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Assessment of structural commonality between tetrahydrocannabinol and anandamide

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Abstract

In order to make further structural comparisons between tetrahydrocannabinol and anandamide, substituents at C1 and C3 of the phenolic ring of tetrahydrocannnabinol were altered. In order to examine the alignment of the phenolic hydroxyl of tetrahydrocannnabinol with the hydroxyl group of anandamide, 1-fluoro-1-deoxy-tetrahydrocannnabinol analogs were prepared. These analogs had low affinity for the CB_1 cannabinoid receptor and were considerably less potent than tetrahydrocannnabinol in producing pharmacological effects in mice. These results suggest that these two oxygen moieties do not overlap. Additionally, the fact that a fluorine group can only accept hydrogen bonds suggest that the phenolic oxygen at the C1 position of tetrahydrocannnabinol donates electrons for hydrogen bonding rather than the hydrogen of the hydroxyl group interacting with the receptor. Additionally, substitution of a fluorine for the hydroxyl group at C1 led to analogs with higher affinity for CB_2 than CB_1 cannabinoid receptors, thereby underscoring a fundamental difference in the binding properties of these two receptor subtypes.

Keywords: Cannabinoid; Anandamide; Tetrahydrocannabinol; Structure–activity relationship; Receptor

1. Introduction

The characterization of cannabinoid receptor binding in rodent brain (Devane et al., 1988) and the subsequent cloning of the CB_1 cannabinoid receptor (Matsuda et al., 1990) provided some of the first direct evidence that the myriad effects of tetrahydrocannabinol are mediated through an endogenous cannabinoid system. However, the isolation and identification of anandamide provided the last crucial element for such a system (Devane et al., 1992). Since its elucidation, anandamide has been shown to mimic the effects on tetrahydrocannnabinol in both in vitro and in vivo systems. It binds to both CB_1 and CB_2 cannabinoid receptors, and it produces tetrahydrocannnabinol-like effects in mice (Fride and Mechoulam, 1993; Smith et al., 1994), rats (Jarbe et al., 1998; Wiley et al., 1995) and dogs (Lichtman et al., 1998), to name just a few studies.

Despite these common pharmacological properties, the structures of tetrahydrocannnabinol and anandamide are dramatically different, with the most notable distinction being that the former is a rigid compound and the latter is highly flexible. There has been intense interest in determining whether a common pharmacophore exists for these two cannabinoids. There has been speculation that a pentyl group attached to an sp2-hybridized carbon is essential for both anandamide and tetrahydrocannnabinol. This notion has been supported by several structure–activity relationship studies that revealed similar changes in the pentyl side chain of tetrahydrocannnabinol and the terminal alkyl position of anandamide led to parallel changes in biological activity (Ryan et al., 1997; Seltzman et al., 1997). Similarly, it has been proposed that the phenolic hydroxyl and the pyran oxygen of tetrahydrocannnabinol can be aligned with the hydroxyl group and carbonyl oxygen of anandamide, respectively (Thomas et al., 1996). This conclusion was based upon a reasonable comparative molecular field analysis correlation between tetrahydrocannnabinol and anandamide analogs when these oxygen atoms and pentyl (or terminal

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aliphatic) groups were superimposed (Thomas et al., 1996). We reasoned that if the hydroxyl groups of both compounds represent a common pharmacophore, then similar substitutions on both tetrahydrocannnabinol and anandamide should produce similar changes in pharmacological potency and receptor affinity. We have previously demonstrated that substituting a fluorine atom for the hydroxyl group in anan-damide increased CB_1 cannabinoid binding affinity approximately 10-fold (Adams et al., 1998). Therefore, we prepared 1-fluoro-tetrahydrocannnabinol to determine whether it would have similar influence on tetrahydrocannnabinol. However, it should be pointed out that the anandamide hydroxyl moiety is not essential for pharmacological activity, because an *N*-alkyl anandamide analogs are also active (Pinto et al., 1994; Sheskin et al., 1997).

