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Structure-Activity Relationships for Cannabinoid Receptor-Binding and Analgesic Activity: Studies of Bicyclic Cannabinoid Analogs

LAWRENCE S. MELVIN, GEORGE M. MILNE, M. ROSS JOHNSON,[1] BABU SUBRAMANIAM,[2] GERALD H. WILKEN, and ALLYN C. HOWLETT

Pfizer Inc., Central Research, Groton, Connecticut 06340 (L.S.M., G.M.M., M.R.J.), and Department of Pharmacological and Physiological Science, St. Louis University School of Medicine, St. Louis, Missouri 63104 (B.S., G.H.W., A.C.H.)

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SUMMARY

Cannabimimetic compounds, such as Δ^9-tetrahydrocannabinol (Δ^9-THC), evoke analgesia in addition to other behavioral responses in humans and animals. The cannabinoid receptor mediating this response has been characterized by its ability to bind the cannabinoid agonist [^{3}H]CP-55,940 and to inhibit adenylyl cyclase via G_i. An investigation of structural requirements for antinociceptive activity of cannabinoid structures led to the development of a simple bicyclic cannabinoid agonist, CP-47,497, that possessed a spectrum of cannabinoid activities in animals that resembled that of Δ^9-THC. The present investigation examines several series of CP-47,497 analogs for their binding affinity at the cannabinoid receptor and their ability to evoke analgesia in rodents. Analogs substituted at the C-3 alkyl side chain exhibited maximal affinity for the cannabinoid receptor with side chains of seven or eight carbons in length. Analgesic potency paralleled the receptor-binding affinity. The cyclohexyl ring was optimized as a six- or seven-membered ring structure for binding as well as analgesic activity. Cyclohexyl alkyl side chain extensions of up to four carbons in length had little influence on the affinity for the receptor or analgesic activity. Hydroxyalkyl side chains exhibited optimal binding affinity and antinociceptive activity at three or four carbon atoms in length; however, polar groups closer to the ring diminished binding to the receptor. The importance of the phenolic and cyclohexyl hydroxyl groups for binding affinity was demonstrated. In general, analgesic activity correlated well with the affinity of these analogs for the cannabinoid receptor. Exceptions could be explained by metabolic transformations likely to occur *in vivo*.

The CBR was defined by the pharmacological characterization of the *in vivo* antinociceptive activity of a series of bicyclic and tricyclic analogs of Δ^9-THC (1). Subsequent studies have described the pharmacology for the biological responses of hypothermia, decreased locomotor activity, and catalepsy that parallel analgesia (2), suggesting that the same receptor is present in neuronal pathways mediating each of these behaviors.

Cannabinoid inhibition of adenylyl cyclase activity occurs via a receptor coupled to G_i a subfamily of G protein heterotrimers characterized by their sensitivity to ADP-ribosylation by pertussis toxin and their ability to functionally inhibit adenylyl cyclase activity (although interaction with other effectors is also possible). The pharmacological profile for inhibition of adenylyl cyclase demonstrated that the receptor for this *in vitro* activity is the same receptor that is involved in the analgesic activities described in animals (3). Using a potent, radiolabeled, bicyclic cannabinoid ligand, [^{3}H]CP-55,940, the CBR in rat brain membrane preparations was identified and characterized (4). Quantitative autoradiographic analysis was used to further characterize the receptor with respect to species distribution (5) and, more specifically, localization and concentration in rat brain and pituitary (6). A cDNA for the CBR in rat brain has been cloned and expressed in CHO-K1 cells, with demonstrated cannabinoid functional inhibition of cAMP accumulation in these cells (7). Finally, a cDNA for the human brain CBR was cloned and expressed in COS-7 and CHO-K1 cells, with functional cannabinoid activity (8, 9).

SAR studies of the analgesic activity of cannabinoid compounds began with the observation that 9-nor-9β-hydroxyhexahydrocannabinol exhibited enhanced biological activity, com-

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[1] Current address: Glaxo Inc., Research Triangle Park, NC 27709.

[2] Current address: University of Washington School of Medicine, Seattle, WA 98195.

ABBREVIATIONS: CBR, cannabinoid receptor; DALN, desacetyllevonantradol; MPE, maximum possible effect; MPE_{50}, 50% of the maximum possible effect; PBQ, phenylbenzoquinone; SAR, structure-activity relationship; THC, tetrahydrocannabinol; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

pared with Δ^9-THC (10). Continued studies at Pfizer Inc. demonstrated that the dimethyldihydropyran ring structure was not required for potent antinociceptive activity (11, 12). This led to the formulation of the hypothesis that cannabinoid compounds interact with their receptor by a three-point contact that includes the equatorial alcohol, the phenol, and the C-3 side chain (13). This hypothesis was tested by the development of a novel bicyclic cannabinoid, CP-47,497, which represented the simplest structure possessing these three binding sites (14). CP-47,497 was shown to be equivalent in analgesic potency to 9-nor-9β-hydroxyhexahydrocannabinol and exhibited other cannabinoid biological activities as well (14–16).

The present investigation was undertaken to describe the interaction of CP-47,497 and related bicyclic cannabinoid ligands with the CBR in rat brain membranes. These studies define the SAR for agonist ligand binding and provide insights into the structural requirements for binding to the CBR and evoking a response.

