerating system (0.4 units/ml G-6-P dehydrogenase plus $NADP^+$ [1.3 mM, except CYP2D6 at 8.2 μM]). Incubations (200 μl) were terminated by the addition of quenching solution (75 μl of 80% acetonitrile (v/v)/ 20% 0.5M Tris base (v/v)).

The assays were performed in white 96-well plates (Greiner, Stonehouse, Gloucestershire, UK) and the fluorescence was measured using a fluorescence plate reader (Victor™, EG&G Wallac, Milton Keynes, UK). The percentage activity (compared to background wells and wells without test compound) was plotted against log [test compound], and IC_{50} values were determined using a four parameter logistic equation (Prism™, GraphPad Software Inc, CA, USA).

Results

Carboxamido Pentyl Side Chain Analogs. There is ample evidence that a wide range of structural alterations at the terminal carbon of a dimethypentyl chain in THC can be made without diminishing cannabinoid pharmacological activity. For example, addition of a cyano moiety at this position greatly enhances CB_1 receptor affinity and pharmacological potency

(Martin et al., 1999). Therefore, a series of carboxamido derivatives were analyzed as shown in Table 1 (structures in left column of Scheme 2). Most of the analogs exhibited high affinity for both CB_1 and CB_2 receptors, were effective in stimulating GTPγS binding and produced *in vivo* cannabinoid effects. The unsubstituted carboxamido (O-2352) exhibited excellent CB_1 receptor affinity and even higher CB_2 receptor affinity. GTPγS binding indicated it was a potent and fully efficacious agonist. In the *in vivo* mouse model, it was slightly more potent than Δ^9-THC. Moreover, its lipophilicity was 35-fold less than that of Δ^9-THC. CB_1 receptor affinity, efficacy (GTPγS binding) and pharmacological potency were retained with carboxamido substitutions containing methyl (O-2490), morpholine (O-2544), homo-piperidine (O-2489) or pyrrolidine (O-2543), although there were several notable differences between these compounds. The CB_2 receptor selectivity was greatly diminished with all substitutions and was actually reversed with the morpholino and homo-piperdino analogs. Interestingly, the homo-piperidino (O-2489) and pyrrolidino (O-2543) analogs were 4-25 fold less potent in producing hypothermia and catalepsy than in producing hypoactivity and analgesia. In addition, O-2490 failed to produce maximal effects in hypothermia and catalepsy assays at a dose (1 mg/kg) that produced maximal effects in the other two measures. A separation in pharmacological potencies of this magnitude is unusual. The lipophilicity was similar for all of the carboxamides with the exception of the homo-piperidinyl (O-2489) analog that was calculated to be 28 times more lipid soluble than the unsubstituted carboxamido (O-2352).

Five carboximido analogs were prepared with the morpholino (O-2372), piperidino (O-2373), pyrrolidino (O-2399), and homo-piperidino (O-2421) compounds having high CB_1 and CB_2 receptor affinity, high efficacy in GTPγS binding, and very high *in vivo* potency in all four pharmacological measures. The most active compounds (O-2372 and O-2373) were 100-200

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times more potent than Δ^9-THC. It is noteworthy that replacement of a piperidine (O-2373) with a basic N-methyl piperazine group (O-2381) decreased CB_1 receptor affinity and pharmacological potency more than a hundred fold. Most of these compounds were slightly less lipophilic than Δ^9-THC. Of the remaining compounds in Table 1, only O-2619 demonstrated reasonable affinity for the CB_1 receptor; however, it was not evaluated *in vivo*.

Compounds O-2352, O-2372, O-2373, O-2399, and O-2544 were converted to hydrochloride salts as confirmed by NMR. However, none of the HCl salts was water-soluble. These analogs were deemed representative of the analogs in Table 1, and therefore no attempts were made to convert the other analogs in this table to HCl salts. Due to lack of water-solubility, all of the analogs in Table 1 were administered to mice using the 1:1:18 emulphor:ethanol:water vehicle.

Imidazole-, Pyrazole-, and Triazole-pentyl Side Chain Analogs. The imidazole substitution (O-2545) on the terminal carbon atom of the side chain resulted in a high affinity CB_1 agonist that was fully efficacious in stimulating GTPγS binding (Table 2). It exhibited even higher affinity for the CB_2 receptor. The free base of this analog, dissolved in 1:1:18 emulphor:ethanol:water vehicle, produced the full spectrum of pharmacological effects in the mouse model and proved to be at least 40-fold more potent than Δ^9-THC in all measures. The hydrochloride salt of O-2545 was dissolved in saline and when administered to mice produced effects that were equivalent to those of the free base. The calculated lipophilicity (cLogP**)** of this analog was 20 times less than that of Δ^9-THC. A single methyl substitution at the 2 position of the imidazole (O-2651) reduced affinity for both CB_1 and CB_2 receptors: however, it was still water soluble. This relatively minor structural alteration had a greater impact on efficacy in that it was only a partial CB_1 receptor agonist in GTPγS binding. When administered in saline to

mice, it was at least 10-fold less potent than the corresponding hydrochloride salt of O-2545. Both pyrazole (O-2715) and triazole (O-2716) analogs had high CB_1 receptor affinity and were fully efficacious in stimulating GTPγS binding. They were also very potent in producing cannabinoid effects in the tetrad model. However, neither hydrochloride salts of these analogs was water-soluble. Merely changing the attachment position from the nitrogen on the imidazole (O-2545) to the carbon 2 position on the imidazole ring (O-2737) dramatically reduced CB_1 and CB_2 receptor affinities. As expected based upon their CB_1 receptor affinity, they exhibited only weak *in vivo* pharmacological activity.