An additional rationale for preparing 1-fluoro-tetrahydrocannnabinol arises from the recently reported selectivity of 1-deoxy-tetrahydrocannnabinol's and 1-alkoxy-tetrahydrocannnabinol's for the CB_2 cannabinoid receptor (Gareau et al., 1996; Huffman et al., 1996; Reggio et al., 1997). Additional substitutions at the C1 position may provide additional insight into the determinants for CB_1 and CB_2 cannabinoid receptor selectivity. A second strategy for assessing the potential overlap of the hydroxyl groups in tetrahydrocannnabinol and anandamide was to transpose the phenolic hydroxyl and side-chain groups of tetrahydrocannnabinol.

2. Materials and methods

2.1. Synthesis

The synthesis of compounds discussed in this paper was carried out using the standard methodology for the synthesis of tetrahydrocannnabinol analogs described by us previously (Crocker et al., 1999). This involved synthesizing the appropriate resorcinol precursors followed by condensation with *cis*-*p*-menth-2-ene-1,8-diol to give a mixture of isomeric products from which the desired analogs were isolated by silica gel chromatography. The fluorinated analogs (**4** to ***7***) were synthesized from 1-fluoro-3,5-dimethoxybenzene after demethylation with boron tribromide to give the corresponding resorcinol. Condensation of this 5-fluororesorcinol with ***cis***-*p*-menth-2-ene-1,8-diol furnished a mixture, from which two isomers were isolated in approximately equal amounts (~ 10% each). These isomers differed by the relative substitution pattern on the aromatic tetrahydrocannnabinol ring, one isomer being the normal 3-fluoro-1-hydroxy-Δ^8-tetrahydrocannnabinol and the other isomer being the abnormal 1-fluoro-3-hydroxy-Δ^8-tetrahydrocannnabinol. The latter isomer was then activated as a triflate and then subjected to a palladium (0)-catalyzed coupling with 1-heptyne to afford 1-deoxy-1-fluoro-3-(1-heptynyl)-Δ^8-tetrahydrocannnabinol (**5**). This in turn was reduced to both the corresponding ***cis***-alkene **6** using Lindlar's catalyst (one drop quinoline/reagent ethanol) and the alkane ***4*** (5% Pd/C/reagent ethanol) in both cases without reducing the Δ^8-double bond. Similarly, treatment of 3-fluoro-1-hydroxy-Δ^8-tetrahydrocannnabinol (as the triflate) with 1-heptyne in the presence of Pd(0) gave compound ***7***. Compound ***11*** was isolated from the tetrahydrocannnabinol mixture obtained in the synthesis of compound ***10***. Treatment of ***11*** with NaN_3/dimethyl sulfoxide (DMSO) formed the azide ***14*** as in the preparation of ***13***. Compound ***15*** was synthesized from ***10*** by treatment with $CH_3I/K_2CO_3/CH_3CN$, to give the corresponding 1-methoxy tetrahydrocannnabinol, followed by treatment with NaN_3/DMSO. Reduction of the acetylene in ***15*** (5% Pd/$BaSO_4$) formed the ***cis***-alkene **16**. Compound ***19*** was isolated during the synthesis of ***18*** as in the preparation of ***11***. The 1-deoxy analog ***20*** was prepared from ***18*** using a literature procedure for deoxygenation of phenols (Chen et al., 1986; Peterson et al., 1987). Thus, compound ***18***, as its triflate, was treated with $Pd(PPh_3)_4$/HCOOH in the presence of Et_3N/DMF at 60 °C for 16 h to furnish the deoxy compound ***20***.

All reactions used in the preparation of these analogs have been described previously (Peterson et al., 1987). All compounds showed appropriate ^{1}H-nuclear magnetic resonances (JEOL Eclipse 300 Hz) and were characterized on the basis of their ^{1}H-nuclear magnetic resonances and elemental analyses.

