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Manipulation of the Tetrahydrocannabinol Side Chain Delineates Agonists, Partial Agonists, and Antagonists[1]

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ABSTRACT

Structure-activity relation studies have established that the alkyl side chain in tetrahydrocannabinol (THC) plays a crucial role in the activation of the cannabinoid receptor. Unfortunately, the flexible nature of this side chain has hampered efforts to elucidate the precise nature of the interaction of THC with its receptors. Therefore, a series of analogs with structurally restrained side chains of varying length was synthesized and evaluated for pharmacological potency in mice and for receptor affinity. The introduction of *cis* double bonds inserted rigid angles, whereas triple bonds developed regions of planarity. Receptor affinity for the acetylenic and saturated side chains were the same, whereas double bond substitution increased affinity 10-fold. Moreover, the relationship between receptor affinity and potency was 10-fold less than that of Δ^8-THC in the case of some acetylenic derivatives, whereas changing the triple bond to a double bond restored the potency/affinity ratio. Additionally, an acetylene at C2–C3 in the octyl and nonyl side chains favored antinociception by as much as 70-fold. Surprisingly, several high-affinity acetylenic derivatives, especially those with cyano substitutions at the terminus of the side chain, were partial agonists or were inactive. Some of these low-efficacy, high-affinity ligands elicited antagonistic activity. The finding that manipulations of the side chain produces high-affinity ligands with either antagonist, partial agonist, or full agonist effects reveals a critical structural feature for receptor activation.

Tetrahydrocannabinol (Δ^9-THC), a component in marijuana, produces characteristic psychotropic responses in humans as well as specific behavioral alterations in laboratory animals, such as depression of motor activity, hypothermia, antinociception, static ataxia, catalepsy, hyperexcitability, anticonvulsant activity, and the ability to produce a discriminative stimulus cue (Dewey, 1986). Early structure-activity relationship studies provided a compelling argument for a cannabinoid receptor (Razdan, 1986). However, these findings more or less relied on the traditional three-ringed benzopyran structure of Δ^9-THC. The introduction of bicyclic analogs with extended branched side chains, such as CP 55,940, reinforced this receptor concept with their enhanced potency (Howlett et al., 1988). Moreover, [^{3}H]CP 55,940 was used to characterize cannabinoid receptor binding (Devane et al., 1988). Whereas CP 55,940 represented a certain degree of structural diversity, the discovery of cannabinoid activity in aminoalkylindoles demonstrated that Δ^9-THC-like effects could be produced by a structurally distinct compound (Pacheco et al., 1991; Compton et al., 1992a). The discovery of the endogenous ligand anandamide (Devane et al., 1992) provided yet another cannabinoid template.

Although there is little question that all of these compounds are capable of interacting with cannabinoid receptors, as evidenced by their ability to compete with [^{3}H]CP 55,940 binding, it does not appear that they are all identical in this regard. The behavioral potencies of Δ^9-THC, CP 55,940, WIN 55,212, and anandamide in relation to production of hypoactivity, hypothermia, antinociception, and catalepsy in mice demonstrated that these agents produced the same pharmacological profile (Compton et al., 1992a,b; Smith et al., 1994). However, these data also revealed some differences among the different agonists in that they were not equipotent in producing these four pharmacological effects. Additionally, the maximum effects of these compounds on each behavior were not identical. Anandamide provided the clearest example in that it was more efficacious than Δ^9-THC in producing catalepsy, but it was only a partial agonist for reducing body temperature (Smith et al., 1994). There are several possible explanations for these discrepancies. Although multiple cannabinoid receptors cannot be

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ABBREVIATIONS: THC, tetrahydrocannabinol; % MPE, percentage of maximum possible effect; SR 141716A, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxyamide.

ruled out, current evidence supports the CB1 receptor as the sole cannabinoid receptor in the central nervous system. The other possibility is that the CB1 receptor has multiple effector systems that can be selectively coupled, depending upon the binding properties of the agonist. Of course, there is ample evidence that the CB1 receptor is coupled to G proteins associated with adenylyl cyclase (Howlett, 1984) and N-type calcium channels (Mackie and Hille, 1992).

Further clarification of the nature of the interaction of cannabinoids with the CB1 receptor may emerge through additional structure-activity relationship studies. There have been numerous attempts to devise a pharmacophore that accommodates all of these diverse cannabinoid templates (Reggio et al., 1991; Thomas et al., 1991, 1996). One of the structural features that still demands considerable attention is the alkyl side chain in THC, or its equivalent in WIN 55,212 and anandamide. First, the profound influence that the side chain has on the pharmacological activity of THC (Razdan, 1986), CP 55,940 (Melvin et al., 1993), WIN 55,212 (Wiley et al., 1998), and anandamide (Ryan et al., 1997; Seltzman et al., 1997) is indisputable. Second, the flexible nature of the side chain has complicated efforts to specifically define its role in receptor recognition and activation. Therefore, the objective of the present study was to restrict the flexibility of the side chain by the systematic incorporation of either double or triple bonds throughout the side chain as well as incorporating functional groups that are known to influence other classes of ligands. A previous publication suggests that receptor affinity is retained when there is unsaturation in the side chain (Busch-Petersen et al., 1996).

Materials and Methods

ICR male mice (Harlan Laboratories, Indianapolis, IN) weighing 24 to 26 g were used in all experiments. Mice were maintained on a 14:10-h light/dark cycle with free access to food and water. Δ^8- and Δ^9-THC were obtained from the National Institute on Drug Abuse (Bethesda, MD). SR 141716A was provided by Pfizer, Inc. (Groton, CT).

Synthesis. The analogs were prepared by standard synthetic methodology. In general, this involved synthesizing the appropriate resorcinol precursors (i.e., possessing the side chains desired for the THCs in the 5-position of the resorcinol), followed by condensation (Razdan et al., 1974) with *cis*/*para*-menth-2-ene-1,8-diol (Firmenich Inc., Plainsboro, NJ) to give a mixture of isomeric products from which the desired THCs were isolated by silica gel chromatography. In a few cases, the condensation product was merely an intermediate in the synthesis of the desired products, and thus additional reactions were performed on the condensation product, in some cases with and in others without protection of the phenolic hydroxyl as its methoxymethyl ether. The resorcinol precursors were synthesized in a protected form, as their bismethyl ethers, and then deprotected (demethylated) with boron tribromide (McOmie and West, 1973) before condensing with menthenediol. The details of the synthesis of these analogs will be the subject of a separate article that will be published elsewhere.

Pharmacological Assays. Cannabinoids were dissolved in a 1:1:18 mixture of ethanol, Emulphor, and saline for i.v. administration. Mice received the analog by tail-vein injection and were evaluated for their ability to produce hypomotility, hypothermia, and antinociception. These pharmacological measures were determined in the same mouse at a time when maximal activity was present (Little et al., 1989). To measure locomotor activity, mice were placed into individual photocell activity chambers (11 inches × 6.5 inches) 5 min after injection. Spontaneous activity was measured during the next 10-min period, and the number of interruptions of 16 photocell beams per chamber was recorded. Antinociception was determined by the tail-flick reaction time to a heat stimulus (Dewey et al., 1970). Before vehicle or drug administration, the baseline latency period (2–3 s) was determined. Twenty minutes after the injection, tail-flick latency was assessed once more, and the differences in control and test latencies were calculated. A 10-s maximum latency was used. Antinociception was expressed as percentage of maximum possible effect (% MPE) as described below. As for hypothermia, rectal temperature was determined before vehicle or drug administration with a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) and a thermistor probe (model YSI 400, Markson, Inc.) inserted at a depth of 2 mm. At 30 min after the injection, rectal temperature was measured again, and the difference between pre- and postinjection values was calculated.

Receptor Binding. [^{3}H]CP 55,940 (K_D = 690 pM) binding to P_2 membranes was conducted as described elsewhere (Compton et al., 1993), except that whole brain (rather than cortex only) was used. Displacement curves were generated by incubating drugs with 1 nM [^{3}H]CP 55,940. The assays were performed in triplicate, and the results represent the combined data from three individual experiments. The K_I values were determined from displacement data with EBDA software (Equilibrium Binding Data Analysis; Biosoft, Milltown, NJ).

Data Analysis. For production of hypomotility and hypothermia, the data were expressed as percentage of control activity and change in °C, respectively. Antinociception was calculated as

$$\%\ \mathrm{MPE} = \left[\frac{(\text{test latency} - \text{control latency})}{(10\ \text{sec} - \text{control latency})}\right] \times 100.$$

At least six animals were treated with each dose so that dose-response relationships could be determined for each analog. ED_{50} values were determined from least-squares unweighted linear regression analysis of the log dose-response plots. Maximal effects for all compounds combined on spontaneous activity, temperature, antinociception, and catalepsy were, respectively, 90% inhibition, −5°C, 100% MPE, and 60% immobility. Thus, the ED_{50} values indicate response levels of 45% inhibition, −2.5°C, 50% MPE, and 30% immobility.