Phenolic esters. The phenolic esters in Table 3 represent an extension of earlier work on the morpholinobutyryloxy derivative of Δ^8-THC (O-1057). As with O-1057, most of these analogs were water soluble upon conversion to hydrochloride salts. Substituting the morpholino with a piperidino (O-2365) or methypiperidino groups (O-2426) slightly increased CB_1 receptor affinity over that of O-1057. Both analogs were effective in stimulating GTPγS binding and very potent in the mouse behavioral assays. A methyl group was added to the carbon 1 position in the butyryl moiety in an attempt to delay hydrolysis and thereby increase duration of action. This addition produced only a slight reduction in CB_1 receptor affinity and pharmacological potency. The greatest difference was seen with lipophilicity which was increased almost 100-fold by the addition of the methyl group to O-2365 but not to O-2426. The N-4'-methylpiperazino analogs (O-2383 and O-2427) exhibited properties similar to the piperidino (0-2365) and 2-methyl-N-piperidino (O-2374) analogs. A series of N-alkylaminobutyryl oxy analogs (O-2484, O-2487, O-2548, O-2650, O-2382, and O-2485) was prepared. For the most part, all of these analogs had good CB_1 receptor affinity, activated GTPγS binding, and were potent when administered to mice (Table 3). The 2-methyl analogs (O-2650 and O-2485) were

somewhat less potent pharmacologically and had lower CB_1 receptor affinity. It is particularly noteworthy that the quaternary analog O-2548 is very potent pharmacologically. Of course, it also has high lipophilicity despite being a quaternary compound.

4-Diisopropylaminobutyrate of Δ^8-THC-3-(1,1-dimethyl-6-morpholin-4-yl-6-oxo-hexyl. The fact that the diisopropylaminobutyrate of Δ^8-THC (O-2382 in Table 3) had high receptor affinity and was extremely potent *in vivo* provided an opportunity to convert one of the water insoluble carboxamido analogs in Table 1 to a water-soluble cannabinoid. Therefore, the morpholinocarboxamido (O-2372) was chosen, because it also has high receptor affinity and excellent *in vivo* potency despite being water-insoluble. The resulting analog, O-2694, was found to be water-soluble, retained high CB1 and CB2 receptor affinity, and exhibited high potency and efficacy in GTPγS binding (Table 4). It was also highly potent *in vivo* despite an almost 200-fold increase in lipophilicity as compared to O-2372. However, according to cLogP values, it is only five-fold less lipophilic than O-2382.

Influence of route of administration. The absorption of Δ^9-THC following oral administration is both poor and erratic. It is assumed that the high lipophilicity of Δ^9-THC contributes to some extent to its low absorption. Therefore, the time course of three analogs with varying cLogP values was examined following their oral administration in mice (data not shown). O-2545 (24 times less lipophilic than Δ^9-THC) was administered at a dose of 10 mg/kg and different groups of mice were tested at 0.5, 1, 2, 4 and 6 hrs. The time course was similar for all four behavioral measures. For example, maximal analgesia in the tail-flick procedure was obtained at 30 min and remained at 85±11 % (Mean ±SEM) at two hours. By four hours, the effects had largely dissipated. O-2716 was 15 fold less lipophilic than Δ^9-THC. Its oral administration at a dose of 10 mg/kg resulted in a time course very similar to that of O-2545.

Maximal effects were observed at 30 min and only a slight decrease in activity was observed at 2 hours. At four hours, few differences were observed between vehicle- and O-2716-treated mice. The lipophilicities of O-2715 and Δ^9-THC were almost identical. As predicted, the oral administration of O-2715 (10 mg/kg) produced negligible effects at one hr (21±6% analgesia), and the effects declined over time.

Selected analogs as hydrochloride salts were also evaluated by direct injection into either the brain or spinal cord using saline as the vehicle (Table 5). These analogs were chosen as representatives of both phenolic esters (O-1057 and O-2383) and pentyl side chain analogs (O-2545, O-2737 and O-2651). The reference compound Δ^9-THC, prepared in the standard vehicle (combination of emulphor, ethanol, and saline), was effective in all four behavioral measures, albeit with relatively weak potency. While several general observations emerge, it is most noteworthy that all of the analogs were active when administered either i.c.v. or i.t. As expected, the analogs were more effective in suppressing spontaneous activity when given i.c.v. than i.t. In addition, the potencies of the analogs after i.c.v. or i.t. were reflective of their potencies following i.v. administration. O-1057, O-2383, and O-2545 were more potent than O-2651 and O-2737 regardless of the route of administration. It is worthy to note that the side chain imidazole analog O-2737 was not active when administered i.v. at doses up to 10 μmoles/mouse but was as potent as Δ^9-THC when injected either i.c.v. or i.t.

Inhibition of cytochrome P450's. As can be observed in Table 6, several analogs were selected for metabolic studies due to their structural diversity. CP 55,940, O-1057 and O-581, the hydrolyzed form of O-1057, served as a comparison group. All three compounds exhibited little inhibitory effects on CYP1A2. However, they inhibited the other CYP's more or less in the same concentration range. In comparison to O-1057, O-581 was five fold more effective in

blocking CYP2C9 and five fold less effective in blocking CYP2D6. O-2545 differed from the above compounds in that it blocked CYP1A2 at 2 μM and was much more effective than the comparison compounds in blocking CYP3A4. The inhibitory profile of O-2715 was quite similar to that of O-581. The primary difference between O-2715 and O-27l6 was the latter's increased potency at blocking CYP1A2.

Plasma and microsomal stability. All the compounds that contain the ester group were rapidly metabolized in the rodent plasma with t1/2 < 5 min (Table 7). The piperidine-containing compound (O-2374) was also rapidly metabolized in human plasma. The other compounds were all stable in human plasma. The control incubations (buffer only) showed that there was some degradation of the compounds during the 37°C incubation, possibly by thermal decomposition (especially O-2548 and O-2487). This is in contrast to the stability in human plasma, indicating that human plasma may act to protect the compounds, perhaps by protein binding. The ester-containing compounds were all rapidly metabolized when incubated with both mouse and human liver microsomes (Table 7). For example, O-2548 was the most stable analog in mouse liver microsomes with a t1/2 of 12 min. Most of these compounds have an element of non P450-mediated metabolism (i.e. turnover in the absence of NADPH), perhaps due to microsomal esterase activity. Although O-2545 was a more stable compound than the other analogs examined, it was still metabolized reasonably quickly in microsomes from both species (t1/2 ~10 min in both human and mouse liver microsomes).