2.2. Pharmacological assays

ICR male mice (Harlan Laboratories, Indianapolis, IN) were maintained on a 14:10-h light/dark cycle with free access to food and water. SR 141716A (*N*-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide·HCl), Δ^8- and Δ^9-tetrahydrocannnabinol were obtained from the National Institute on Drug Abuse (Bethesda, MD). Cannabinoids were dissolved in a 1:1:18 mixture of ethanol, emulphor and saline for i.v. administration. Mice received the analogs by tail-vein injection and then were evaluated for their ability to produce hypomotility, hypothermia, and antinociception as described previously (Little et al., 1989). These pharmacological measures were determined in the same mouse at a time when maximal activity was present. At least six animals were treated with each dose. ED_{50} values were determined from least-squares unweighted linear regression analysis of the log dose–response plots.

2.3. Receptor binding

[^{3}H]CP-55,940 {(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol} was obtained from Amersham Pharmacia Biotech (Piscataway, NJ). [^{3}H]CP-55,940 binding to P_2 membranes prepared from whole brain was used to determine CB_1 cannabinoid receptor affinity as described earlier (Compton et al., 1993). CB_2 cannabinoid receptor affinity was estab-

lished with [^{3}H]CP-55,940 binding to P2 membranes prepared from human embryonic kidney 293 cells transfected with CB_2pcDNA3 as described recently (Huffman et al., 2001). Displacement curves were generated by incubating drugs with 1 nM of [^{3}H]CP-55,940. The addition of 1 μM unlabelled CP-55,940 was used to assess nonspecific binding. 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 using EBDA (Equilibrium Binding Data Analysis; Biosoft, Milltown, NJ).

3. Results

We had reported earlier (Martin et al., 1999) that substitution of a heptyl group (analog **1**) for the pentyl side chain of tetrahydrocannnabinol increased both CB_1 cannabinoid receptor affinity and pharmacological potency as illustrated in Table 1. When rigidity was imposed on the heptyl side chain by introducing either a triple bond (analog ***2***) or a double bond (analog ***3***), the result was a respective decrease and increase in CB_1 cannabinoid receptor affinity and pharmacological potency. Substitution of a fluorine for the phenolic hydroxyl in 3-heptyl-Δ^8-tetrahydrocannnabinol (***1***) led to analog ***4*** and a substantial reduction in CB_1 cannabinoid receptor affinity and pharmacological activity. However, this fluorine derivative was a weak partial agonist in depressing spontaneous activity and producing antinociception and a full agonist for producing hypothermia. The same C1-fluorine substitution in the heptynyl analog (***2***) led to analog ***5*** that had a CB_1 cannabinoid receptor affinity greater than 10,000 nM and no pharmacological activity at a dose of 30 mg/kg. Likewise, the 1-fluorine analog (***6***) of heptenyl-Δ^8-tetrahydrocannnabinol (analog ***3***) had a 300-fold reduction in CB_1 cannabinoid receptor affinity. Despite this dramatic reduction in receptor affinity, analog ***6*** was capable of producing in vivo cannabinoid effects. It was approximately 30–60 times less potent than the corresponding analog (***3***) that contained a phenolic hydroxyl group. However, it did produce maximal antinociception and hypothermia.

Transposing the C1 fluorine and C3 heptynyl group (analog **7**) restored neither CB_1 cannabinoid receptor affinity nor pharmacological activity. In the hexyl side chain series (analogs ***8***–***11***), we reported previously that a C2 acetylene with (analog ***10***) or without (analog ***9***) a bromine substitution on the terminal carbon atom increased receptor affinity while decreasing pharmacological potency and efficacy (Martin et al., 1999). In the present study, however, interchanging the 6′-bromo-2′-hexynyl moiety and the hydroxyl groups reduced receptor affinity more than 600-fold and dramatically reduced pharmacological activity. Altering the terminal substituent (azido) on the side chain did not effect this outcome (analogs ***12***–***14***).