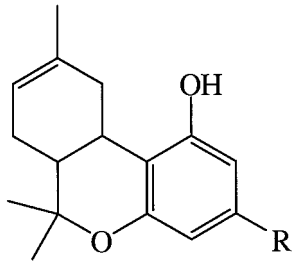
Results

The analogs depicted in Table 1 were designed for the purpose of determining the consequences of incorporating rigidity into the alkyl side chain in THC. A region of rigidity was achieved either by inserting acetylenic linkages throughout the side chain or by inserting *cis* double bonds at the same acetylenic regions. The latter introduces a bend or angle in the side chain. An additional objective was to determine the chain link at which unsaturation exerted the greatest influence. As an initial target, we chose to introduce *cis* double bonds in the chain, because they are present in the arachidonic acid portion of the endogenous ligand anandamide. The receptor affinity of Δ^8- and Δ^8-THC was found to be approximately 40 nM (Table 1). Neither Δ^8-THC nor Δ^9-THC exhibited any pharmacological selectivity in that their ED_{50} values were comparable for all three pharmacological measures. Δ^8-THC was three to six times less potent than Δ^9-THC. With regard to efficacy, both produced maximal effects in depression of spontaneous activity and production of antinociception at a dose of 30 mg/kg. Δ^8-THC was somewhat less efficacious than Δ^9-THC in its hypothermic effects (Table 1). Dose-response curves for Δ^8- and Δ^9-THC are presented in

TABLE 1

Influence of side chain constraint and length on receptor affinity, pharmacological potency, and selectivity

Spontaneous activity (SA), tail-flick response (TF), and rectal temperature (RT) are expressed as ED_{50} values (mg/kg). A blank space indicates that calculation of an ED_{50} was not possible. The maximal effect with the dose in parentheses is given under the ED_{50} value. The K_D values represent means ± S.E. of at least three separate experiments.



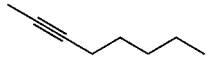
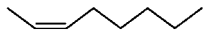
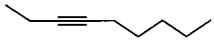
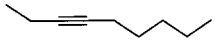
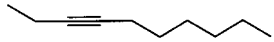
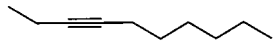
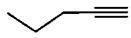
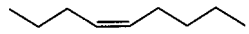
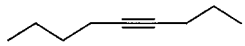
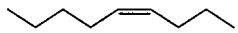
No.[a]	Chemical Name	R	K_D	ED_{50}		
				SA	TF	RT
			nM		*mg/kg*	
	Δ^9-THC		41 ± 1.7	1.1 94 (30)	1.1 100 (30)	1.0 –5.4 (10)
	Δ^8-THC		45 ± 12	3.0 94 (30)	2.6 100 (30)	5.9 –4.8 (30)
1 JWH 091	3-Heptyl-Δ^8-THC		22 ± 3.9	0.14 80 (30)	0.61 100 (10)	0.16 –4.0 (30)
2 O-964	3-(1-Heptynyl)-Δ^8-THC		36 ± 0.8	3.69 74 (30)	3.25 100 (30)	2.97 –5.6 (10)
3 O-1317	*cis*-3-(1-Heptenyl)-Δ^8-THC		0.86 ± 0.09	0.09 87 (1)	0.09 100 (1)	0.13 –6.3 (1)
4 JWH 124	3-Hexyl-Δ^8-THC		41 ± 3.8	1.16 91 (10)	1.79 98 (10)	0.10 –5.3 (10)
5 O-615	3-(2-Hexynyl)-Δ^8-THC		11 ± 1.0	47.7 63 (100)	3.08 71 (10)	14.8 –3.2 (100)
6 JWH 138	3-Octyl-Δ^8-THC		8.5 ± 1.4	0.39 82 (3)	0.34 100 (3)	0.24 –6.6 (3)
7 O-584	3-(2-Octynyl)-Δ^8-THC		4.9 ± 2.0	0.51 89 (10)	0.03 97 (10)	2.14 –4.2 (10)
8[b] O-1314	*O*-Methyl-3-(2-octynyl)-Δ^8-THC		189 ± 20	16.2 60 (30)	15.1 70 (30)	50.8 –2.7 (30)
9 O-1318	*cis*-3-(2-Octenyl)-Δ^8-THC		3.19 ± 0.92	0.05 92 (1)	0.11 100 (1)	0.07 –6.0 (1)

TABLE 1—(*Continued*)

No.[a]	Chemical Name	R	K_D	ED_{50}		
				SA	TF	RT
			nM		*mg/kg*	
10 O-630	3-(2-Nonynyl)-Δ^8-THC		3.7 ± 1.3	2.22 81 (30)	0.31 100 (10)	1.91 –4.1 (10)
11 O-629	3-(2-Nonynyl)-Δ^9-THC		6.0 ± 2.0	5.73 71 (30)	1.39 100 (10)	5.28 –5.3 (30)
12 JWH 130	3-Butyl-Δ^8-THC		65 ± 13	9.04 80 (30)	10.1 80 (30)	6.29 –4.6 (30)
13 O-1004	3-(3-Butynyl)-Δ^8-THC		367 ± 23	28 (30)	11 (30)	–1.0 (30)
14 O-1020	3-(3-Octynyl)-Δ^8-THC		9.0 ± 1.3	2.39 75 (30)	1.46 85 (30)	1.14 –3.4 (30)
15 O-1319	3-(3-Octenyl)-Δ^8-THC		3.36 ± 0.91	0.09 88 (1)	0.16 96 (1)	0.19 –6.1 (1)
16 O-1052	3-(4-Octynyl)-Δ^8-THC		19 ± 1.3	2.88 75 (30)	3.26 82 (30)	4.22 –4.5 (30)
17 O-1083	*cis*-3-(4-Octenyl)-Δ^8-THC		11 ± 3.2	0.22 89 (1)	0.12 97 (3)	0.57 –4.9 (10)

[a] Listed below the boldface analog number is the reference number assigned by either Organix, Inc. (O) or Dr. Huffman (J.W.H.).
[b] The phenolic hydroxyl group is methylated.

Fig. 1 which demonstrate that both are full agonists in all three pharmacological tests.

Substitution of a seven-carbon side chain for the traditional pentyl group in Δ^8-THC resulted in an analog (**1**) with a K_D approximately one half-that of Δ^8-THC (Table 1). This analog was considerably more potent than Δ^8-THC in all three pharmacological measures (Table 1), although it appeared to be somewhat less potent in producing antinociception than in reducing spontaneous activity and rectal temperature. Its antinociceptive efficacy was similar to that of Δ^8-THC, whereas that for depression of spontaneous activity was somewhat less (Fig. 1). In addition, the hypothermic response was somewhat variable and resulted in a maximal effect of only −4.0°C at a dose of 30 mg/kg. Insertion of an acetylene at the first carbon atom (C1) of this heptyl side chain (analog **2**) resulted in receptor affinity, pharmacological potency, and efficacy similar to that of Δ^8-THC. The only exception appeared to be its effects on spontaneous activity in that a dose of 30 mg/kg produced no greater than a 74% effect (Fig. 1). Changing the acetylenic structure to a *cis* double bond (analog **3**) eliminated the colinearity of the first three carbon atoms of the side chain and thereby dramatically enhanced both receptor affinity and potency to comparable degrees. Moreover, doses as low as 1 mg/kg were fully efficacious (Fig. 1). The receptor affinity/potency ratios were approximately 10 and similar to those of Δ^8-THC.

To examine the C2–C3 positions, the saturated hexyl side chain derivative (**4**) was prepared and found to have receptor affinity and pharmacological potencies in the spontaneous activity and tail-flick assays comparable with those of Δ^8-THC. It was somewhat exceptional in that **4** was almost 60-fold more potent than Δ^8-THC in producing hypothermia. To examine the linearity of positions C2–C4 in the side chain, an acetylene was inserted into the hexyl side chain to form analog **5**. As shown in Table 1, this analog had an affinity for the receptor greater than that of Δ^8-THC, yet was consider-