Discussion

Extensive structure-activity relationship studies began almost immediately after the structure of Δ^9-THC was established (Gaoni and Mechoulam, 1964; Edery et al., 1971; Razdan,

1986). These initial studies firmly established the importance of three structural features of Δ^9-THC: the C9 position, phenolic hydroxyl group, and pentyl side chain. These regions of the molecule continue to be the focus of structural modifications of THC. Elimination or structural modification of the phenolic hydroxyl group rendered THC inactive at the CB_1 receptor (Razdan, 1986). Recently, a series of desoxy-Δ^8--THC analogs were found to lack CB_1 receptor affinity but retained their ability to interact with CB_2 receptors (Huffman et al., 2002). The side chain can easily be manipulated to increase agonist potency or to reduce or eliminate agonist efficacy (Martin et al., 1999). In addition, the terminal carbon atom of the side chain can tolerate a wide range of substituents (Martin et al., 1999). Therefore, structural modifications at the phenolic hydroxyl and side chain represent logical sites for expanding the structure-activity relationship of Δ^8-THC and for developing water-soluble ligands.

As we had previously shown (Zitko et al., 1972; Pertwee et al., 2000), the addition of a basic functional group through an ester linkage at the phenolic hydroxyl group provides a means for preparing hydrochloride salts that are water-soluble. It is now evident that hydrochloride salts can be readily prepared when the terminal group in the butyryloxy group is either morpholino, piperidino or piperazino moieties. Since a free phenolic group is essential for interaction with the CB_1 receptor, it would appear that all of the compounds are readily hydrolyzed since they are highly potent when administered either i.v., i.c.v. or i.t. to mice. In an effort to retard hydrolysis, we incorporated a methyl group on the alpha carbon of several analogs. While we have no direct evidence of the degree of hydrolysis that occurs in the receptor binding assays, it is noteworthy that the CB_1 receptor affinity decreased with the methyl addition, whereas binding to the CB_2 receptor was less affected. These observations are consistent with decreased hydrolysis, since a free hydroxyl is essential for CB_1 but not CB_2 receptor binding. It is also evident that an alkyl

substituted amino or ammonium group forms a hydrochloride salt that is in turn water-soluble. Alpha methylation in the butyryloxy group had mixed results on CB_1 and CB_2 binding. In the case of the ammoniumbutyryloxy analogs (O-2548 and O-2650, Table 3), binding was increased for both receptor subtypes. However, binding was increased only for the CB_1 receptor with the dimethyaminobutyryloxy (O-2484 and O-2487) and diisopropylaminobutyryloxy (O-2382 and O-2485) analogs. In the latter case, CB_1 receptor binding was increased 15-fold with the alpha methyl addition. There was also considerable diminution in the pharmacological potency of the methyl derivative, again suggestive of decreased hydrolysis.

The second strategy for developing water-soluble derivatives was incorporation of nitrogenous constituents in the side chain that could be converted to hydrochloride salts. Earlier studies had demonstrated that a wide range of small substituents (hydroxy, bromo, cyano, azido, acetamido, etc.) could be incorporated into the terminus of the side chain (Charalambous et al., 1992; Martin et al., 1993; Martin et al., 1999). Importantly, these studies demonstrated that the nitrogen containing substituents increased CB_1 receptor affinity and pharmacological potency. Therefore, it was not unexpected that the carboxamido (O-2352) and N-methylcarboxamido (O-2490) analogs would have reasonable receptor affinity and be pharmacologically active. The finding that the incorporation of morpholino, piperidino and homo-piperidino and pyrrolidino groups on the side chain terminus retains pharmacological activity indicates that the receptor pharmacophore readily accommodates bulky substituents at this position. Similar findings were observed in the carboximido series with the exception that these analogs exhibited higher receptor affinity and greater pharmacological potency than the corresponding analogs in the carboxamido series. It is interesting to note that the homo-piperidino analog in the carboxamido series (O-2489, Table 1) was considerably less potent than the corresponding analog in the

carboximido series (O-2421). In addition, the methylpiperidino analog in the carboximido series was a very weak agonist. These two observations suggest that electrostatic influences are more important than steric effects at this position. On the other hand, the loss in activity of the di-morpholino-acetamide (O-2620) is highly likely to be due to steric hinderance. While most of the carboxamido and carboximido analogs exhibited high affinity and excellent pharmacological potency, it was disappointing that none of the hydrochloride salts was water-soluble.

In contrast to the carboxamido and carboximido analogs, the imidazole analogs readily formed hydrochloride salts that were water-soluble. The fact that the imidazole-1-yl analog (O-2545, Table 3) had high receptor affinity, efficacy similar to CP 55,940 in stimulating GTPγS binding and high potency in the mouse behavioral assays make it an ideal water-soluble cannabinoid agonist. It is also 24-fold less lipophilic than Δ^9-THC and does not require metabolic conversion to an active constituent. Moreover, it has excellent stability in buffer and plasma and microsomal enzymes as compared to most of the analogs. The pyrazole and triazole analogs also exhibited excellent receptor affinity and high potency *in vivo*. The dramatic decrease in receptor affinity and pharmacological potency that occurred with the imidazole-2-yl analog (O-2737) demonstrates the importance of electrostatic influences at the side chain terminus. On the other hand, the observation that some of these imidazole derivatives have greater potency when administered i.c.v. suggests that they may have difficulty penetrating the central nervous system.

The question arises as to whether incorporation of both a phenolic ester and a side chain carboxamide (O-2694, Table 4) would provide even greater water solubility. Clearly, this analog retained high affinity and excellent *in vivo* potency.

High lipophilicity is not the only challenge that cannabinoids present. Δ^9-THC is also rapidly metabolized by cytochrome P450 enzymes (Bornheim et al., 1992) and is tightly bound to plasma proteins (Widman et al., 1974). The structural changes that rendered the analogs water-soluble have the potential of altering both the metabolic pattern as well as plasma protein binding. The two enzymes most sensitive to Δ^9-THC, CYP2C9 and CPY2C19, were inhibited more or less to the same degree by the side chain substituted analogs. Since the major metabolic pathway for Δ^9-THC is C11 hydroxylation via CYP2C9 (Bornheim et al., 1992), it is anticipated that the metabolic profile of the analogs will exhibit some similarity to that of Δ^9-THC. The morpholino derivative O-1057 was somewhat less effective in inhibiting these enzymes. Δ^9-THC was considerably less effective than the analogs in inhibiting CYP2D6, the enzyme that is involved in the metabolism of several centrally acting drugs. It is reasonable to speculate that the analogs may have greater potential than Δ^9-THC in inhibiting the CYP2D6 metabolism of other drugs. All of the compounds exhibited short half-lives in the presence of microsomal enzymes with the exception of O-2545 and O-2548. There was a dramatic difference between rat and human plasma stability for all of the compounds with the exception of O-2545 and O-2374, a difference that serves as a reminder for use of caution in extrapolating animal data to humans.