Replacement of the phenolic hydroxyl with a methoxy group represents another strategy for assessing C1 substitutions. By converting the 6′-azido-hexynyl analog (**12**) to the corresponding C1 methoxy derivative ***15***, CB_1 cannabinoid receptor affinity was reduced approximately 40-fold with a corresponding decrease in potency. The methoxy group (in analog ***16***) exerted an even greater influence on the hexenyl analog ***13*** in that the resulting analog 16 had 70-fold decrease in receptor affinity and elicited only 44% reduction in spontaneous activity and 16% antinociception at a dose of 30 mg/kg.

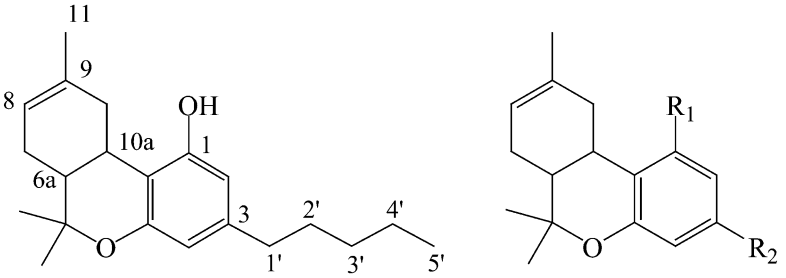
The last series of compounds involved phenolic hydroxyl alterations in compounds with an octyl side chain (analog **17**). These compounds were chosen because of their similarity to the hexyl series. Introduction of a triple bond in this eight-carbon side chain did not reduce receptor affinity but led to a compound (analog ***18***) that exhibited differing potencies in the pharmacological measures in mice. Transposing the 2′-octynyl and phenolic hydroxyl groups (analog ***19***) reduced receptor affinity 70-fold and less than 50% pharmacological activity could be detected at a 30 mg/kg dose. When the phenolic hydroxyl group of analog ***18*** was merely eliminated, the resulting analog ***20*** was 10, 30 and 200 times less potent in hypothermic, spontaneous activity and antinociceptive measures, respectively. However, it is important to point out that this deoxy analog was capable of producing pharmacological effects in this cannabinoid model.

The 1-fluoro-analogs were also evaluated for CB_2 cannabinoid receptor affinity. The 1-fluoro-analogs with heptyl (O-1191), heptynyl (O-1189) and heptenyl (O-1190) side chains exhibited greater receptor affinity for CB_2 cannabinoid receptors. The corresponding heptenyl analog with a C1 hydroxyl had high affinity for both CB_1 and CB_2 cannabinoid receptors that was also comparable. On the other hand, the heptynyl analog with a C1 hydroxyl exhibited 10-fold selectivity for CB_1 cannabinoid receptors. The 6-azido-2-hexynyl (O-1184) and hexenyl (O-1238) analogs were found to have high affinity for both receptor subtypes. However, substitution of a methoxy group for the hydroxyl at C1 in these two analogs resulted in analogs O-2027 and O-2028 that demonstrated modest selectivity for the CB_2 cannabinoid receptor.

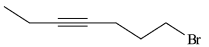
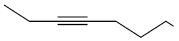
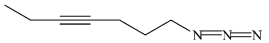
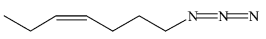
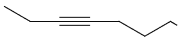
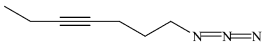
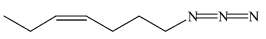
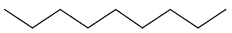
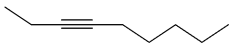
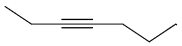
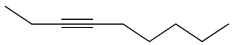
4. Discussion