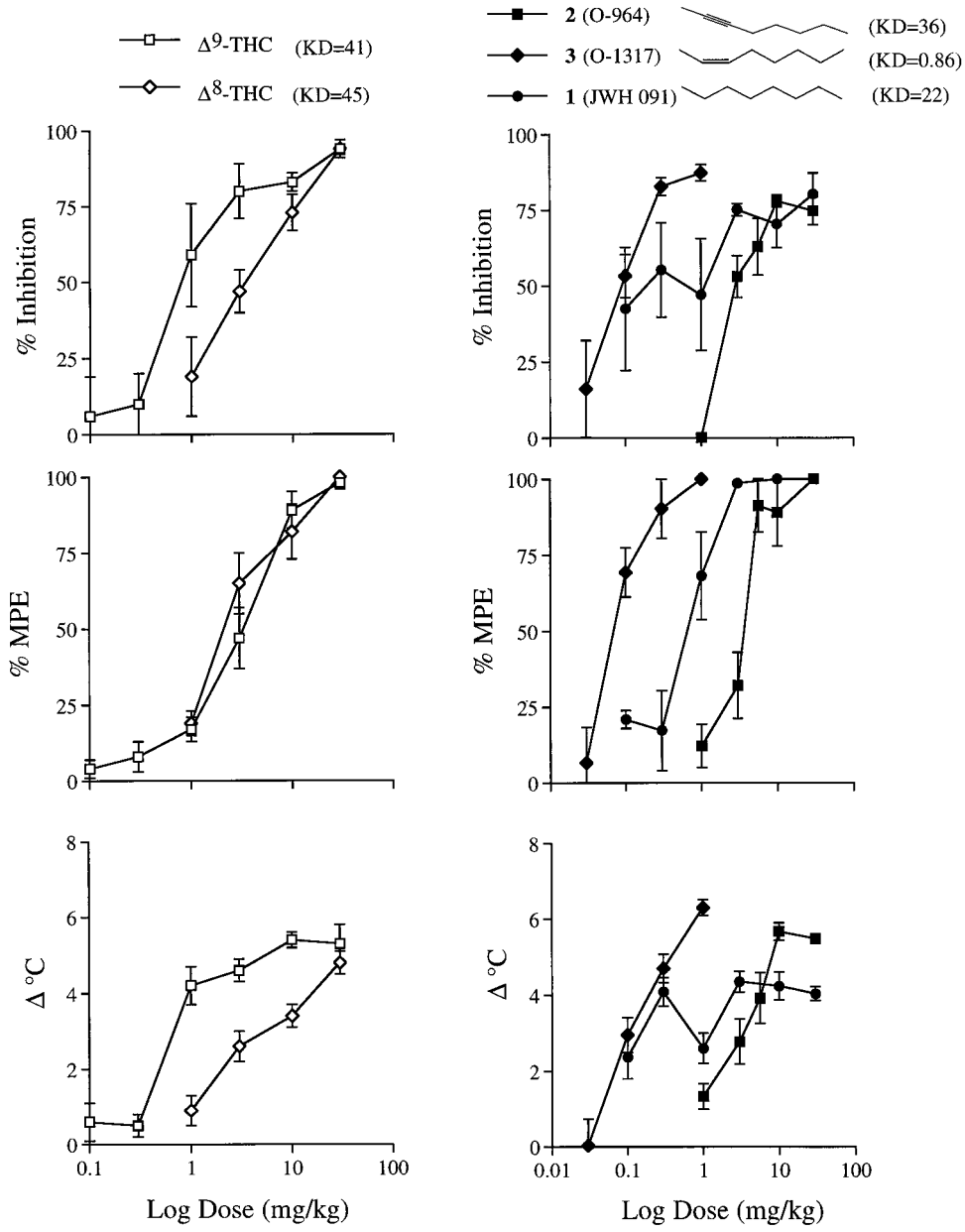


Fig. 1. Comparison of Δ^9- and Δ^8-THC dose-response curves to those of analogs with varying degrees of unsaturation at C1 of a heptyl side chain. Inhibition of spontaneous activity, antinociception, and hypothermia is presented in the top, middle, and bottom panels, respectively. All data are presented as means ± S.E. for at least six mice per group.

ably less potent and less efficacious than Δ^8-THC. A dose of 100 mg/kg was unable to produce maximal effects on spontaneous activity and hypothermia, whereas 71% MPE was obtained with a 10-mg/kg dose. Estimating the ED_{50} values revealed that this partial agonist is almost 15 times less potent than Δ^8-THC in its effects on spontaneous activity and approximately 3-fold less potent in producing hypothermia. In contrast to these potency differences, **5** was equipotent to Δ^8-THC in producing antinociception. This analog is unique for being a partial agonist as well as for antinociceptive selectivity. It is obvious that linearity about the C2–C4 region alters the pharmacological profile in that it is unusual for a cannabinoid to exhibit antinociceptive potency that is 5- to 15-fold greater than the other effects in this model. Extending the side chain by two additional carbons atoms resulted in analog **6,** which had receptor affinity 5-fold greater than that of Δ^8-THC and pharmacological potencies 10 to 20 times greater than those of Δ^8-THC. Incorporation of an acetylene between C2 and C3 led to analog **7**. The striking feature of **7** is its antinociceptive selectivity. Potency in the tail-flick procedure was 17 and 71 times that for producing hypoactivity and hypothermia, respectively (Fig. 2). To determine whether modifications in other parts of the molecule would influence antinociceptive selectivity, the phenyl hydroxyl of analog **7** was methylated (analog **8**), which produced an almost 50-fold attenuation in receptor affinity. Not only was pharmacological potency reduced, but antinociceptive selectivity was practically eliminated. Analog **8** is clearly a partial agonist in all three pharmacological tests.

Changing the triple bond in analog **7** to a *cis* double bond led to analog **9** with little change in receptor affinity. However, as with the previous change from a triple bond to a double bond (analogs **2** and **3**), there was a substantial increase in potency for production of both hypoactivity and hypothermia (Table 1). In contrast, antinociceptive potency decreased somewhat as pharmacological selectivity was elim-

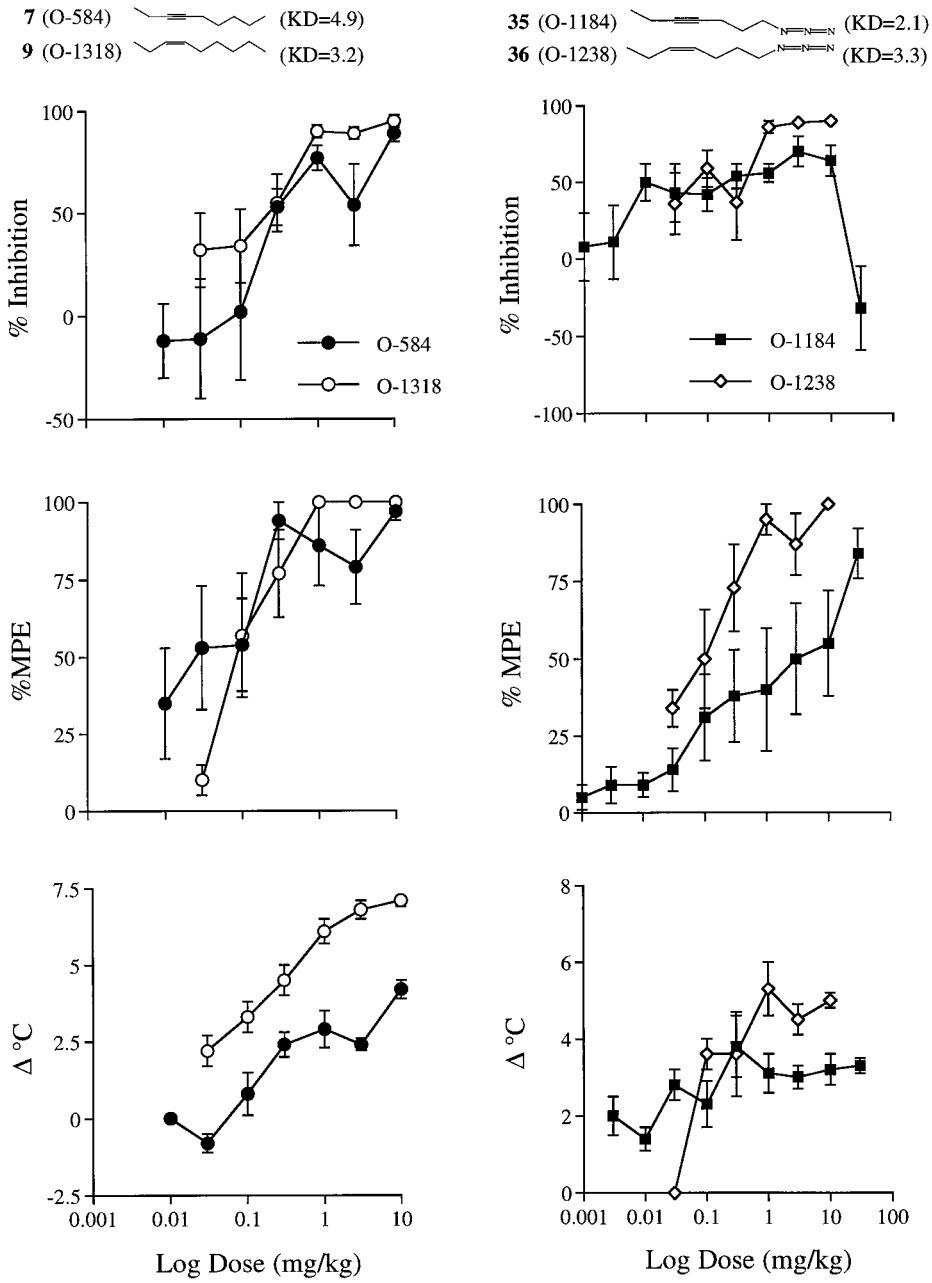


Fig. 2. Comparison of C2–C3 double- and triple-bond side chains with and without azido substitutions. Inhibition of spontaneous activity, antinociception, and hypothermia is presented in the top, middle, and bottom panels, respectively. All data are presented as means ± S.E. for at least six mice per group.

inated. However, there is a definite distinction between analogs **7** and **9** with regard to their efficacy, with **9** being a full agonist at very low doses in all three pharmacological measures (Fig. 2).

Extending the chain length to nine carbons in the 2′-acetylenic series yielded analog **10,** which had receptor affinity similar to that of the 2-octynyl analog (**7**) but with a somewhat modified pharmacological profile. The hypothermic potencies of analogs **7** and **10** were similar, the hypoactivity potency of **10** was about 4-fold higher, and the antinociceptive potency of **10** was increased 10-fold. Nevertheless, analog **10** still retained some antinociceptive selectivity. The maximal effects produced by **7** and **10** were similar. Preparation of an analogous Δ^9-THC derivative resulted in an analog (**11**) with properties very similar to those of **10**.