In summary, there are numerous effective means of synthesizing water-soluble cannabinoids. The conversion of the phenolic hydroxyl group to an ester that is readily hydrolyzed *in vivo* is an effective strategy. Despite the requirement for metabolic activation, these compounds are active when injected directly into brain or spinal cord. In addition, incorporation of nitrogen-containing rings at the terminal carbon atom of the side chain allows for the preparation of hydrochloride salts that are readily water-soluble. The advantage of these

compounds is that they do not require metabolic activation. It is now possible to use these same synthetic strategies to develop water-soluble CB_1 and CB_2 selective agonist and antagonists. The necessity of using solubilizing agents in order to evaluate the pharmacological properties of an agent poses challenges to the investigator. There is always the possibility of a pharmacological interaction between vehicle and test agent. More likely, the vehicle will influence the pharmacokinetics of the test substance that adds an additional challenge when comparing data generated from different labs that utilize various vehicles. Elimination of solubilizing agents as a vehicle eliminates possible artifacts arising from these substances. Incorporation of nitrogen-containing rings at the terminal carbon atom of the THC side chain allows for the first time the synthesis of CB_1 receptor agonists that do not require metabolic conversion in order to be water-soluble.

References

Blicke FF, Wright WB, Jr. and Zienty MF (1941) The action of heat on γ-alkoxybutyryl chlorides. *J. Amer. Chem. Soc.* **63**:2488-2490.

Bornheim LM, Lasker JM and Raucy JL (1992) Human hepatic microsomal metabolism of Δ^1-tetrahydrocannabinol. *Drug Metab. Dispos.* **20**:241-246.

Charalambous A, Yan G, Houston DB, Howlett AC, Compton DR, Martin BR and Makriyannis A (1992) 5'-Azido-Δ^8-THC: A novel photoaffinity label for cannabinoid receptor. *J. Med. Chem.* **35**:3076-3079.

Compton DR and Martin BR (1990) Pharmacological evaluation of water soluble cannabinoids and related analogs. *Life Sci.* **46**:1575-1585.

Cruickshank PA and Sheehan JC (1961) Intramolecular assistance of decarboxylative acylation. *J. Amer. Chem. Soc.* **83**:2891-2895.

Deng H, Gifford AN, Zvonok AM, Cui G, Li X, Fan P, Deschamps JR, Flippen-Anderson JL, Gatley SJ and Makriyannis A (2005) Potent cannabinergic indole analogues as radioiodinatable brain imaging agents for the CB1 cannabinoid receptor. *J Med Chem* **48**:6386-6392.

Dhanak D, Christmann LT, Darcy MG, Keenan RM, Knight SD, Lee J, Ridgers LH, Sarau HM, Shah DH, White JR and Zhang L (2001) Discovery of potent and selective phenylalanine derived CCR2 antagonists. Part1. *Bioorg. Med. Chem. Lett.* **11**:1445-1450.

Edery H, Grunfeld Y, Ben-Zvi Z and Mechoulam R (1971) Structural requirements for cannabinoid activity. *Ann. N.Y. Acad. Sci.* **191**:40-53.

ElSohly MA, Harland EC, Benigni DA and Waller CW (1984) Cannabinoids in glaucoma II: The effect of different cannabinoids on intraocular pressure of the rabbit. *Curr. Eye Res.* **3**:841-850.

Gaoni Y and Mechoulam R (1964) Isolation, structure, and partial synthesis of an active constituent of hashish. *J. Amer. Chem. Soc.* **86**:1646-1647.

Gatley SJ, Lan R, Volkow ND, Pappas N, King P, Wong CT, Gifford AN, Pyatt B, Dewey SL and Makriyannis A (1998) Imaging the brain marijuana receptor: development of a radioligand that binds to cannabinoid CB1 receptors in vivo. *J Neurochem* **70**:417-423.

Harris LS, Carchman RA and Martin BR (1978) Evidence for the existence of specific cannabinoid binding sites. *Life Sci.* **22**:1131-1138.

Huffman JW, Bushell SM, Miller JR, Wiley JL and Martin BR (2002) 1-Methoxy-, 1-deoxy-11-hydroxy- and 11-Hydroxy-1-methoxy-Delta(8)- tetrahydrocannabinols: new selective ligands for the CB(2) receptor. *Bioorg Med Chem* **10**:4119-4129.

Hylden JL and Wilcox GL (1980) Intrathecal morphine in mice: a new technique. *Eur. J. Pharmacol.* **67**:313-316.

Juntunen J, Huuskonen J, Laine K, Niemi R, Taipale H, Nevalainen T, Pate DW and Jarvinen T (2003a) Anandamide prodrugs. 1. Water-soluble phosphate esters of arachidonylethanolamide and R-methanandamide. *Eur J Pharm Sci* **19**:37-43.

Juntunen J, Vepsalainen J, Niemi R, Laine K and Jarvinen T (2003b) Synthesis, in vitro evaluation, and intraocular pressure effects of water-soluble prodrugs of endocannabinoid noladin ether. *J Med Chem* **46**:5083-5086.

Khanolkar AD, Palmer SL and Makriyannis A (2000) Molecular probes for the cannabinoid receptors. *Chem. Phys. Lipids* **108**:37-52.

Kumar JS, Prabhakaran J, Arango V, Parsey RV, Underwood MD, Simpson NR, Kassir SA, Majo VJ, Van Heertum RL and Mann JJ (2004) Synthesis of [O-methyl-11C]1-(2-chlorophenyl)-5-(4-methoxyphenyl)-4-methyl-1H-pyrazole- 3-carboxylic acid piperidin-1-ylamide: a potential PET ligand for CB1 receptors. *Bioorg Med Chem Lett* **14**:2393-2396.

Lichtman AH and Martin BR (1991) Spinal and supraspinal mechanisms of cannabinoid-induced antinociception. *J. Pharmacol. Exp. Ther.* **258**:517-523.