Early structure–activity relationship findings led Binder and Franke (1982) to formally propose a receptor model with the minimal three point attachments being an alkyl at C9, an hydroxyl at C1, and a C3 alkyl group. Since that time considerable attention has been directed toward these functional groups. There have been several attempts to determine whether the C1 hydroxyl group serves as an electron donor or acceptor (Reggio et al., 1989, 1990; Semus and Martin, 1990). The fact that a fluorine atom can only accept hydrogen bonds prompted us to substitute a fluorine for the phenolic hydroxyl group. This substitution resulted in

Table 1
Influence of C1-substitutions and side-chain modifications on pharmacological potency and selectivity[a]



No.[b]	Chemical name	R1	R2	CB1 KD (nM)	CB2 KD (nM)	SA	TF	RT
						ED50 (mg/kg)		
	Δ^9-THC[c]	OH		41±1.7		1.1 94(30)	1.1 100(30)	1.0 -5.4(10)
	Δ^8-THC[c]	OH		45±12		3.0 94(30)	2.6 100(30)	5.9 -4.8(30)
1 JWH 091	3-Heptyl-Δ^8-THC[c]	OH		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[c]	OH		36±0.8	444±37	3.69 74(30)	3.25 100(30)	2.97 -5.6(10)
3 O-1317	*cis*-3-(1'-Heptenyl)-Δ^8-THC[c]	OH		0.86±0.09	1.80±0.04	0.09 87(1)	0.09 100(1)	0.13 - 6.3(1)
4 O-1191	1-Fluoro-1-deoxy-3-heptyl-Δ^8-THC	F		1560±203	45±3.2	31 45(30)	21.5 63(30)	6.9 -5.2(30)
5 O-1189	1-Fluoro-1-deoxy-3-(1-heptynyl)-Δ^8-THC	F		>10,000	1640±95	 8(30)	 4(30)	 -2.2(30)
6 O-1190	1-Fluoro-1-deoxy-3-(1'-heptenyl)-Δ^8-THC	F		284±35	39.5±7.9	0.51 74(30)	5.8 100(30)	4.1 -6.6(30)
7 O-1188	1-Deoxy-1-(1'-heptynyl)-3-fluoro-Δ^8-THC		F	>10,000		 27(30)	 12(0)	 -2.4(30)
8 JWH 124	3-Hexyl-Δ^8-THC[c]	OH		41±3.8		1.16 91(10)	1.79 98(10)	0.10 -5.3(10)
9 O-615	3-(2'-Hexynyl)-Δ^8-THC[c]	OH		11±1.0		47.7 63(100)	3.08 71(10)	14.8 -3.2 (100)

10 O-806	3-(6'-Bromo-2'-hexynyl)-Δ^8-THC[c]	OH		1.2±0.1		0(30)	4.78 60(30)	29 -3.8(30)
11 O-1786	1-Deoxy-1-(6'-bromo-2'-hexynyl)-3-hydroxy-Δ^8-THC		OH	684±142		10(30)	1(30)	-0.7(30)
12 O-1184	3-(6-Azido-2-hexynyl)-Δ^8-THC[c]	OH		2.14±0.44	1.12±0.02	9.8 64(10)	1.8 84(30)	0.17 -3.3(30)
13 O-1238	*cis*-3-(6-Azido-2-hexenyl)-Δ^8-THC[c]	OH		3.32±0.59	1.90±0.14	0.02 75(1)	0.08 100(1)	0.16 -6.1(1)
14 O-1807	1-Deoxy-1-(6'-azido-2'-hexenyl)-3-hydroxy-Δ^8-THC		OH	315±39		59(30)	13(30)	-0.6(30)
15 O-2027	1-Methoxy-3-(6'-azido-2'-hexynyl)-Δ^8-THC	OCH3		82.5±3.86	11.2±0.14	63(30)	33.4 61(30)	31/3 -3.0(30)
16 O-2028	1-Methoxy-3-(6'-azido-2'-hexenyl)-Δ^8-THC	OCH3		247±14	18.8±2.18	44%@30	16%@30	0@30
17 JWH 138	3-Octyl-Δ^8-THC[c]	OH		8.5±1.4		0.39 82(3)	0.34 100(3)	0.24 -6.6(3)
18 O-584	3-(2'-Octynyl)-Δ^8-THC[c]	OH		4.9±2.0		0.51 89(10)	0.03 97(10)	2.14 -4.2(10)
19 O-1316	1-Deoxy-1-(2'-octynyl)-3-hydroxy-Δ^8-THC		OH	337±37		43(30)	34(30)	-2.4(30)
20 O-1315	1-Deoxy-3-(2'-octenyl)-Δ^8-THC			172±28		15.6 55(30)	7.85 91(30)	22.5 -3.1(30)