Two derivatives were prepared in which the traditional pentyl side chain was reduced to a butyl (analogs **12** and **13**). As expected, both receptor affinity and pharmacological potency were reduced. To examine the influence of linearity at the terminus of the side chain, the 3′-butyne analog **13** was prepared. As indicated in Table 1, this analog had very little affinity for the receptor and was practically devoid of pharmacological effects. However, increasing the chain length to eight carbons (analog **14**) restored both receptor affinity and pharmacological activity for the C3–C4 acetylene series. However, this 3′-octyne analog did not show any antinociceptive selectivity. As was noted with analogs **2** and **5**, the receptor affinity/potency ratios of 4 to 8 for **14** were in the range that is somewhat lower than that for Δ^8-THC. As with several of the acetylenic derivatives described above, a high dose of 30 mg/kg failed to produce maximal effects (Fig. 3). Reducing the 3-octynyl (**14**) to the corresponding 3-octenyl

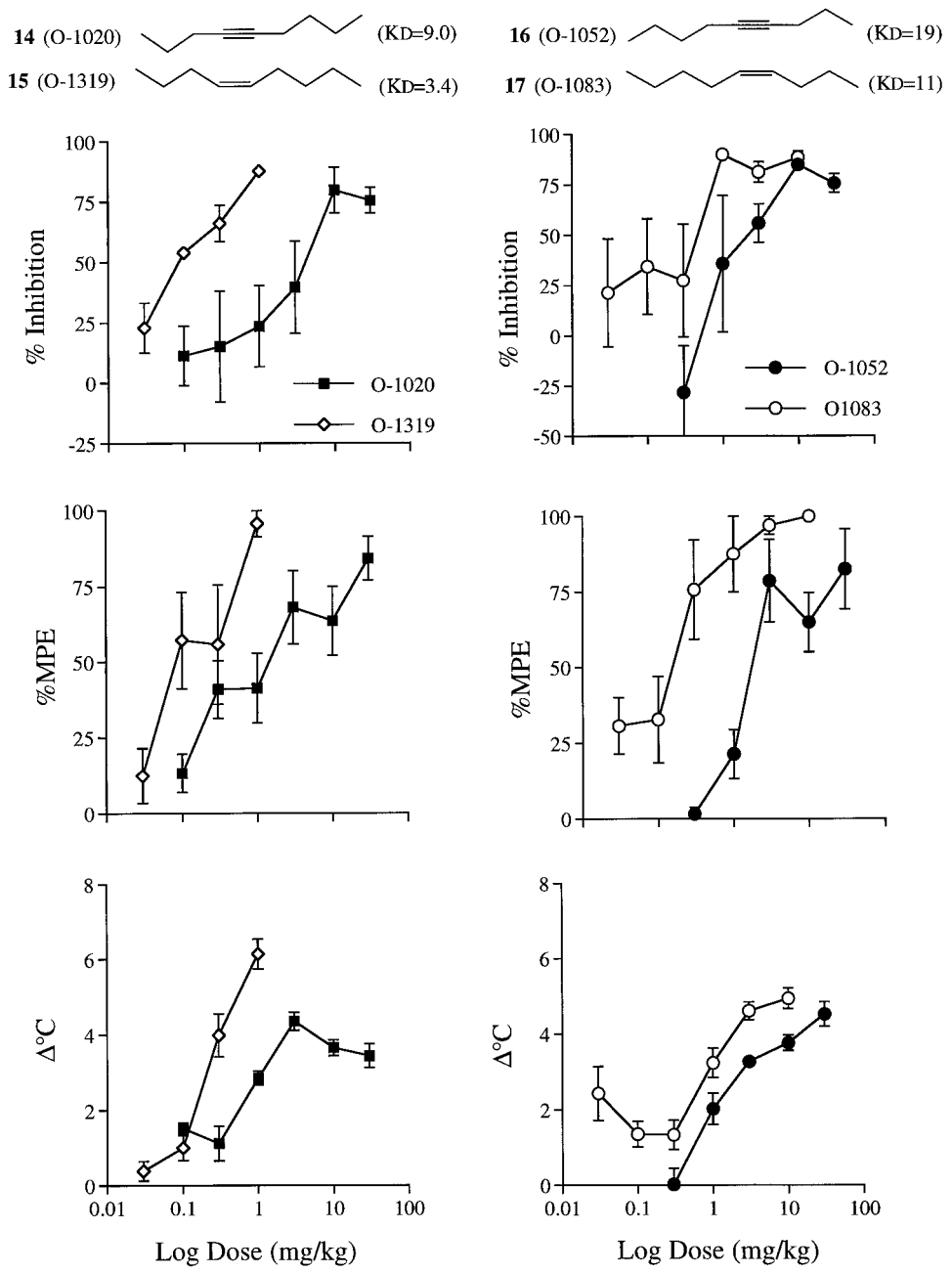


Fig. 3. Comparison of Δ^8-THC analogs with double and triple bonds at either position C3 or C4 of an octyl side chain. Inhibition of spontaneous activity, antinociception, and hypothermia is presented in the top, middle, and bottom panels, respectively. All data are presented as means ± S.E. for at least six mice per group.

analog (**15**) restored potency to levels commensurate with high receptor affinity. Moreover, a dose of 1 mg/kg produced greater maximal effects than that produced by high doses of **14** (Fig. 3).

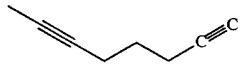
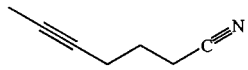
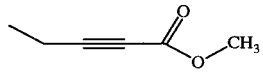
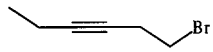
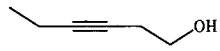
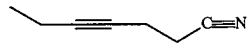
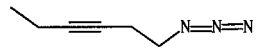
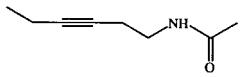
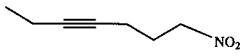
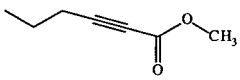
Positioning the acetylene between carbons 4 and 5 produced a compound (**16**) that was very similar to analog **14** with regard to maximal effects. There was a slight reduction in receptor affinity and potency. Analog **16** also did not exhibit any antinociceptive selectivity. When the corresponding 4-octenyl analog (**17**) was prepared, potency was increased as much as 30-fold with only a modest increase in receptor affinity. Efficacy of **17** was enhanced for both hypokinesia and antinociception, but not hypothermia.

The results in Table 1 clearly demonstrate that the localization of the acetylene and the length of the side chain influence potency and pharmacological selectivity. To determine whether substitution at the terminus of the side chain would have an impact on this profile, several analogs were prepared as depicted in Table 2. Two analogs were prepared with an acetylene at C1–C2 and either an acetylene (**18**) or a cyano (**19**) at the end of the side chain. Neither analog had high-affinity for the receptor, and neither produced more than only minimal effects at high doses. There appears to be some separation in activities for analog **18** in that a dose of 30 mg/kg only produced 12% inhibition of spontaneous activity, whereas this dose was capable of eliciting 57% antinociception and almost a 3°C decrease in body temperature. Failure of these compounds to exert high receptor affinity, coupled with the lack of influence that a C1–C2 acetylene had on the unsubstituted side chain (analog **2**), prompted us to pursue the C2–C3 acetylene series. Analog **20**, with a C2–C3 propynyl side chain and a carbomethoxy substitution on the ter-

TABLE 2

Effects of side chain length and substitutions in the terminal carbon on receptor affinity and pharmacological potency

Spontaneous activity (SA), tail-flick response (TF), and rectal tempereature (RT) are expressed as ED_{50} values (mg/kg). A blank space indicates that calculation of an ED_{50} was not possible. The maximal effect with the dose in parentheses is given under the ED_{50} value. The K_D values represent means ± S.E. of at least three separate experiments.

No.[a]	Chemical Name	R	K_D	ED_{50}		
				SA	TF	RT
			nM		*mg/kg*	
18 O-1021	3-(1,6-Heptadiynyl-Δ^8-THC		460 ± 79	 12 (30)	31 57 (30)	37 –2.9 (30)
19 O-1068	3-(5-Cyano-1-pentynyl)-Δ^8-THC		104 ± 12	 34 (60)	33.6 53 (60)	15.1 –4.3 (60)
20 O-1351	3-(3-Carbomethoxy-2-propynyl)-Δ^8-THC		731 ± 139	 29 (30)	 31 (30)	 –1.6 (30)
21 O-1187	3-(4-Bromo-2-butynyl)-Δ^8-THC		143 ± 31	 60 (30)	 38 (30)	 –2.6 (30)
22 O-1105	3-(5-Bromo-2-pentynyl)-Δ^8-THC		25 ± 4.2	14.1 63 (60)	23.7 52 (30)	11.4 –4.2 (60)
23 O-1359	3-(5-Hydroxy-2-pentynyl)-Δ^8-THC		448 ± 75	3.77 91 (30)	 25 (30)	22.8 –3.1 (30)
24 O-1106	3-(5-Cyano-2-pentynyl)-Δ^8-THC		31 ± 8.3	 45 (60)	 53 (60)	 –3.3 (60)
25 O-1355	3-(5-Azido-2-pentynyl)-Δ^8-THC		6.15 ± 0.44	1.54 82 (30)	7.56 53 (10)	2.76 –4.5 (10)
26 O-1356	3-(5-Acetamido-2-pentynyl)-Δ^8-THC		307 ± 24	4.69 71 (30)	 46 (30)	30.2 –3.2 (30)
27 O-1358	3-(6-Nitro-2-hexynyl)-Δ^8-THC		5.34 ± 0.75	 50 (30)	 14 (30)	 –3.0 (30)
28 O-1357	3-(4-Carbomethoxy-3-butynyl)-Δ^8-THC		>10,000	 61 (30)	 41 (30)	 –3.2 (30)