Experimental Procedures

Materials. CP compounds were developed and synthesized at Pfizer Inc., Central Research Division. Compounds were prepared as 10 mM stock solutions in absolute ethanol and were stored at −20° until use. For assays, 10-μl aliquots were dried under N_2 gas and rapidly resuspended as a 100 μM suspension in 50 mg/ml fatty acid-deficient bovine serum albumin, in Regisil-treated glass tubes. Subsequent dilutions were made in 20 mM Tris, 3 mM $MgCl_2$, 1 mM EDTA solution containing 1 mg/ml bovine serum albumin. Pipetting was performed using siliconized plastic pipet tips. [^{3}H]CP-55,940 was custom-tritiated by DuPont-NEN, from a precursor developed and synthesized at Pfizer Inc. (4).

CBR binding in rat forebrain membranes. Brains were dissected from male Sprague-Dawley rats weighing between 300 and 400 g. A P_2 membrane preparation was made from the brain minus cerebellum and brainstem, as described previously (4), and was standardized for protein according to the method of Bradford (17). CBR binding was determined in a 1-ml volume containing (final concentrations) 50 mM Tris·HCl, pH 7.4, 1 mM Tris-EDTA, 3 mM $MgCl_2$, 0.15 mg/ml fatty acid-deficient bovine serum albumin, 100 fmol/ml [^{3}H]CP-55,940, 30 μg/ml protein P_2 brain membranes, and the indicated compounds. Each assay included tubes containing 100 nM DALN to calculate nonspecific binding. Incubations were performed in siliconized plastic microfuge tubes for 60 min at 30° and were stopped by sedimentation at 16,000 × g for 10 min. The free concentration of [^{3}H]CP-55,940 was determined in a 500-μl aliquot of the supernatant, and the bound ligand was determined in the pellet.

Triplicate determinations were made for each experimental point within an assay, and the vehicle control and nonspecific binding values were determined with no fewer than six tubes per assay. Each compound was assayed at least three times. The concentration of compounds displacing [^{3}H]CP-55,940 by 50% (IC_{50}) was determined by linear regression analysis using the Hill equation (18). The K_i was calculated using the assumptions of Cheng and Prusoff (19). Reported values are the means ± standard errors calculated from three to five experiments. Graphic displays for figures were developed using GraphPAD InPlot 4.0, with values for [^{3}H]CP-55,940 displacement at each drug concentration from all of the three to five experiments. Nonlinear regression analysis was performed using the equation:

$$y = A + [(B - A)/(1 + (10^c/10^x)^{n_H})]$$

where B is the constant 100% bound (vehicle), A is the constant 0% bound (plus 100 nM DALN), c is the variable IC_{50} of the displacing compound, and n_H is the variable slope factor of the displacement curve, using the Marquardt algorithm as performed by InPlot 4.0 (GraphPAD, Inc.). IC_{50} and slope factor values determined by this method did not differ from those obtained by averaging the IC_{50} values derived from linear regression analysis for each experiment.

Adenylyl cyclase assay in N18TG2 cell membranes. Cultured neuroblastoma cells were maintained and membranes were prepared as described previously (20). The assay of adenylyl cyclase activity and the dilution of cannabinoid compounds were performed as described previously (20), except that the buffer used was 50 mM Tris·HCl, pH 7.4, the $MgCl_2$ concentration was 3 mM, and 50 μM rolipram was present as the phosphodiesterase inhibitor. The buffer used for the single experiment shown in Fig. 3, *inset*, was 50 mM Na-HEPES, pH. 8.0, and 5 mM $MgCl_2$ was present.

Antinociceptive activity. The PBQ stretching test in mice was used to determine antinociceptive activity (21). Compounds were administered subcutaneously and testing was performed after 1 hr. Data were calculated as the percentage of the MPE, and an MPE_{50} value was calculated using least squares regression analysis over a range of doses.

Results

SARs for the tricyclic cannabinoid class of drugs highlight the requirement for a 1,3 relationship of a phenolic hydroxyl group and medium-length (C_5-C_7) lipophilic side chain (22). Most often, these structural fragments were optimized for or by incorporation into tricyclic tetrahydro- and hexahydro-6*H*-dibenzo[*b*,*d*]pyran ring systems (22). These SAR points have also been described for related rigid tricyclic ring systems such as octahydrophenanthridin-1,9-diols, octahydrophenanthren-1,9-diols, and tetrahydro-5*H*-[1]benzopyrano[4,3-*c*]pyridines (1, 12). We wished to extend the SAR of tricyclic cannabinoids to one of the simplest nonrigid AC-bicyclic cannabinoids (1), CP-47,497, and expand the scope of SAR for the latter (14). Use of this simplified prototype nucleus for SAR studies was desirable, not only for ease of access to synthetic materials but also because this minimal structure was envisioned as providing the most stringent test of key SAR conclusions. Compounds were tested for cannabinoid activity *in vitro* by measuring potencies for displacement of [^{3}H]CP-55,940 in the CBR binding assay. *In vivo* activity was assessed using the mouse PBQ-induced stretching test for analgesia.