Little PJ, Kaplan NC and Martin BR (1987) Pharmacological profile of Δ^9-THC carbamate. *Alc. Drug Res.* **7**:517-523.

Martin B, Compton D, Semus S, Lin S, Marciniak G, Grzybowska J, Charalambous A and Makriyannis A (1993) Pharmacological evaluation of iodo and nitro analogs of Δ^8-THC and Δ^9-THC. *Pharmacol. Biochem. Behav.* **46**:295-301.

Martin BR, Jefferson R, Winckler R, Wiley JL, Huffman JW, Crocker PJ, Saha B and Razdan RK (1999) Manipulation of the tetrahydrocannabinol side chain delineastes agonists, partial agonists, and antagonists. *J. Pharmacol. Exp. Ther.* **290**:1065-1079.

Nye JS, Voglmaier S, Martenson RE and Snyder SH (1988) Myelin basic protein is an endogenous inhibitor of the high-affinity cannabinoid binding site in brain. *J. Neurochem.* **50**:1170-1178.

Pedigo NW, Dewey WL and Harris LS (1975) Determination and characterization of the antinociceptive activity of intraventricularly administered acetylcholine in mice. *J Pharmacol Exp Ther* **193**:845-852.

Pertwee RG, Gibson TM, Stevenson LA, Ross RA, Banner WK, Saha B, Razdan RK and Martin BR (2000) O-1057, a potent water-soluble cannabinoid receptor agonist with antinociceptive properties. *Br. J. Pharmacol.* **129**:1577-1584.

Razdan RK (1986) Structure-activity relationships in cannabinoids. *Pharmacol. Rev.* **38**:75-149.

Razdan RK, Terris BZ, Pars HG, Plotnikoff NP, Dodge PW, Dren AT, Kyncl J and Somani P (1976) Drugs derived from cannabinoids: 2. Basic esters of nitrogen and carbocyclic analogs. *J. Med. Chem.* **19**:454-461.

Singer M, Ryan WJ, Saha B, Martin BR and Razdan RK (1998) Potent cyano and carboxamide side-chain analogus of 1',1'-dimethyl-Δ^8-tetrahydrocannabinol. *J. Med. Chem.* **41**:4400-4407.

Widman M, Agurell S, Ehrnebo M and Jones G (1974) Binding of (+) and (-)-Δ^1-tetrahydrocannabinols and (-)-7-hydroxy-Δ^1-tetrahydrocannabinol to blood cells and plasma proteins in man. *J. Pharm. Pharmacol.* **26**:914-916.

Willis PG, Pavlova OA, Chefer SI, Vaupel DB, Mukhin AG and Horti AG (2005) Synthesis and structure-activity relationship of a novel series of aminoalkylindoles with potential for imaging the neuronal cannabinoid receptor by positron emission tomography. *J Med Chem* **48**:5813-5822.

Zitko BA, Howes JF, Razdan RK, Dalzell BC, Dalzell HC, Sheehan JC and Pars HG (1972) Water-soluble derivatives of Δ^1-tetrahydrocannabinol. *Science* **177**:442-444.

Footnote.

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TABLE 1. Carboxamido Pentyl Side Chain Analogs

CB_1 and CB_2 receptor binding was carried out in CHO and HEK cells, respectively, with the exception of THC and CP 55,940 CB_1 binding which were performed in rat brain membranes. GTPγS binding was carried out in CHO cells and maximal binding was expressed as a percentage of stimulation produced by CP 55,940. For the *in vivo* studies, the ED50 values are provided as μmoles/kg for reducing spontaneous activity (S.A.), producing antinociception in the tail-flick procedure (T.F), lowering rectal temperature (R.T.) and producing relative immobility (R.I.) following i.v. administration in mice. Analogs were dissolved in emulphor:ethanol:saline 1:1:18) since they were insoluble in saline.

O#	Name	CB_1 Ki (nM)	CB_2 Ki (nM)	CB_1 EC50	CB_1 %CP Stim	S.A. (μmol/kg)	T.F. (μmol/kg)	R.T. (μmol/kg)	R.I. (μmol/kg)	cLogP
	Δ^9-THC	41±3	94±4	ND	ND	2.23	2.77	2.23		7.238
	CP 55,940	4.0±0.3	2.5±0.1	ND	100	0.11	2.77	0.93	0.92	5.819
O-2352	Carboxamido	13±0.61	0.84±0.05	42 ± 4.0	95 ± 0.30	0.16	0.84	1.72	0.93	5.699
O-2490	N-(Methyl)carboxamido	19±0.58	3.2 ± 0.75	64±11	93±7.8	0.49	1.37	>1	>1	5.735
O-2544	N-(Morpholin-1-yl)-carboxamido	6.0±0.65	11±0.91	40±11	116±3.7	0.17	0.48	0.25	0.63	5.860
O-2489	N-(Homo-piperidin-1-yl) carboxamido	16±0.44	35 ± 4.5	87 ± 17	112 ± 2.3	2.07	2.53	8.50	20.5	7.153
O-2543	N-(Pyrrolidin-yl)-carboxamido	23±3.4	11±0.08	81±40	71±4.2	0.71	0.75	8.19	25.8	6.035
O-2372	Morpholino-carboxamido	1.3±0.12	0.57±0.04	5.9 ± 0.42	120 ± 1.1	0.01	0.01	0.07	0.03	6.000
O-2373	Piperidino-carboxamido	0.96±0.11	0.96±0.01	14 ± 1.8	119 ± 2.1	0.02	0.03	0.03	0.04	6.794
O-2381	Methylepiperazino-carboxamido	112±14	389±46	ND	ND	21.4	34.0	51.1	182	6.561
O-2399	Pyrrolidino-carboxamido	2.9±0.52	2.9±0.68	275 ± 87	117 ± 4.9	0.03	0.10	0.23	0.15	6.235

O-2421	Homopiperidino-carboxamido	4.2±1.0	3.5±0.58	23 ± 2.1	81 ± 9.0	0.15	0.16	0.44	0.86	7.353
O-2589	2,4-Dimethyl-thiazole-5-carboxamide	244±29	38±7.6	ND	ND	ND	ND	ND	ND	7.437
O-2590	5-Methyl-2-phenyl-oxazol-4-yl-acetamide	890±161	169±39	ND	ND	ND	ND	ND	ND	7.499
O-2619	Morpholino-1-carboxylic acid amide	19±3.9	2.3±0.38	86±3.1	116±7.9	ND	ND	ND	ND	6.160
O-2620	Di(morpholino-1-carboxylic acid) amide	3020±579	772±60	ND	ND	ND	ND	ND	ND	7.925