[a] Listed below the boldface analog number is the reference number assigned by either Organix, Inc. (O) or Dr. Huffman (J.W.H.).

minal carbon, had both low receptor affinity and low efficacy. Analog **21**, which contains a C2–C3 butyne with a bromine substitution on the terminal carbon atom, had somewhat higher receptor affinity; however, its effects were not dose-responsive, and it failed to elicit full agonist activity at doses as high as 30 mg/kg. Increasing the length of the side chain of **21** by one carbon led to analog **22**, which had considerably higher receptor affinity. The effects of the latter were dose-responsive, although full agonist effects were not obtained with doses up to 60 mg/kg. This analog is markedly different

from Δ^8-THC in that it has a 2-fold higher affinity, yet it is much less potent and efficacious. It was surprising that substitution of a hydroxy for the bromo group (analog **23**) decreased receptor affinity approximately 20-fold and resulted in a compound with either partial (antinociception and hypothermia) or full agonist (spontaneous activity) effects, depending upon the pharmacological assay. Substituting a cyano group for the bromine in analog **22** led to the formation of analog **24,** which had receptor affinity comparable with that of both Δ^8-THC and analog **22**, but it failed to produce half-maximal effects even at doses as high as 60 mg/kg. Substitution of an azido rather than a cyano in the pentynyl side chain, which led to analog **25**, had a significant influence on both receptor affinity (8-fold greater than that of Δ^8-THC) and pharmacological profile. Despite the high receptor affinity, **25** produced hypoactivity and hypothermia comparable with those of Δ^8-THC, but it was only a partial antinociceptive agonist. Substitution of an acetamido (**26**) for the cyano resulted in both very low receptor affinity and low potency. Conversely, the 6-nitro-hexynyl analog (**27**) was similar to some of the above derivatives in that it has high receptor affinity with little pharmacological activity. The final compound in Table 2 contains a carbomethoxy-3-butynyl side chain (**28**) that has both low affinity and potency, characteristics similar to those of the carbomethoxy-2-propynyl analog (**20**).

The compounds described in Table 2 revealed that substitutions on the terminal carbon atom can have a profound influence on both receptor affinity and pharmacological potency of analogs with the C2–C3 acetylene side chain. These findings, along with the observations in Table 1, suggested that increasing the chain length would magnify these effects. Therefore, a third series of analogs was developed in which various substituents were incorporated into the terminus of either 2′-hexenyl or 2′-hexynyl side chains. Initially, a terminal carboxylic acid group was attached to a 1,2-hexadienyl side chain, which resulted in an analog (**29**) with receptor affinity >3000 nM. Despite this very low receptor affinity, **29** did produce modest antinociception and hypothermia. Effects on spontaneous activity were erratic in that a dose of 3 mg/kg produced 31% motor stimulation, a dose of 10 mg/kg was without effect, and a dose of 30 mg/kg attenuated activity by 80%. Efforts were then redirected toward the C2–C3 acetylene derivatives where incorporation of a terminal amino group (**30**) produced a low-affinity analog that failed to achieve even half-maximal effects at doses up to 30 mg/kg. However, incorporation of an isothiocyanate group (**31**) into the side chain enhanced receptor affinity more than 100-fold over that of the amino derivative (Table 3). Whereas the receptor affinity of **31** was approximately 4 times higher than that of Δ^8-THC, its pharmacological potencies were considerably lower, and it failed to produce maximal effects at a dose of 30 mg/kg. There was slight antinociceptive selectivity.

When the isothiocyanate of **31** was replaced with an acetylene, the resulting analog (**32**) had excellent receptor affinity but a mixed pharmacological profile. It produced 74% antinociception and a 4.6°C drop in rectal temperature at a dose of 30 mg/kg; its effects on spontaneous activity were biphasic, with maximal effects occurring at 3 mg/kg. Based upon the findings in Table 2 that addition of a cyano in the side chain enhanced potency, the acetylene group of **32** was changed to a cyano moiety, resulting in a high-affinity ligand (**33**) with a K_D less than 1 nM (Table 3). Despite this high affinity for the receptor, **33** was almost devoid of pharmacological activity. The results were variable, and doses of 30 mg/kg failed to produce even half-maximal effects (Fig. 4). However, simply replacing the triple bond with a double bond fully restored agonist activity, so that analog **34** was a high-affinity/high-potency agonist. As with the 2-octenyl derivatives in Table 1, **34** lacked pharmacological selectivity. Comparison of the dose-response curves for **33** and **34** in Fig. 4 demonstrates the profound differences engendered by the triple and double bonds.

To determine whether the pharmacological profiles of **33** and **34** were unique for the cyano substituent, other terminal substitutions were carried out, the first of which were azido analogs (**35** and **36**). The 6-azido-2-hexynyl analog (**35**) retained high receptor affinity and, like its cyano counterpart (**33**), was a partial agonist. Analog **35** is unique in that at low doses it is more effective in reducing body temperature than in producing antinociception and hypomotility. Moreover, **35** produced considerable motor stimulation at a dose of 30 mg/kg (Fig. 2). As with the analogs discussed earlier, when a double bond is incorporated into the side chain (analog **36**) rather than a triple bond, receptor affinity is relatively unaltered, pharmacological selectivity is attenuated, and potency is dramatically increased. However, in this instance the alteration in pharmacological profile involves a 20- and a 50-fold increase in potency for antinociception and depression of spontaneous activity, respectively, and little or no changes in hypothermia potency (Fig. 2).

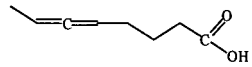
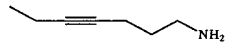
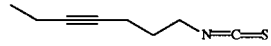
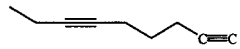
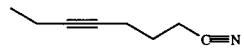
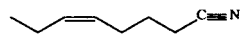
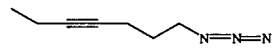
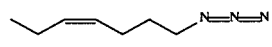
The last comparison of double and triple bond compounds involves analogs **37** to **38** with a bromo substitution on the terminal carbon atom of the side chain (Table 3). The 6-bromo-2-hexynyl analog (**37**) exhibited high receptor affinity. Its effects on spontaneous activity were biphasic in that low doses produced some hypoactivity, whereas higher doses were without effect (Fig. 4). It was a partial agonist in the other two tests, producing maximal effects of 57% MPE in the tail-flick test and a 3.8°C decrease in body temperature. ED_{50} values in the respective tests were estimated to be 4.78 and 29 mg/kg. However, the corresponding 6′-bromo-2′-hexenyl analog **38** was a very potent full agonist with high receptor affinity (Fig. 4). The affinity/potencies ratios were in the range of 10 to 30. To determine whether substituents other than a bromine would exert full agonist activity when added at the C6 position of a 2′-hexenyl side chain, analogs **39** to **42** were synthesized. The fluoro analog (**39**) was somewhat less potent than the bromo derivative. Hydroxylation (**40**) further reduced receptor affinity so that its binding was comparable with that of Δ^8-THC. The potency of **40** was also in the range of that of Δ^8-THC, albeit somewhat less potent in producing hypothermia. In contrast, the methoxy derivative (**41**) was more potent and had a higher receptor affinity than did the hydroxyl analog. The acetyl-amino analog (**42**) was also quite potent in producing hypoactivity and antinociception but was only weakly effective regarding hypothermia.

The potency of **42** suggests that the receptor can accommodate a longer side chain with a polar substituent in the terminal position. The synthesis of a 7′-nitro hept-2′-enyl (**43**) and subsequent pharmacological evaluation confirmed this observation. Analog **43** had a high receptor affinity and was very potent in producing hypoactivity, antinociception, and hypothermia.

TABLE 3

Effects of side chain length and substitutions in the terminal carbon on receptor affinity and pharmacological potency

Spontaneous activity (SA), tail-flick response (TF), and rectal temperature (RT) are expressed as ED_{50} values (mg/kg). A blank space indicates that calculation of an ED_{50} was not possible. The maximal effect with the dose in parentheses is given under the ED_{50} value. The K_D values represent means ± S.E. of at least three separate experiments.