The previously described synthesis and pharmacological studies of CP-47,497 have shown it to be approximately equipotent with Δ^9-THC (14). However, the nucleus of CP-47,497 differs significantly from Δ^9-THC, because it lacks the rigid fused hexahydro-6*H*-dibenzo[*b*,*d*]pyran ring system. Although the two rings of the hydroxycyclohexylphenol in CP-47,497 are freely rotating, a spatial conformational relationship approximating that found in Δ^9-THC is relatively accessible (14). The 1,1-dimethylheptyl side chain of CP-47,497 was derived from previous cannabinoid SAR studies, based on its ability to enhance potency in this series (23). The cannabinoids closest in structure to CP-47,497 are the synthetic ketone derivative nabilone and its equatorial alcohol derived by reduction (24).

The lipophilic C-3 side chain of CP-47,497 was modified through one-carbon atom homologation that provided the compounds of Fig. 1 and Table 1 (14). A significant relationship is apparent between side chain length and potency *in vitro* for binding to the CBR, as well as analgesic activity *in vivo* in the mouse. In the CBR binding assay, peak potency occurs for the 1,1-dimethyloctyl derivative, which *in vivo* is equipotent to the most common 1,1-dimethylheptyl derivative. Potency rapidly diminishes on both sides of the 1,1-dimethyloctyl maximum, suggesting a high degree of specificity for this structural grouping.

The cyclohexane ring incorporated in the initial AC-bicyclic prototype, CP-47,497, was chosen based on direct dissection from the common tricyclic structures of naturally occurring and synthetic cannabinoids (14). The correctness of this choice was probed by the ring size analogs of Table 2. Cycloalkanol rings that can adopt a conformation approximating that of the cyclohexane ring (i.e., cycloheptanol **11**) retain similar *in vitro* and *in vivo* activities and potencies. Even the cyclooctanol analog **12** exhibits significant potency, with only a 7- and 5-fold reduction of *in vitro* and *in vivo* potencies, respectively. However, the compounds with least constrained overlap with the cyclohexanol ring of CP-47,497, cyclopentanol **10** and the seco-diastereomers **13** and **14**, exhibit only very weak potencies. Together, these data suggest a desirable conformation for the C-ring approximated by CP-47,497, most tricyclic cannabinoids, and the cycloheptyl derivative **11**. Loss of six-member ring overlap or conformational control (e.g., an eight-member ring or acyclic compound) is contraindicated for potent receptor-binding and *in vivo* functional activity.

Once it was concluded that the pyran portion of the Δ^9-THC nucleus is not a key structural element for cannabinoid activity, SAR studies were developed for the prototype CP-47,497 that explored the effect of substitution at position C-4 of the cyclohexanol ring. This site was chosen for initial studies because it approximated a region in space that was occupied by the pyran ring of tricyclic compounds and that seemed to offer the opportunity for structural elaboration with possible separation of the various cannabinoid biological activities. Table 3 lists the C-4 derivatives that were synthesized and tested for both *in vitro* and *in vivo* activities (14), and Fig. 2 shows the log dose-response curves for this series of compounds.

By inspection, there is a trend for increased potency with shorter lipophilic aliphatic chains, with the maximum being at C_3 (i.e., **17**) (Fig. 2A). A reverse trend is suggested for longer, more hydrophilic, hydroxyaliphatic chains, with the maximum occurring at C_4-C_5 [i.e., (±)-CP-55,940 and **22**] (Fig. 2B). This apparent reciprocal relationship between more and less lipophilic chains suggests that receptor binding through the C-4 position can be moderated by more than one type of productive ligand-receptor interaction. With the exception of **19**, absolute potencies for this group of derivatives are similar, indicating that an optimal effect cannot be discerned from this group of compounds.

Three additional compounds with C-4 substituents exhibit significant receptor-binding potency, i.e., compounds **23**, **25**, and **30**. Compound **23** has a methoxypropyl chain five atoms in length and is similar in potency to (±)-CP-55,940 and **22**, rather than **19** (aliphatic chain of five atoms). Likewise, **25** has a propionic acid carboxamide group of four atoms in length, with binding potency similar to that of the three-atom-length hydroxyalkyl **21**. The more polar methoxy- and carboxamidoalkyl groups seem to interact at the receptor like the ω-hydroxyalkyl groups.

In spite of the relatively high affinity for the CBR exhibited by compound **25**, little biological activity was observed *in vivo* (MPE_{50} approximating 56 mg/kg). This finding suggests that this compound might be an antagonist at the CBR. To test this

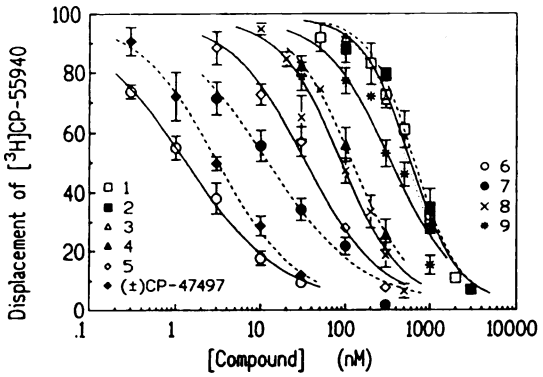
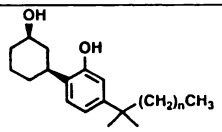


Fig. 1. Log dose-response curves for binding of C-3 alkyl chain analogs to the CBR. Heterologous dissociation curves were generated and data were analyzed as described in the text. The *curves* include data from three or four displacement experiments for each compound.