[a]Spontaneous activity (SA), tail-flick response (TF), and rectal temperature (RT) are expressed as ED50 values (mg/kg). A indicates that calculation of an ED50 was not possible. The maximal effect with the dose in parenthesis is given under the ED50 value. The KD values represent means ± S.E. of at least three separate experiments.

[b]Listed below the boldface analog number is the reference number assigned by either Organix (O) or by Dr. John Huffman (JWH).

[c]Reported previously (Martin et al., 1999).

a dramatic reduction in both CB_1 cannabinoid receptor affinity and pharmacological potency which suggests that the C1 hydroxyl group must be able to donate electrons for hydrogen bonding rather than the hydrogen of the hydroxyl group binding to the receptor. Fluorine's van der Waals radius (1.47 Å) lies between oxygen's (1.57 Å) and hydrogen's (1.2 Å). Therefore, steric consequences of this atom substitution would not be anticipated to be sufficient to explain the decrease in pharmacological activity. With regard to electrostatic or hydrogen bond interactions, there are two effects of the substitution of fluoride for the phenolic hydroxyl that could preclude receptor interactions that promote high affinity binding. The first is the absence of the phenolic proton in the fluoro analog, which would prevent the molecule from acting as a hydrogen donor. (e.g., hydrogen bonding or electrostatic interactions). The alternative possibility is based upon the assumption that electronegativity of the hydrogen acceptor atom (fluoride/oxygen) correlates with the strength of the hydrogen bond or electrostatic interaction. Other investigators have discussed the electrostatic effects of fluorine for oxygen substitutions and noted that despite an additional lone pair of electrons, the higher electronegativity and lower polarisibility of fluorine attenuates its electrostatic influence in comparison to oxygen (O'Hagan and Rzepa, 1997). Theoretical calculations discussed by Howard et al. (1996) also indicate the strength of a F···H bond to be between 2 to 3.2 kcal/mol as compared to the O—H hydrogen bond, which is typically between 5 and 10 kcal/mol. Thus, the decrease in biological activity in the Δ^8-tetrahydrocannnabinol analogs could also result from the replacement of an atom capable of strong electrostatic interactions (the oxygen) with an atom with less electrostatic potential (fluorine).

The other impetus for examining the phenolic hydroxyl comes from attempts to devise a common pharmacophore for anandamide and tetrahydrocannnabinol. In our previous model, we had overlaid the hydroxyl and pentyl side chains of both compounds (Thomas et al., 1996). Although a hydroxy group is not essential for anandamide's activity (Pinto et al., 1994; Sheskin et al., 1997), similar alterations at the C1 position of tetrahydrocannabinol and the N-terminus of anandamide represent a logical strategy for exploring structural commonalities between these two compounds. While substitution of a fluorine atom for the terminal hydroxy in anandamide increased CB_1 cannabinoid receptor affinity and pharmacological potency (Adams et al., 1995), the comparable substitution of the phenolic hydroxyl in tetrahydrocannnabinol did not. One would expect similar trends in pharmacological activity to result from the fluorine substitution, particularly when the magnitude in the drop in atom point charges and electrostatic potentials is similar between the two series. Therefore, it would appear that the hydroxyl groups are not binding at a common receptor site. However, it has also been noted in theoretical calculations described by Howard et al. (1996) that the $C(sp^3)$–F···H interaction is stronger than the $C(sp^2)$–F···H interaction, both of which are weaker than O···H hydrogen bonds. Therefore, the lesser ability of the fluorine in the 1-fluoro-1-deoxy-Δ^8-tetrahydrocannnabinol analogs to form as strong a hydrogen bond as the fluorine in the fluoroanandamide, combined with the greater conformational flexibility in the fluoroanandamide analog, suggests that confor- mational accommodation cannot be ruled out. Thus, further studies will need to be performed to determine if the flexible fluoroanandamide analogs can conformationally realign the fluorine and carbonyl groups and reestablish an optimal interaction with the receptor's H-bond donor sites, which may not be possible with the conformationally restrained Δ^8-tetrahydrocannnabinol analogs.