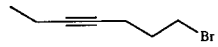
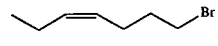
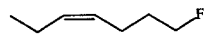
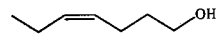
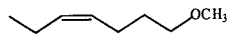
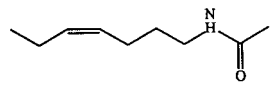
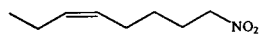
No.[a]	Chemical Name	Structure	K_D	ED_{50}		
				SA	TF	RT
			nM		*mg/kg*	
29 O-1174	3-(6-Carboxy-1,2-hexadienyl)-Δ^8-THC		3170 ± 105	0 (10)	24.3 53 (30)	23.9 –3.4 (30)
30 O-1175	3-(6-Amino-2-hexynyl)-Δ^8-THC		1300 ± 180	40 (30)	40 (30)	–1.7 (30)
31 O-1176	3-(6-Isothiocyano-2-hexynyl)-Δ^8-THC		11.5 ± 2.3	10 65 (30)	4.3 87 (30)	24.2 –3.0 (30)
32 O-965	3-(2,7-Octadiynyl)-Δ^8-THC		4.7 ± 0.4	77 (3)	2.92 74 (30)	1.06 –4.6 (30)
33 O-823	3-(6-Cyano-2-hexynyl)-Δ^8-THC		0.77 ± 0.05	43 (30)	45 (30)	–2.3 (30)
34 O-1237	*cis*-3-(6-Cyano-2-hexenyl)-Δ^8-THC		1.25 ± 0.51	0.17 91 (1)	0.08 100 (1)	0.15 –5.0 (1)
35 O-1184	3-(6-Azido-2-hexynyl)-Δ^8-THC		2.14 ± 0.44	9.8 64 (10)	1.8 84 (30)	0.17 –3.3 (30)
36 O-1238	*cis*-3-(6-Azido-2-hexenyl)-Δ^8-THC		3.32 ± 0.59	0.02 75 (1)	0.08 100 (1)	0.16 –6.1 (1)

Discussion

The systematic evaluation of structural alterations of the THC side chain underscores the importance of their structural feature with regard to receptor affinity, as well as pharmacological potency and efficacy. A review (Mechoulam and Edery, 1973) of the early literature revealed that 3′-alkyl substituents smaller than pentyl were either inactive or considerably less potent than THC, whereas longer chain substituents tended to be slightly more potent. However, the most dramatic influence on the side chain came with alkyl branching (Mechoulam and Edery, 1973). Therefore, efforts to enhance the potency of cannabinoid analogs usually rely on replacement of the pentyl side chain with a dimethylalkyl group such as dimethylheptyl. Unfortunately, the dimethylalkyl group retains considerable flexibility that limits the analysis of side chain orientation in receptor interactions.

There are several ways of constraining the side chain, including cyclization to form an additional ring. Unfortunately, this latter approach eliminated pharmacological activity. There have been several attempts to reduce flexibility through introduction of unsaturated alkyl side chains. Loev et al. (1973) and Razdan et al. (1976) prepared 1-heptenyl and 1-octenyl derivatives, respectively, both of which had increased pharmacological potency. Unfortunately, there were no means of assessing receptor affinity at that time. More recently, Busch-Petersen et al. (1996) compared recep-

TABLE 3—(*Continued*)

No.[a]	Chemical Name	Structure	K_D	ED_{50}		
				SA	TF	RT
			nM		*mg/kg*	
37 O-806	3-(6-Bromo-2-hexynyl)-Δ^8-THC		1.2 ± 0.1	0 (30)	4.78 60 (30)	29 –3.8 (30)
38 O-1236	*cis*-3-(6-Bromo-2-hexenyl)-Δ^8-THC		1.66 ± 0.66	0.16 86 (1)	0.13 100 (1)	0.05 –5.7 (1)
39 O-1310	*cis*-3-(6-Fluoro-2-hexenyl)-Δ^8-THC		20.9 ± 1.91	0.48 80 (1)	0.26 100 (1)	0.42 –5.2 (1)
40 O-1311	*cis*-3-(6-Hydroxy-2-hexenyl)-Δ^8-THC		53.7 ± 6.5	0.8 80 (10)	2.1 74 (10)	4.2 –3.5 (10)
41 O-1312	*cis*-3-(6-Methoxy-2-hexenyl)-Δ^8-THC		11.5 ± 0.16	0.16 74 (1)	0.16 92 (1)	0.41 –4.2 (1)
42 O-1309	*cis*-3-(6-Acetamido-2-hexenyl)-Δ^8-THC		16.7 ± 1.51	0.67 82 (3)	0.84 90 (3)	10.5 –3.1 (3)
43 O-1313	*cis*-3-(7-Nitro-2-heptenyl)-Δ^8-THC		3.56 ± 2.18	0.05 83 (1)	0.09 92 (1)	0.10 –3.4 (1)

[a] Listed below the boldface analog number is the reference number assigned by either Organix, Inc. (O) or Dr. Huffman (J.W.H.).

tor affinities of 11-hydrohexahydro-THCs with heptyl side chains that contained either a double or a triple bond at C1–C2 in the side chain. Their findings that receptor affinity for the acetylenic and saturated side chains were the same and that double bond substitution increased affinity 10-fold are identical with the receptor affinities reported herein for analogs **1–3**. However, it is intriguing that our findings show the presence of this differential receptor affinity regardless of the location of the double/triple bond and length of the side chain. Although molecular modeling studies are beyond the scope of the present investigation, it would appear that electrostatic charges are more crucial than conformational constraint induced by either double or triple bonds.

A notable distinction between the present study and most previous ones is the opportunity to relate pharmacological selectivity, potency, and efficacy to receptor affinity. In a preliminary study, we had reported that analogs 2-hexenyl, 2-octenyl, and 2-nonenyl all had high receptor affinity but pharmacological potencies that were lower than expected (Ryan et al., 1995). The correlation between pharmacological potency and receptor affinity for all agonists and partial agonists in this study (Fig. 5) was considerably lower than that reported previously for a series of bicyclic cannabinoids (Compton et al., 1993). In order to identify the contribution that each group of analogs made to the relationship between potency and receptor affinity, additional correlation analyses were carried out (Table 4). The addition of a substituent at the terminal end of the side chain reduced the correlation between affinity and antinociceptive potency considerably but had relatively little influence on the correlation with the other two pharmacological effects. When considering the degree of unsaturation, the correlation between antinociceptive potency and receptor affinity was also poorer with triple bond analogs (Table 4). However, the influence on the correlation between receptor affinity and depression of spontaneous activity was affected the most dramatically, there being almost no correlation when there was a triple bond in the side chain.

The demonstration that affinity for [^{3}H]CP 55,940 binding was highly correlated with the three behavioral measures discussed herein provided a model for characterizing the

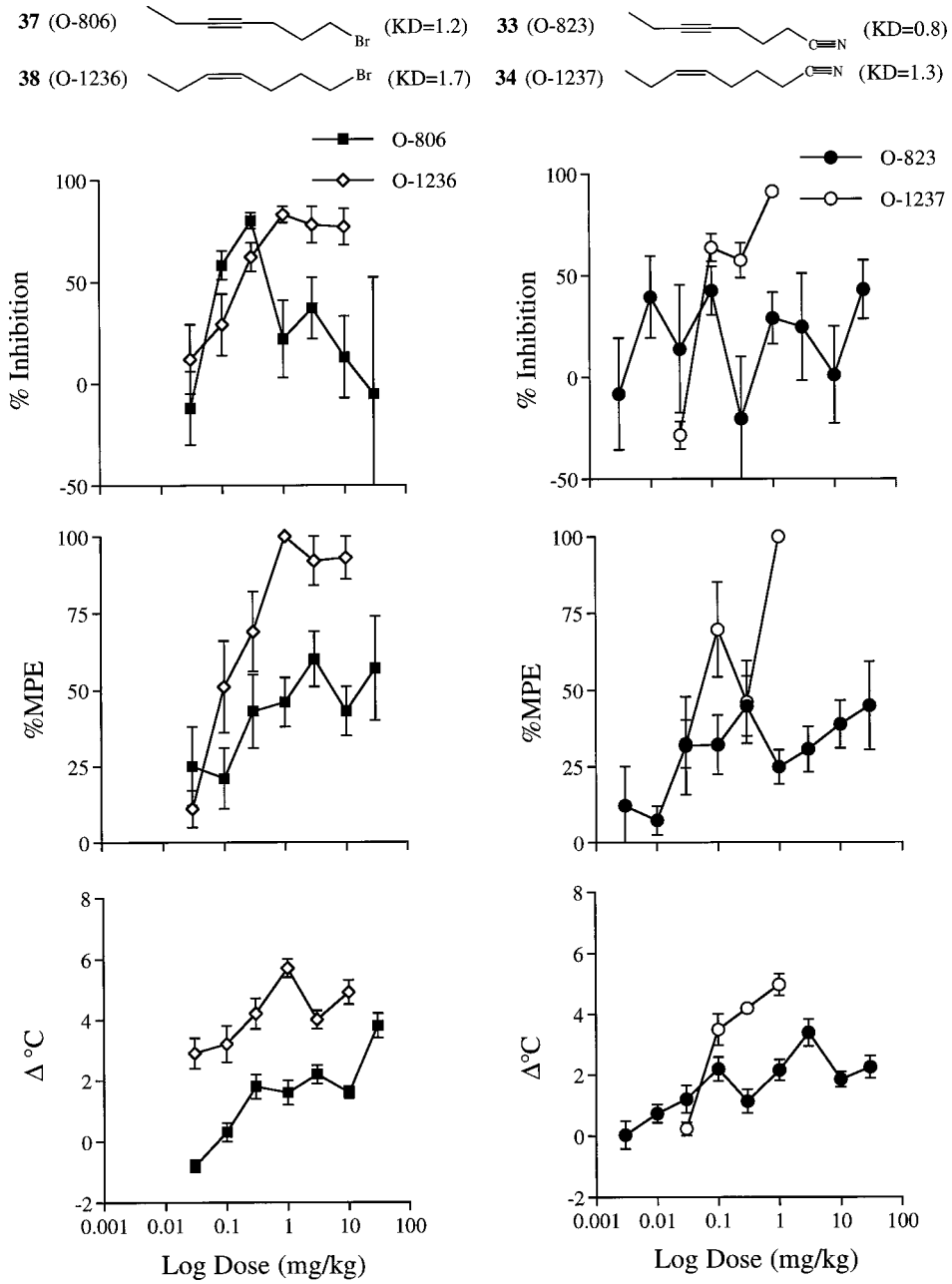


Fig. 4. Comparison of C2–C3 double- and triple-bond side chains with bromo and cyano terminal substitutions. Inhibition of spontaneous activity, antinociception, and hypothermia is presented in the top, middle, and bottom panels, respectively. All data are presented as means ± S.E. for at least six mice per group.