TABLE 1

Role of CP-47,497 C-3 side chain length in CBR binding

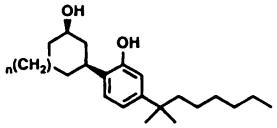


Compound	n	CBR binding, K_i[a]	Mouse PBQ stretching MPE_{50}[b] or inhibition
		nM	
1	0	300 ± 43.8	12% (56)[c]
2	1	406 ± 70.4	25% (56)[c]
3	2	326 ± 27.2	16% (56)[c]
4	3	74.9 ± 10.4	24% (56)[c]
5	4	19.0 ± 1.76	11.9 mg/kg (4.07–20.0)[d]
(±)-CP-47,497	5	2.20 ± 0.47	1.0 mg/kg (0.35–1.62)[d]
6	6	0.834 ± 0.10	1.2 mg/kg (1.05–1.38)[d]
7	7	7.66 ± 0.90	15.2 mg/kg (11.5–19.2)[d]
8	8	47.0 ± 6.04	15% (56)[c]
9	9	171 ± 24.0	18% (56)[c]

[a] Values are mean ± standard error.
[b] Subcutaneous, 1 hr.
[c] Dose (mg/kg).
[d] 95% confidence interval.

TABLE 2

Role of ring size in CBR binding

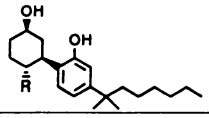


Compound	n[a]	CBR binding K_i[b]	Mouse PBQ stretching MPE_{50}[c] or inhibition
		nM	
10	0	19.9 ± 3.5	51% (56)[d]
(±)-CP-47,497	1	2.2 ± 0.47	1.0 mg/kg (0.35–1.62)[e]
11	2	8.95 ± 3.28	1.44 mg/kg (1.45–1.52)[e]
12	3	14.2 ± 3.5	4.61 mg/kg (1.56–8.69)[e]
13′		134 ± 17	2% (56)[d]
14′		39.2 ± 9.8	24–54% (32–100)[d]

[a] DMHP, 4-(1,1-dimethylheptyl)-2-hydroxyphenyl.
[b] Values are mean ± standard error.
[c] Subcutaneous, 1 hr.
[d] Dose (mg/kg).
[e] 95% confidence interval.
[f] Single diastereomer.

TABLE 3

Role of CP-47,497 cyclohexyl side chain in CBR binding



Compound	R	CBR binding k_i[a]	Mouse PBQ stretching MPE_{50}[b] or inhibition
		nM	
(±)-CP-47,497	H	2.20 ± 0.47	1.0 mg/kg (0.35–1.62)[c]
15	CH_3	2.07 ± 0.79	1.02 mg/kg (0.81–1.25)[c]
16	CH_2CH_3	2.04 ± 0.81	1.75 mg/kg (1.12–2.34)[c]
17	$CH_2CH_2CH_3$	1.30 ± 0.57	0.16 mg/kg (0.00–0.74)[c]
18	$CH_2CH_2CH_2\ CH_3$	2.55 ± 1.29	0.83 mg/kg (0.73–0.94)[c]
19	$CH_2CH_2CH_2CH_2CH_3$	51.8 ± 15.1	62% (56)[d]
20	CH_2OH	14.4 ± 5.35	1.77 mg/kg (1.38–2.07)[c]
21	CH_2CH_2OH	5.65 ± 0.66	2.58 mg/kg (1.27–5.10)[c]
(±)-CP-55,940	$CH_2CH_2CH_2OH$	1.14 ± 0.04	0.29 mg/kg (0.27–0.32)[c]
22	$CH_2CH_2CH_2CH_2OH$	1.23 ± 0.17	0.23 mg/kg (0.02–0.50)[c]
23	$CH_2CH_2CH_2OCH_3$	3.29 ± 1.29	0.37 mg/kg (0.24–0.52)[c]
24	$CH_2CH_2CH_2N(CH_3)_2 \cdot HCl$	44.4 ± 6.62	6.38 mg/kg (2.66–10.8)[c]
25	$CH_2CH_2CONH_2$	4.81 ± 1.06	50% (56)[d]
26	CH_2CH_2COOH	180 ± 23.4	66% (56)[d]
27	CH_2COOH	188 ± 31.2	0% (56)[d]
28	CH_2CHO	202 ± 49.8	1.21 mg/kg (0.19–2.19)[c]
29	CHO	35.2 ± 7.61	2.59 mg/kg (0.00–8.65)[c]
30	CH═NOH	6.16 ± 0.67	3.22 mg/kg (2.79–3.69)[c]
31	$CH_2N(CH_3)_2 \cdot HCl$	82 ± 22.1	50% (56)[d]
32	$CH_2CH_2NH_2 \cdot HCl$	264 ± 137	8% (56)[d]
33	CH_2NH_2	119 ± 9.81	61% (56)[d]

[a] Values are mean ± standard error.
[b] Subcutaneous, 1 hr.
[c] 95% confidence interval.
[d] Dose (mg/kg).