TABLE 2. Imidazole, Pyrazole, Triazole and Morpholine Pentyl Side Chain Analogs

CB_1 and CB_2 receptor binding was carried out in CHO and HEK cells, respectively, with the exception of THC and CP 55,940 CB_1 binding which were performed in rat brain membranes. GTPγS binding was carried out in CHO cells and maximal binding was expressed as a percentage of maximal stimulation produced by CP 55,940. For the *in vivo* studies, the drugs were dissolved in either saline or emulphor:ethanol:saline (E:E:S) as indicated below. The ED50 values are provided as μmoles/kg for reducing spontaneous activity (S.A.), producing antinociception in the tail-flick procedure (T.F), lowering rectal temperature (R.T.) and producing relative immobility (R.I.) following i.v. administration in mice. Solubility was determined by dissolving 1 mg of analog in 1 ml of water.

O#	Name	CB_1 Ki (nM)	CB_2 Ki (nM)	CB_1 EC50	CB_1 %CP Stim	Vehicle	S.A. (μmol/kg)	T.F. (μmol/kg)	R.T. (μmol/kg)	R.I. (μmol/kg)	Soluble	cLogP
	Δ^9-THC	41±3	94±4				2.23	2.77	2.23			7.238
	CP 55,940	4.0±0.3	2.5±0.1		100		0.11	2.77	0.93	0.92		5.819
O-2545	Imidazol-1-yl	1.3±0.17	0.12±0.003	29±3.3	107±6.2	E:E:S	0.01	0.07	0.04	0.13	No	5.860
O-2545	Imidazol-1-yl	1.5±0.22	0.32±0.02	4.6±0.72	84±5.0	Saline	0.09	0.16	0.29	0.14	Yes	5.860
O-2651	2-Methyl-imidazol-1-yl	14 ±0.83	1.2±0.28	83±45	33±3.4	Saline	1.46	1.75	3.58	4.29	Yes	7.069
O-2715	Pyrazol-1-yl	1.9±0.29	1.5±0.31	4.0±0.28	93±1.8	E:E:S	0.004	0.06	0.14	0.07	No	7.070
O-2716	1,2,4-Triazol-1-yl	3.4±0.16	0.92±0.07	37±4.2	93±1.7	E:E:S	0.03	0.09	0.15	0.20	No	6.070
O-2737	1H-Imidazol-2-yl	54±4.9	15±1.1	ND	ND	Saline	>2.2	>2.2	>2.2	>2.2	Yes	6.646
O-2737	1H-Imidazol-2-yl	54±4.9	15±1.1	ND	ND	E:E:S	>10	>10	>10	>10	Yes	6.646
O-3226	Morpholin-4-yl	2.8±0.35	1.0 ±0.16	ND	ND	Saline	0.04	0.04	0.08	ND	Yes	6.980

TABLE 3. Pharmacological Activity of Phenolic Esters

CB_1 and CB_2 receptor binding was carried out in CHO HEK cells, respectively, with the exception of THC and CP 55,940 CB_1 binding which were performed in rat brain membranes. GTPγS binding was carried out in CHO cells and maximal binding was expressed as a percentage of maximal stimulation produced by CP 55,940. For the *in vivo* studies, the drugs were dissolved in either saline or emulphor:ethanol:saline (E:E:S) as indicated below. The ED50 values are provided as μmoles/kg for reducing spontaneous activity (S.A.), producing antinociception in the tail-flick procedure (T.F), lowering rectal temperature (R.T.) and producing relative immobility (R.I.) following i.v. administration in mice. Solubility (Sol) was determined by dissolving 1 mg of analog in 1 ml of water. N.D. means not determined.

O#	Name	CB_1 Ki (nM)	CB_2 Ki (nM)	CB_1 EC50	CB_1 %CP Stim	Vehicle	S.A. (μmol/kg)	T.F. (μmol/kg)	R.T. (μmol/kg)	R.I. (μmol/kg)	Sol	cLogP
	Δ^9-THC	41±3	94±4				2.23	2.77	2.23			7.238
	CP 55,940	4.0±0.3	2.5±0.1		100		0.11	0.23	0.93	0.92		5.819
O-1057	morpholinobutyryloxy	15 ± 5.0	N.D.	N.D.	N.D.	E:E:S	0.03	0.03	0.10		Yes	6.934
O-2365	1-(4-N-piperidinobutyryloxy)	2.7±0.83	0.20±0.07	8.4±2.3	93±0.68	Saline	0.02	0.07	0.03	0.10	Yes	6.419
O-2374	1-(2-methyl-4-N-piperidinobutyryloxy)	4.6±2.2	1.10±0.1	17±7.0	81±3.7	Saline	0.18	0.41	0.72	0.57	Yes	8.457
O-2426	1-(4-N-2-methylpiperidino-butyryloxy)	2.6±0.55	0.70±0.09	22±7.8	77±5.9	Saline	0.05	0.04	0.10	0.11	Yes	8.523
O-2486	1-2-methyl-(4-N-2'-methylpiperidino-butyryloxy)	11±1.4	0.34±0.04	37±6.0	81±18	Saline	0.07	0.33	2.76	0.94	Yes	8.832
O-2383	1-(4-N(4'-methylpiperazino)-butyryloxy)	2.0±0.14	0.78±0.13	16±3.1	82±5.8	Saline	0.03	0.04	0.09	0.09	Yes	6.031