pharmacological properties of the cannabinoid receptor, presumably the CB_1 receptor, while at the same time providing a means for identifying ligands with selective pharmacological profiles. It is intriguing that the correlation between any two pharmacological effects exceeds that between the pharmacological effect and receptor affinity (Table 4). However, this analysis is somewhat skewed by the fact that analogs producing effects in one assay but not in another are eliminated from the comparison. Closer inspection of the data reveals that there are indeed several analogs with some pharmacological selectivity. In an earlier study, it was noted that the 2′-acetylenic compounds appeared to exhibit some pharmacological selectivity with regard to antinociception (Ryan et al., 1995). With the exception of an occasional compound, the potency differences in the four mouse behavioral assays always differ by less than 10-fold for cannabinoid agonists (Martin et al., 1987). In the present study, analogs with double bonds in the side chain exhibited little or no selectivity in these pharmacological assays. In contrast, placing an acetylene at C2′–C3′ in the octyl and nonyl side chains favored antinociception by as much as 70-fold. Acetylenes at any of the other positions did not result in significant selectivity. This antinociceptive selectivity was largely eliminated with the incorporation of an additional functional group in the terminal end of a C2–C3 acetylenic side chain. Actually, there appears to be a trend toward greater depression of spontaneous activity than for either antinociception or hypothermia for these latter side chain derivatives; however, this generalization should be viewed cautiously because of the low efficacy of these compounds.

In addition to their attenuated pharmacological potency, the acetylenic derivatives are unique with regard to efficacy. It is important to point out that these pharmacological tests are not readily amenable to efficacy determinations because

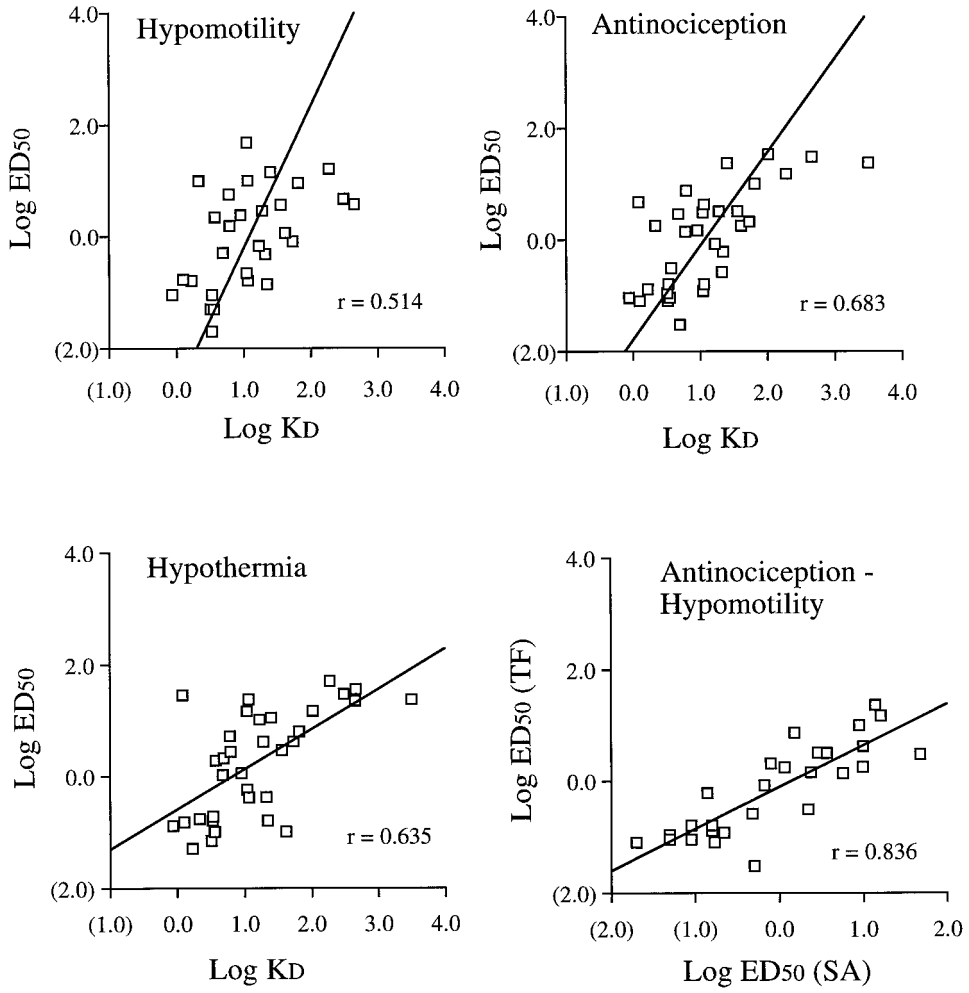


Fig. 5. Correlation between hypomotility potency and receptor affinity (upper left), antinociceptive potency and receptor affinity (upper right), hypothermia potency and receptor affinity (lower left), and antinociceptive and hypomotility potencies (lower right) for all analogs.

TABLE 4
Correlation between receptor affinity and pharmacological effects of agonists and partial agonists

Class	Analog	*N*	Correlation	Correlation Coefficient
Unsubstituted	**1–17**	15	Receptor affinity—spontaneous activity	0.586
Substituted	**18–43**	14	Receptor affinity—spontaneous activity	0.475
All	**1–43**	28	Receptor affinity—spontaneous activity	0.514
Unsubstituted	**1–17**	15	Receptor affinity—tail-flick activity	0.812
Substituted	**18–43**	17	Receptor affinity—tail-flick activity	0.635
All	**1–43**	32	Receptor affinity—tail-flick activity	0.683
Unsubstituted	**1–17**	15	Receptor affinity—rectal temperature	0.523
Substituted	**18–43**	19	Receptor affinity—rectal temperature	0.680
All	**1–43**	34	Receptor affinity—rectal temperature	0.635
Unsubstituted	**1–17**	15	Spontaneous activity—tail flick activity	0.802
Substituted	**18–43**	12	Spontaneous activity—tail flick activity	0.889
All	**1–43**	27	Spontaneous activity—tail flick activity	0.836
Unsubstituted	**1–17**	15	Tail-flick activity—rectal temperature	0.645
Substituted	**18–43**	18	Tail-flick activity—rectal temperature	0.853
All	**1–43**	32	Tail-flick activity—rectal temperature	0.774
Unsubstituted	**1–17**	15	Spontaneous activity—rectal temperature	0.887
Substituted	**18–43**	14	Spontaneous activity—rectal temperature	0.708
All	**1–43**	28	Spontaneous activity—rectal temperature	0.772
Double bonds		12	Receptor affinity—spontaneous activity	0.677
Triple bonds		17	Receptor affinity—spontaneous activity	0.129
Double bonds		12	Receptor affinity—tail flick	0.810
Triple bonds		18	Receptor affinity—tail flick	0.600
Double bonds		12	Receptor affinity—rectal temperature	0.794
Triple bonds		22	Receptor affinity—rectal temperature	0.465

of the truncate nature of the models. The lack of irreversible antagonists precludes depletion of spare receptors that would benefit efficacy comparisons. However, even under these circumstances, several acetylenic derivatives were obviously incapable of eliciting maximal responses, particularly the 2′-hexynyl and 3′-octynyl analogs. There is always the possibility that pharmacokinetics could play a role. However, all these analogs are highly lipophilic and should readily penetrate the central nervous system. Moreover, analogs such as O-806 and O-1020 produced biphasic effects, i.e., diminished effects at higher doses, which argues against pharmacokinetics as an explanation for the less than maximal effects of these particular compounds.

THC derivatives have been prepared with structural modifications in the terminal end of the side chain. Several halogen, azido, and amino substitutions have been made with relatively modest effects on pharmacological profile and receptor affinity (Charalambous et al., 1991, 1992; Martin et al., 1993). In contrast, placement of a cyano or carboxamido group in this position in a dimethylheptyl side chain tends to enhance potency (Singer et al., 1998). Similar additions to acetylenic side chains augmented the unique characteristics of these compounds. These multiple alterations, such as the 6-cyano-2-hexynyl analog, **33,** led to further reduction in efficacy so that partial agonistic activity was definitive.