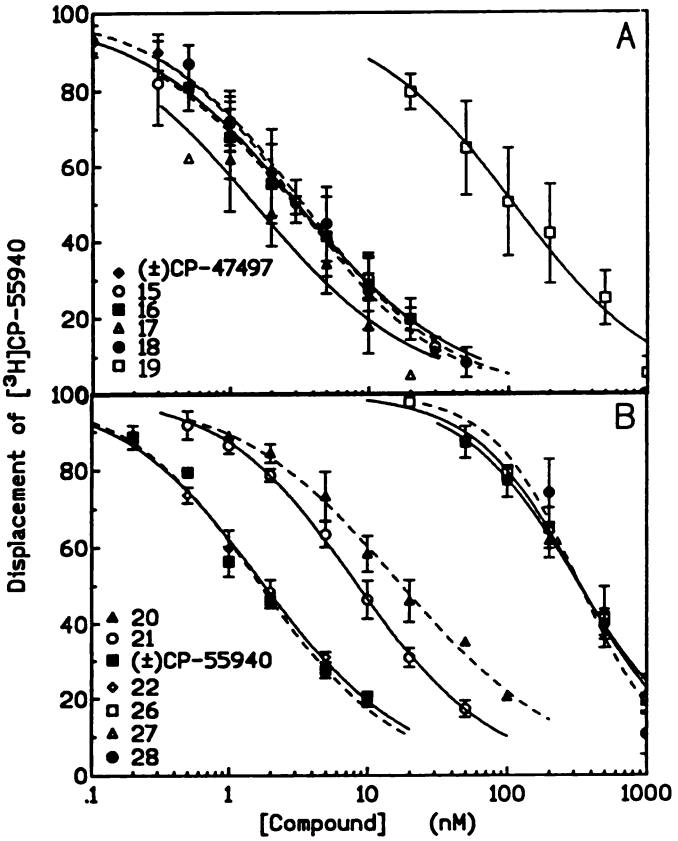


Fig. 2. Log dose-response curves for CBR binding of cyclohexyl alkyl (A) and hydroxy- or carboxyalkyl (B) side chain analogs of CP-47,497. As described in the text, heterologous dissociation curves were generated from the data from three or four separate ligand-binding experiments. The nonlinear regression *lines* were determined using the InPlot program.

hypothesis, the ability of **25** to inhibit hormone-stimulated adenylyl cyclase was determined (Fig. 3). As shown, the maximal inhibition produced by **25** was identical to that produced by a maximally inhibitory concentration of DALN. Furthermore, the results with combined submaximal concentrations of **25** plus DALN were additive and never exceeded the maximal inhibition attained with either compound alone. No antagonism of the DALN response by **25** was found using several different conditions for the assay (varied Na^+, Mg^{2+}, and pH). These data demonstrate that **25** behaves as a full agonist in the regulation of the second messenger response *in vitro*. Alternative explanations for the poor biological activity *in vivo* may be metabolism of the compound in the intact animal or a poor ability of the compound to cross the blood-brain barrier. Consistent with the former possibility, it should be noted that metabolic deamidation of **25** would yield **26**, which binds poorly to the receptor.

Compound **30**, the oxime of **29**, has binding activity similar in potency to that of the three-atom-length hydroxyalkyl **21**. The potency of **30** is interesting because at equilibrium it may exist as the cyclic isomer **30A** (Fig. 4),[3] which contains the natural tricyclic cannabinoid nucleus without a free phenol. It is assumed that the equilibrium between these isomers provides sufficient free phenol to elicit receptor-binding and functional activity (see below).

The aldehydes **28** and **29** probably also are in equilibrium with their hemiacetals, **28A** and **29A**, respectively (Fig. 4).[3] Compound **28** has significantly reduced receptor-binding potency, whereas **29** is somewhat more potent but still 31-fold

[3] Spectroscopic analysis by IR and proton NMR spectroscopy, in chloroform or deuteriochloroform, respectively, supports the structural assignments of **29A** and **30A**. Similar analysis in chloroform suggests that **28A** and **38A** are in equilibrium with **28** and **38**, respectively.