O-2427	1-2-methyl-4-N(4'-methyl-piperazino)butyryloxy)	6.7±1.8	1.56±0.47	16±7.8	98±10	Saline	0.23	0.17	0.40	0.22	Yes	6.314
O-2484	1-(4-N,N-dimethylaminobutyryloxy)	2.6±0.83	0.23±0.03	17±1.7	126±11	Saline	0.07	0.09	0.35	0.16	Yes	6.988
O-2487	1-(2-methyl-4,N,N-dimethylaminobutyryloxy)	5.9±0.79	0.44±0.07	16±6.0	119±12	E:E:S	0.40	0.26	0.40	0.59	Yes	7.297
O-2548	1-(4-N,N,N-trimethyl-ammoniumbutyryloxy)	15±4.0	0.35±0.03	60±13	112±1.2	E:E:S	0.02	0.05	0.09	0.08	Yes	7.845
O-2650	1-(2-methyl-4-N,N,N-trimethylammonium-butyryloxy)	45 ±3.8	7.4±0.24	138±36	113±4.7	Saline	0.002	1.99	3.82	14.81	Yes	8.154
O-2382	1-(4-N,N-diisopropylaminobutyryloxy)	5.6±1.9	1.9±0.08	18±1.9	78±5.6	Saline	0.04	0.05	0.85	0.06	Yes	8.989
O-2485	1-(2-methyl-4-N,N-diisopropylaminobutyryloxy)	82±11	1.9±0.24	ND	ND	Saline	0.45	1.00	1.90	1.92	Yes	8.973

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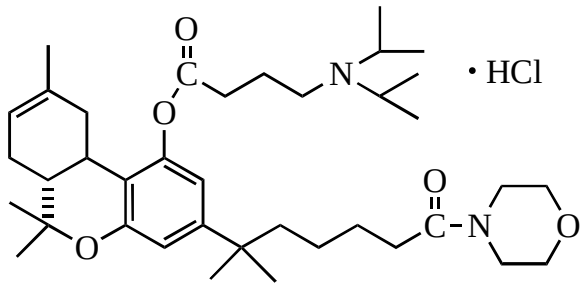
TABLE 4. 4-Diisopropylaminobutyrate of Δ^8-THC-3-(1,1-dimethyl-6-morpholin-4-yl-6-oxo-hexyl)

This analog was evaluated as described in Table 3. For the in vivo studies, the drug was dissolved in saline. The ED50 values are provided as µmoles/kg for reducing spontaneous activity (S.A.), producing antinociception in the tail-flick procedure (T.F), lowering rectal temperature (R.T.) and producing relative immoility (R.I.) following i.v. administration in mice. Solubility was determined by dissolving 1 mg of this analog in 1 ml of water.



O#	CB_1 Ki (nM)	CB_2 Ki (nM)	CB_1 EC50	CB_1 %CP Stim	S.A. (µmol/kg)	T.F. (µmol/kg)	R.T. (µmol/kg)	R.I. (µmol/kg)	Soluble	cLogP
O-2694	3.7±0.43	2.8±0.44	28±3.5	120±8.0	0.045	0.035	0.12	0.11	Yes	8.245

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TABLE 5. Pharmacological activity of analogs administered i.c.v. or i.t.

Hydrochloride salts of the O-analogs were prepared in saline, whereas THC was prepared in emulphor:ethanol:saline (1:1:18) and administered either i.c.v. or i.t. The ED50 values are provided as nmoles/mouse for reducing spontaneous activity (S.A.), producing antinociception in the tail-flick procedure (T.F), lowering rectal temperature (R.T.) and producing relative immobility (R.I.) in mice.

Compound	S.A.		T.F.		R.T.		R.I.	
	ICV	IT	ICV	IT	ICV	IT	ICV	IT
THC	3.0	16	63	70	57	70	21	16
O-1057	0.09	0.96	4.2	2.8	0.87	1.3	0.44	1.3
O-2383	0.28	0.84	4.5	2.6	0.65	1.4	0.56	2.6
O-2545	0.37	7.6	7.8	16	1.6	9.3	0.61	5.9
O-2651	2.8	17	78	63	33	82	9.7	35
O-2737	7.1	61	107	73	76	104	2.1	95

TABLE 6. Inhibition of cytochrome P450 enzymes

Compound	IC_{50} (μM)				
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
Δ^9-THC	7.1	0.3	1.1	34	28
CP 55,940	> 75	1.2	1.3	3.0	13
O-581	> 75	0.6	3.1	15	4.9
O-1057	Activation*	2.8	3.1	2.9	4.4
O-2545	2.0	0.4	1.1	1.0	0.5
O-2715	> 75	0.5	3.8	9.4	4.7
O-2716	4.9	0.6	1.6	18	1.9

* 15% at 10μM

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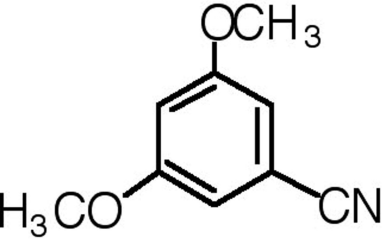
TABLE 7. Microsomal and plasma stability of selected analogs

		Plasma stability (half-life, min)		Microsomal stability (half-life, min)	
Compound	Buffer	Rat	Human	Mouse	Human
O-581	> 150	Not tested	> 150	< 5	13
O-1057	65	< 5	250	< 5 (non-P450)	13 (non-P450)
O-2383	93	< 5	> 150	< 5 (non-P450)	< 5 (non-P450)
O-2374	92	< 5	< 5	< 5 (non-P450)	< 5
O-2427	105	5	> 150	< 5 (non-P450)	< 5
O-2487	45	< 5	> 150	< 5 (non-P450)	< 5 (non-P450)
O-2545	135	> 150	> 150	11	9
O-2548	32	< 5	> 150	12	< 5

Scheme 1



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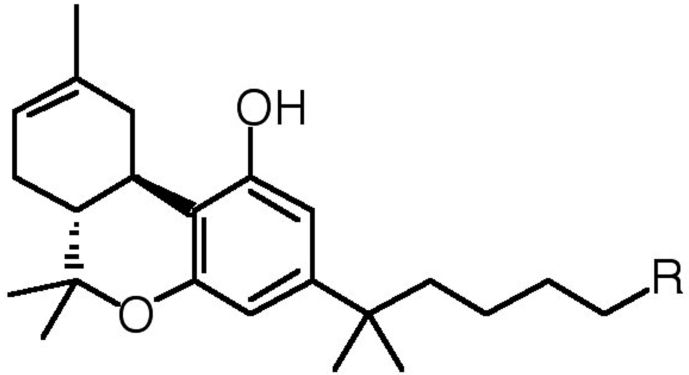
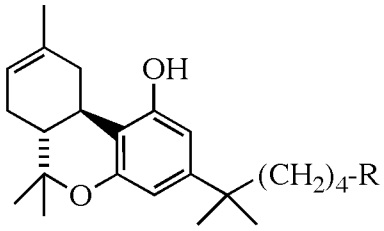


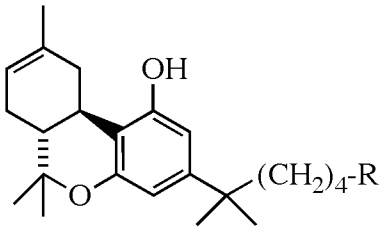
2a R = Br
2b R = CN
2c R = COOH

Compounds listed in Tables 1, 2, 3, and 4.