In our model, we had also superimposed the alkyl side chain of tetrahydrocannnabinol and the terminal alkyl groups in anandamide. However, anandamide is so highly flexible that the terminal alkyl chain can assume numerous conformations. Since it is far easier to fix the location of the side chain in tetrahydrocannnabinol, the transposition of the side chain and the phenolic hydroxyl in tetrahydrocannnabinol were carried out. However, this maneuver failed to restore receptor affinity in the fluoro-substituted analogs. Likewise, the C3 hydroxy compounds were inactive regardless of the side chain placed at C1. These findings provide additional insight into the common binding properties of anandamide and tetrahydrocannnabinol.

We purposefully chose analogs with side chains that made them highly potent in order to manipulate the C1 position. The decrease in receptor affinity/pharmacological potency in the C1 methoxy derivatives is consistent with the notion that electron donation is important. The hydrogens in the methoxy are available for bond formation, whereas the lone pair of electrons on the methoxy oxygen are less readily shared compared to those in the hydroxyl group. Of course, we cannot rule out steric bulk as a contributor to the influence of the methoxy group. Certainly, the importance of the C1 hydroxyl group was underscored when a very potent analog became weakly active with the removal of the phenolic hydroxyl, an observation that is well documented (Razdan, 1986). There has also been considerable interest in the structural features of analogs that delineate between receptor recognition and activation. Our earlier report on the introduction of an acetylene into the side chain of tetrahydrocannabinol clearly demonstrated a retention of receptor affinity but a dramatic reduction in potency and efficacy (Martin et al., 1999). Analogs with a double bond tended to have concordant CB_1 cannabinoid receptor affinity and pharmacological potency. The present results suggest that alterations at C1 does not provide any additional separation of receptor recognition and activation. On the contrary, substitution of a fluoro for the C1 hydroxy in compound **6** reduced CB_1 cannabinoid receptor affinity to a greater extent than pharmacological potency. Caution must be used in interpreting results from a single compound. Similar results obtained with a systematic alterations of an unsaturated side chain of 1-fluoro-tetrahydrocannabinoidmight suggest that these com-

pounds are acting at sites other than the CB_1 cannabinoid receptor.

Finally, the discovery of the CB_2 cannabinoid receptor subtype necessitates the development of a new structure–activity relationship with an emphasis on discovering compounds that are selective for either of the cannabinoid receptors. We had previously reported that the C1 hydroxy is not needed for CB_2 cannabinoid receptor affinity (Huffman et al., 1996). The 1-fluoro-tetrahydrocannnabinols do not have greater CB_2 cannabinoid receptor selectivity than the 1-deoxy- and 1-alkoxy-tetrahydrocannnabinol derivatives. However, the substitution of a fluorine for the hydroxyl group at C1 led to analogs with higher affinity for CB_2 than CB_1 cannabinoid receptors, thereby underscoring a fundamental difference in the binding properties of these two receptor subtypes for both tetrahydrocannnabinol and anandamide.

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