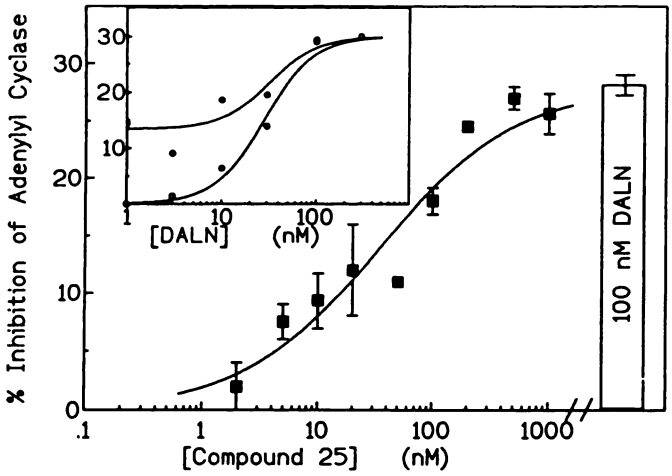


Fig. 3. Inhibition of adenylyl cyclase by **25**. Adenylyl cyclase activity was determined in N18TG2 cell membranes in the presence of 750 nM secretin, to stimulate activity, plus the indicated concentrations of **25**. The data points are the percentage inhibition of hormone-stimulated activity (mean ± standard error for four experiments). The nonlinear regression *line* generated from these data exhibited a K_{inh} of 35 nM and a slope factor of 0.7. Shown for comparison is the mean inhibition exhibited by 100 nM DALN in the four experiments. *Inset*, percentage inhibition of secretin-stimulated adenylyl cyclase by various concentrations of DALN is shown in the absence (○) and presence (●) of 100 nM **25**. Compound **25** alone exhibited 14% inhibition of adenylyl cyclase. The data are the means of triplicate determinations from a single experiment representative of three that examined additivity of the DALN and **25** responses.

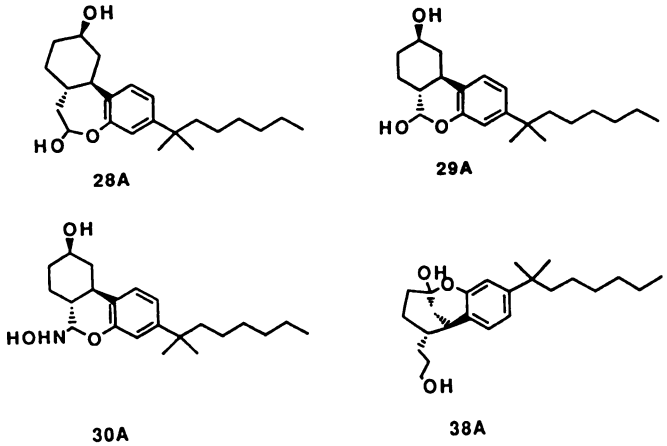


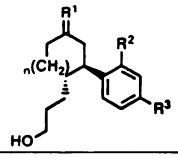
Fig. 4. Structures of cyclic isomers of compounds **28**, **29**, **30**, and **38**.[3]

less potent than (±)-CP-55,940. However, both aldehydes exhibit significant *in vivo* potency, suggesting that at least *in vivo* the hemiacetals are in equilibrium with free phenols. Under the conditions of the receptor-binding assay, equilibrium conditions may not exist for sufficiently rapid interconversion of the phenolic aldehyde and hemiacetal. Alternatively, a very plausible explanation for these results is that metabolic reduction *in vivo* of compounds **28** and **29** leads to compounds **21** and **20**, respectively, which have reasonably high affinity for the receptor and would account for the antinociceptive potency observed for these compounds. The extent of intrinsic activity of the structural isomers, the hemiacetals and **30A**, cannot be ascertained.

Basic primary amines (e.g., **32** and **33**) do not substitute for

TABLE 4

Effect of modification of (±)-CP-55,940 on CBR binding



Compound	*n*	R^1	R^2	R^3[a]	CBR binding K_i[b]	Mouse PBQ stretching MPE_{60}[c] or inhibition
					nM	
(±)-CP-55,940	1		OH	DMH	1.14 ± 0.04	0.29 mg/kg (0.27–0.32)[d]
(−)-CP-55,940					0.137 ± 0.038	0.059 mg/kg (0.017–0.113)[d]
(+)-CP-55,940					12.0 ± 1.36	14.6 mg/kg (3.87–22.8)[d]
(±)-**34**	2		OH	DMH	0.696 ± 0.16	0.15 mg/kg (0.07–0.23)[d]
(−)-**34**					0.17 ± 0.04	0.06 mg/kg (0.04–0.09)[d]
(+)-**34**					18.3 ± 0.58	45% (10)[e]
35	1		H	DMH	40.2 ± 13.5	49% (56)[e]
36	1		OH	H	441 ± 113	26% (56)[e]
37	2		OH	DMH	2.13 ± 0.54	0.16 mg/kg (0.025–0.30)[d]
38	1		OH	DMH	94.0 ± 27.3	0.38 mg/kg (0.20–0.59)[d]
39	1		OH	DMH	NA[f]	0.93 mg/kg (0.49–1.42)[d]

[a] DMH, 1,1-dimethylheptyl.
[b] Values are means ± standard error.
[c] Subcutaneous, 1 hr.
[d] 95% confidence interval.
[e] Dose (mg/kg).
[f] NA, not available.

ω-hydroxyalkyls, whereas tertiary amines (e.g., **24** and **31**) do exhibit some binding potency and *in vivo* activity. Carboxylic acids (e.g., **26** and **27**) generally exhibit weaker receptor-binding activity and seem not to be replacements for ω-hydroxyalkyls.

Overall, for the functional groups in Table 3 there is a very reasonable correlation for compounds exhibiting good receptor-binding and *in vivo* potencies. The one exception, carboxamide **25**, may result from different pharmacokinetics or tissue distribution, both of which have been assumed to be equal for all derivatives in this analysis.