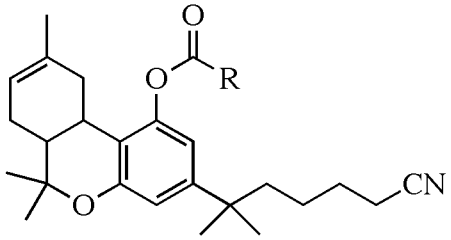
SCHEME 2. Structures of Analogs



Carboxamido



Imidazole, Pyrazole
Triazole, Morpholine



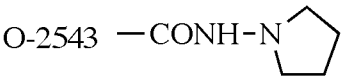
Phenoxlic esters

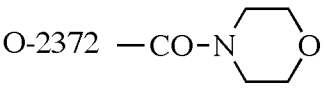
O-2352 —$CONH_2$

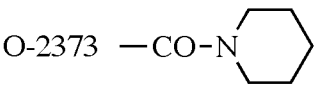
O-2490 —$CONHCH_3$

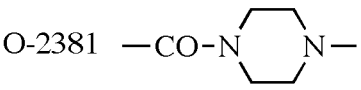

O-2544 —CONH—N O

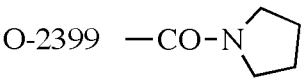

O-2489 —CONH—N


O-2543 —CONH—N


O-2372 —CO—N O


O-2373 —CO—N


O-2381 —CO—N N—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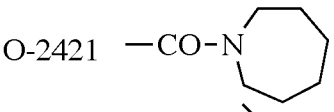
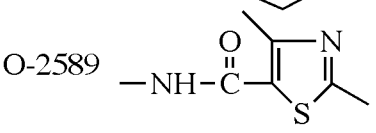
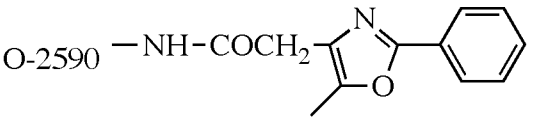
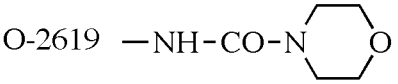
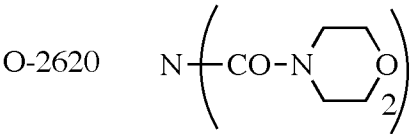
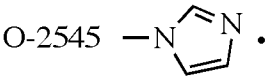

O-2421 —CO—N

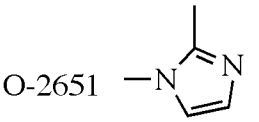

O-2589 —NH—C(=O) N S


O-2590 —NH—$COCH_2$ N O


O-2619 —NH—CO—N O


O-2620 N—CO—N O 2


O-2545 —N N • HCl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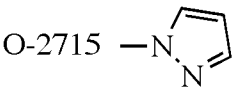
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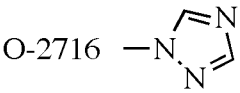
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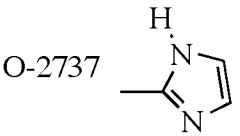
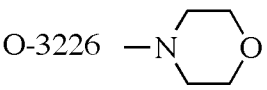
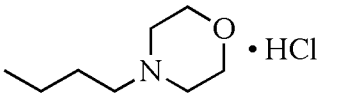
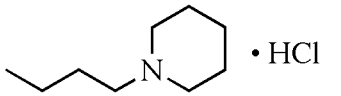
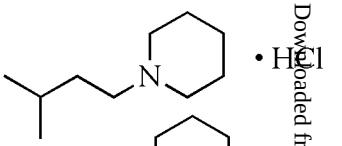

O-2737 H N N • HCl


O-3226 —N O


O-1057 O N • HCl


O-2365 N • HCl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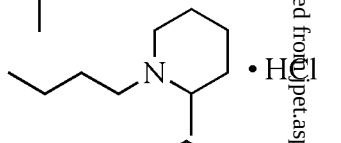
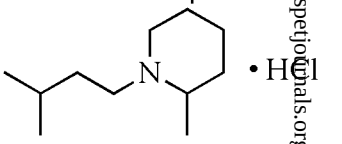

O-2426 N • HCl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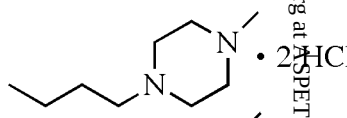
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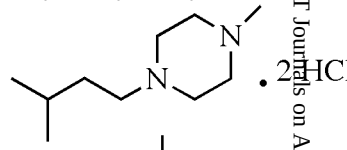
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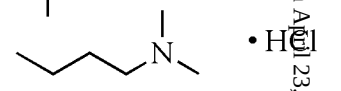
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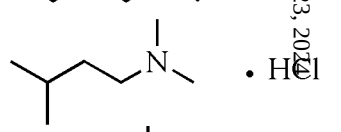
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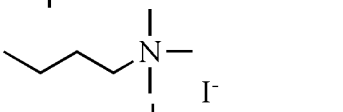
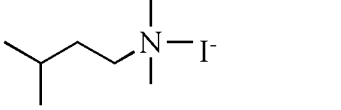
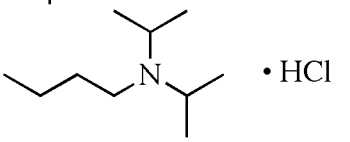

O-2548 N— I-


O-2650 N— I-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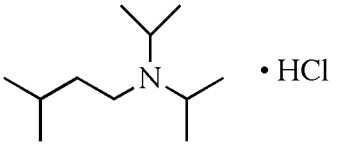
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