Establishing structural criteria based upon correlations between potency and receptor affinity was complicated in the present study because of the unusual finding that more than a third of the high-affinity analogs failed to produce significant pharmacological effects. The low efficacy of these high-affinity ligands prompted us to evaluate some of these compounds for antagonistic activity. In an earlier study, we found that the 6-cyano-2-hexynyl analog antagonized the effects of cannabinoids in the guinea pig ileum (Pertwee et al., 1996). In a follow-up study, we examined four 6-substituted hexynyl (O-806, O-823, O-1176, and O-1184) analogs and the 2-octynyl analog O-584 in the GTPγS binding assay and found them to be devoid of agonist effects (Griffin et al., 1999). However, these compounds were effective antagonists in that they blocked the agonist effects of several potent cannabinoids in this assay. Unfortunately, these analogs were not very effective in blocking the pharmacological effects of Δ^9-THC in vivo (data not presented). Pretreatment with low doses of these compounds was without efficacy on the in vivo effects of THC in mice, whereas high doses tended to augment, rather than diminish, the effects of Δ^9-THC. It appears that these compounds have sufficient, albeit very weak, agonist effects that mask their antagonistic effects.

The side chain derivatives are the first compounds structurally related to THC that have partial agonist/antagonist properties. At present, an explanation for these unique effects is lacking. As for the pharmacological selectivity exhibited by some analogs, there are several possible explanations. It is reasonable to speculate that multiple transduction pathways for the CB1 may account for agonist specificity. This notion is supported by recent suggestions of CB1 receptor coupling to both G_S and $G_{i/o}$ proteins (Glass and Felder, 1997), a notion that is consistent with earlier findings that cannabinoids both stimulate and inhibit cAMP production (Howlett and Fleming, 1984). Conversely, there is the possibility that these agonists are interacting with as-yet-unidentified receptors. It seems unlikely that these derivatives are acting at CB2 receptors because of their questionable presence in brain and the fact that cannabinoids that bind selectively to CB2 receptors do not produce hypoactivity, antinociception, and hypothermia (our unpublished observations).

In conclusion, unsaturation in the side chain produces dramatic effects on both receptor affinity and pharmacological potency. Introduction of double bonds at several locations increased both receptor affinity and pharmacological potency, whereas incorporation of a triple bond in the side chain produced less predictable results. Pharmacological selectivity also resulted from placing an acetylene at C2′–C3′ in the octyl and nonyl side chains, which favored antinociception by as much as 70-fold. The combination of substitutions at the terminal end of the side chain, particularly a cyano group, resulted in either partial agonists or antagonists. These analogs provide new opportunities for exploring receptor subtypes as well as receptor-effector coupling.

References

Busch-Petersen J, Hill WA, Fan P, Khanolkar A, Xie X, Tius MA and Makriyannis A (1996) Unsaturated side chain β-11-hydroxyhexahydrocannabinol analogs. *J Med Chem* **39:**3790–3796.

Charalambous A, Lin S, Marciniak G, Banijamali A, Friend FL, Compton DR, Martin BR and Makriyannis A (1991) Pharmacological evaluation of halogenated Δ^9-THC analogs. *Pharmacol Biochem Behav* **40:**509–512.

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, Gold LH, Ward SJ, Balster RL and Martin BR (1992a) Aminoalkylindole analogs: Cannabimimetic activity of a class of compounds structurally distinct from Δ^9-tetrahydrocannabinol. *J Pharmacol Exp Ther* **263:**1118–1126.

Compton DR, Johnson MR, Melvin LS and Martin BR (1992b) Pharmacological profile of a series of bicyclic cannabinoid analogs: Classification as cannabimimetic agents. *J Pharmacol Exp Ther* **260:**201–209.

Compton DR, Rice KC, De Costa BR, Razdan RK, Melvin LS, Johnson MR and Martin BR (1993) Cannabinoid structure-activity relationships: Correlation of receptor binding and in vivo activities. *J Pharmacol Exp Ther* **265:**218–226.

Devane WA, Dysarz FA, Johnson MR, Melvin LS and Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* **34:**605–613.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A and Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258:**1946–1949.

Dewey WL (1986) Cannabinoid pharmacology. *Pharmacol Rev* **38:**151–178.

Dewey WL, Harris LS, Howes JF and Nuite JA (1970) The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. *J Pharmacol Exp Ther* **175:**435–442.

Glass M and Felder CC (1997) Concurrent stimulation of cannabinoid CB1 and Dopamine D2 receptors augments cAMP accumulation in striatal neurons: Evidence for a G_s linkage to the CB1 receptor. *J Neurosci* **17:**5327–5333.

Griffin G, Wray EJ, Rorrer WK, Crocker PJ, Ryan WJ, Saha B, Razdan RK, Martin BR and Abood ME (1999) An investigation into the structural determinants of cannabinoid receptor ligand efficacy. *Br J Pharmacol* **126:**1575–1584.

Howlett AC (1984) Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nantradol compounds. *Life Sci* **35:**1803–1810.

Howlett AC and Fleming RM (1984) Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Mol Pharmacol* **26:**532–538.

Howlett AC, Johnson MR, Melvin LS and Milne GM (1988) Nonclassical cannabinoid analgetics inhibit adenylate cyclase: Development of a cannabinoid receptor model. *Mol Pharmacol* **33:**297–302.

Little PJ, Compton DR, Mechoulam R and Martin BR (1989) Stereochemical effects of 11-OH-dimethylheptyl-Δ^8-tetrahydrocannabinol. *Pharmacol Biochem Behav* **32:** 661–666.

Loev B, Bender PE, Dowalo F, Macko E and Fowler PJ (1973) Cannabinoids: Structure-activity studies related to 1,2-dimethylheptyl derivatives. *J Med Chem* **16:**1200–1206.

Mackie K and Hille B (1992) Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci USA* **89:**3825–3829.

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, Compton DR, Little PJ, Martin TJ and Beardsley PM (1987) Pharmacological evaluation of agonistic and antagonistic activity of cannabinoids, in *Structure-Activity Relationships of Cannabinoids* (Rapaka RS and Makriyannis A eds) pp 108–122, U.S. Government Printing Office, Washington, DC

McOmie JFW and West DE (1973) 3,3′-Dihydroxybiphenyl, *in Organic Syntheses Collective* (Baumgarten HE ed) vol 5, pp 412–414, John Wiley & Sons, Inc., New York.

Mechoulam R and Edery H (1973) Structure-activity relationships in the cannabinoid series, in *Marijuana: Chemistry, Pharmacology, Metabolism, and Clinical Effects* (Mechoulam R ed) pp 101–136, Academic Press, New York.

Melvin L, Milne G, Johnson M, Subramaniam B, Wilken G and Howlett A (1993) Structure-activity relationships for cannabinoid receptor-binding and analgesic activity: Studies of bicyclic cannabinoid analogs. *Mol Pharmacol* **44:**1008–1015.

Pacheco M, Childers SR, Arnold R, Casiano F and Ward SJ (1991) Aminoalkylindoles: Actions on specific G-protein-linked receptors. *J Pharmacol Exp Ther* **257:**170–183.

Pertwee RG, Fernando SR, Griffin G, Ryan W, Razdan RK, Compton DR and Martin BR (1996) Agonist-antagonist characterization of 6′-cyanohex-2′-yne-Δ^8-tetrahydrocannabinol in two isolated tissue preparations. *Eur J Pharmacol* **315:** 195–201.

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

Razdan RK, Dalzell HC and Handrick GR (1974) Hashish. A simple one-step synthesis of (−)-Δ^1-tetraydrocannabinol (THC) from p-mentha-2,8-dien-1-ol and olivetol. *J Am Chem Soc* **96:**5860.

Razdan RK, Dalzell HC, Herlihy P and Howes JF (1976) Hashish: Unsaturated side-chain analogues of Δ^8-tetrahydrocannabinol with potent biological activity. *J Med Chem* **19:**1328–1330.

Reggio PH, McGaughey GB, Odear DF, Seltzman HH, Compton DR and Martin BR (1991) A rational search for the separation of psychoactivity and analgesia in cannabinoids. *Pharmacol Biochem Behav* **40:**479–486.

Ryan W, Singer M, Razdan R, Compton D and Martin B (1995) A novel class of potent tetrahydrocannabinols (THCS): 2′-YNE-Δ^8- and Δ^9-THCS. *Life Sci* **56:**2013–2020.

Ryan WJ, Banner WK, Wiley JL, Martin BR and Razdan RK (1997) Potent anandamide analogs: The effect of changing the length and branching of the end pentyl chain. *J Med Chem* **40:**3617–3625.

Seltzman HH, Fleming DN, Thomas BF, Gilliam AF, McCallion DS, Pertwee RG, Compton DR and Martin BR (1997) Synthesis and pharmacological comparison of dimethylheptyl and pentyl analogs of anandamide. *J Med Chem* **40:**3626–3634.

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

Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R and Martin BR (1994) The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. *J Pharmacol Exp Ther* **270:**219–227.

Thomas BF, Adams IB, Mascarella SW, Martin BR and Razdan RK (1996) Structure-activity analysis of anandamide analogs: Relationship to a cannabinoid pharmacophore. *J Med Chem* **39:**471–479.

Thomas BF, Compton DR, Martin BR and Semus SF (1991) Modeling the cannabinoid receptor: A three-dimensional quantitative structure-activity analysis. *Mol Pharmacol* **40:**656–665.

Wiley JL, Compton DR, Dai D, Lainton JAH, Phillips M, Huffman JW and Martin BR (1998) Structure-activity relationships of indole- and pyrrole-derived cannabinoids. *J Pharmacol Exp Ther* **285:**995–1004.

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