The most potent analog of CP-47,497, CP-55,940, was chosen to explore additional SAR points, as summarized in Table 4. Testing of the single enantiomers of (±)-CP-55,940 for receptor binding shows an 88-fold potency advantage for (−)-CP-55,940. This enantioselectivity for (−)-CP-55,940 is expressed functionally *in vivo*, with the mouse PBQ stretching test showing a potency ratio of 240:1 for the two enantiomers. Compound **34**, the cycloheptyl analog of CP-55,940, showed relative potency ratios for enantiomers of 108:1 and >170:1 for *in vitro* and *in vivo* tests, respectively, indicating a similar degree of enantioselectivity in this larger ring.

Most SARs for cannabinoids define a key relationship between phenolic functionality and expression of cannabinoid activity. This relationship holds for the CP-55,940 series, wherein replacement of the phenolic hydroxyl group by hydrogen (compound **35**) provides a 35-fold drop in receptor-binding activity and an approximately 200-fold loss of *in vivo* potency. Nevertheless, it is surprising, based on earlier literature, that a larger decrement in potency is not observed for **35**. For example, studies with the tetrahydro-6*H*-dibenzo[*b*,*d*]pyran series usually found a loss of *in vivo* activity upon replacement of the phenolic hydroxyl group with hydrogen (12). There is one report of a desphenolic 1,2-dimethylheptyl side chain analog that retains some activity (25, 26). A plausible explanation for our observation is that the additional functionality on CP-55,940 (i.e., hydroxypropyl) adds sufficient binding entropy to partially offset loss of the phenol. This hypothesis is testable using the appropriate SAR probes, and this work is now in progress.

The second key SAR feature of active cannabinoids is the presence of a C-3 lipophilic side chain. The data of Table 1 demonstrated the dependence of activity on a narrow range of alkyl side chain length, and that conclusion is strengthened by the results obtained with **36**. Compound **36**, lacking a 1,1-dimethylheptyl side chain, was prepared and test results confirm the expected significant decrease in activity (390-fold decrease for CBR binding and >200-fold decreased potency *in vivo*). Interestingly, compound **36** suffered a significantly larger loss in receptor-binding potency than did the desphenol derivative **35**. Combined, these findings suggest a relatively greater importance of the side chain versus the phenolic hydroxyl group for receptor-binding and cannabinoid activity.

Replacement of the cyclohexyl hydroxyl group with a bioisosteric acetamido group (compound **37**) caused only a 3-fold loss of binding potency and a negligible effect *in vivo*. This functional group exchange indicates that receptor binding may be accommodated by a range of functionality at this position. The ketone **38** exhibits significantly reduced receptor binding (82-fold) but unexpectedly retains full *in vivo* functional activity. The *in vivo* potency of **38** may result from metabolic reduction of the ketone to an alcohol, the more potent receptor binder CP-55,940. Thus, the results may suggest that a ketone is not an acceptable replacement for the hydroxyl group at the C-1 cyclohexyl position. Alternatively, this case is similar to the result obtained with the aldehydes **28** and **29**. Compound **38** may exist as the hemiketal **38A**, which would be in equilibrium with free ketone and phenol. It is possible that the equilibrium between **38** and **38A** is not established under the assay conditions and thus no free phenol exists to bind the receptor. Compound **39** is devoid of substitution at the position of the C-1 cyclohexyl hydroxyl group in CP-55,940; however, only a 3-fold loss of *in vivo* potency is seen. Although receptor binding data for this compound are not available, this result again suggests that substitution at this position is not an absolute necessity for cannabinoid activity.

Discussion

The present study has provided a comprehensive investigation of a series of bicyclic cannabinoid compounds for their ability to bind to the CBR and to produce a response (analgesia) *in vivo*. Although *in vivo* SAR studies have been performed on numerous series of cannabinoid compounds (1, 22), this study focuses on understanding the structural attributes required for agonist interaction with the CBR itself.

The phenolic ring C-3 nonpolar side chain stands out as being critical for both binding to the receptor and biological activity. The analysis of the bicyclic compounds in this study suggests that a hydrophobic interaction with the receptor requires the extension of a minimum chain length equivalent to five carbon atoms; the most potent binding and dose-dependent antinociceptive activity occurred at chain lengths of six to nine carbons. Even inclusion of additional functional groups for potential receptor-binding interaction, such as the cyclohexyl ring hydroxypropyl moiety of CP-55,940, fails to compensate for the loss of the C-3 alkyl side chain (compound **36**) for binding affinity. A similar rank order for receptor binding, albeit with different absolute K_i values, of these C-3 side chain analogs was recently reported for a modified receptor-binding assay (27).

Decreasing the side chain length of Δ^9-THC diminished the "high" reported in human subjects (28) and decreased the catalepsy response in mice (29). For the series of CP-47,497 side chain analogs, a biological activity profile in mice, including decreased locomotor activity, antinociception (tail flick), hypothermia, and immobility (ring stand), has been reported (30). Consistent with the present report, compounds having chain lengths of six to nine carbons exhibited a spectrum of biological activities resembling those of Δ^9-THC, and those compounds having fewer or more carbons had limited or no activity. It is of interest that the poorly active or inactive compounds in this series exhibited an increased latency in the tail-flick test. However, this occurred at high concentrations, was not dose dependent, and never reached the maximal efficacy attained by Δ^9-THC and the active compounds of this series. As noted for the binding data, the addition of the hydroxypropyl side chain extending from the cyclohexyl ring could not overcome the loss of activity resulting from the removal of the C-3 alkyl side chain in compound **36**. Compton *et al.* (30) also determined that, of those compounds tested, none behaved as antagonists toward the action of Δ^9-THC. According to the present data, this finding is due to the poor affinity of these compounds for the CBR, rather than strong binding but failure to evoke a response.

The minimum five-carbon length of the C-3 side chain might represent a critical distance between a single point of hydrophobic interaction and the remaining points of interaction on the structure. Alternatively, a more extensive hydrophobic domain or pocket within the receptor may surround this region of the molecule and contribute to the binding along the entire chain. The latter is supported by the observation that hydroxylation of Δ^9-THC at various positions along the chain decreased the potency for inhibition of adenylate cyclase in neuroblastoma cell membranes (31). However, in *in vivo* behavioral tests, hydroxylation of Δ^9-THC or Δ^8-THC at various positions resulted in either decreased or increased potency (32–35). It should be noted that, although the relative lipophilicity of the CP-47,497 side chain series increases with chain length (36), binding to the CBR fails to increase in parallel beyond the eight-carbon length. It can be speculated that the profound loss of affinity exhibited by compounds with chain lengths of more than nine carbon atoms may result from steric hindrance of the longer chains with the receptor protein, thus disturbing the proper orientation of remaining points of ligand-receptor interaction.

Hydrogen bonding may be an important source of CBR interaction with both the cyclohexyl and phenolic hydroxyl groups. The relative maintenance of binding activity with acetamido substitution at the cyclohexanol position (compound **37**) and the disruption of binding by the formation of hemiacetal structures (e.g., compounds **28A** and **29A**), which effectively masks the phenolic moiety under *in vitro* conditions, are consistent with this reasoning. Apparently the removal of the cyclohexyl hydroxyl group (compound **35**) is not as detrimental to biological potency as is the removal of the phenolic hydroxyl group (compound **39**). However, this finding may be an artifact of potential metabolic hydroxylation at the cyclohexyl position in the intact animal.

An additional site for potential hydrogen-bonding interaction appears to be created by extension of a three- or four-carbon hydroxyalkyl chain from the cyclohexyl ring (compare **22** with **19**). However, for short chain lengths, the receptor-binding affinity was decreased by incorporation of the hydroxyl moiety (compare **20** and **21** with **16** and **17**, having the same chain length). In general, polar groups in close proximity to the cyclohexyl ring in this position appear to diminish ligand-receptor interactions.

Our data fail to distinguish the regions of the cannabinoid compounds that are critical for conferring intrinsic efficacy, as opposed to conferring affinity for the receptor. Only a single compound (**25**) appeared to exhibit high affinity binding to the receptor but failed to possess high antinociceptive potency (see Fig. 5). Compound **25** was subsequently shown to confer the signal transduction response as a full agonist and with a potency that correlated well with the ratios of K_i for binding to K_{inh} for inhibition of adenylyl cyclase demonstrated for other compounds in this series. Thus, it must be concluded that compound **25** has no potential as a cannabinoid antagonist.

An interesting observation from the data reported here is that compounds exhibiting K_i values for binding to the receptor that exceed about 50 nM fail to exhibit analgesic activity at maximal drug doses (56 mg/kg) (see Fig. 5). These compounds also fail to inhibit adenylyl cyclase activity at concentrations that would be expected to be water soluble. It also appears that modifications of structure that confer greater or lesser antinociceptive activity generally produce parallel changes in affinity for the receptor (see Fig. 5). These observations suggest that the analysis of receptor-binding and functional (*in vitro* and *in vivo*) activity data must be considered in future attempts to develop rational SARs and especially in the search for cannabinoid antagonist ligands.

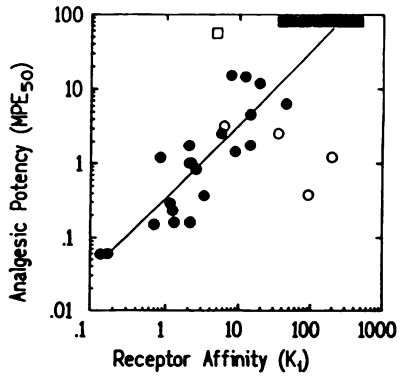


Fig. 5. Correlation between analgesic potency and binding affinity for the CBR. A linear regression analysis was performed with the compounds listed in Tables 1–4 for which an MPE_{50} could be determined for analgesic potency and which were not subject to structural alterations in solution (●). The correlation coefficient was 0.85. *Horizontal bar*, range of binding affinities for which analgesic activity was absent or poor at the highest dosage administered (56 mg/kg). Compounds exhibiting potential metabolism (**25**) (□) or hemiacetal conformations (**28**, **29**, **30**, and **38**) (○) are shown but were not included in the regression analysis.

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Send reprint requests to: Allyn C. Howlett, Department of Pharmacological and Physiological Science, St. Louis University School of Medicine, 1402 South Grand Boulevard, St. Louis, MO 63104.