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The Role of Fluorine Substitution in the Structure-Activity Relationships (SAR) of Classical Cannabinoids.

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Abstract

A facile synthesis of 1-fluoro-1-deoxy-Δ^8-THC analogs with side-chains seven carbons in length, in the alkane/ene/yne-series (**6**, **5** and **4**), was achieved from 1-fluoro-3,5-dimethoxybenzene (**1**). In vitro studies show that substitution by a fluorine has a significant detrimental effect on CB1 binding which is supported by in vivo testing. The implications of these results on the SAR of classical cannabinoids is discussed.

Keywords

Fluorine substitution; tetrahydrocannabinols; CB1 binding affinity

Fluorine's high electronegativity and small size are among the special properties that contribute to its importance in medicinal chemistry.[1] The effects of fluorine substitution on the biological behaviour of biologically active molecules have been used effectively in drug design, especially after the successful use in steroids and the anticancer drug 5-fluorouracil.[1] As a result, the presence of fluorine in drugs is now quite common. We were therefore interested in examining the role of fluorine substitution in classical cannabinoids. It is well known in the SAR of classical cannabinoids that substitutions in the C-1, C-3 and C-9 positions play an important role[2, 3] in the interaction with CB1 cannabinoid receptors. Hydrogen bonding interactions of the hydroxyl group at C-1 and the presence of a hydroxymethyl at C-9 are of particular interest in this respect. The effect of substituting fluorine for a hydroxyl is especially interesting since the fluorine can only accept hydrogen bonds, whereas hydroxyl groups can both accept and donate hydrogen bonds. Our molecular modeling studies[4] suggested that the phenolic hydroxyl of THC corresponded to the terminal hydroxyl of anandamide. Another

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Selected spectroscopic data; ^{1}HNMR (100 MHz, $CDCl_3$) (a) compound **2**. δ 1.11 (s, 3H), 1.37 (s, 3H), 1.70 (br s, 3H), 2.57–3.07(m, 2H), 4.92 (s, 1H), 5.43 (br d, *J* = 4 Hz, 1H), 6.04–6.23 (m, 2H); (C_6D_6) δ 6.09 (dd, *J* = 12.0, 2.5 Hz, 1H), 6.18 (d, *J* = 3 Hz, 1H); TLC, R_f 0.4 (1:4 EtOAc-hexanes); (b) compound **4**. δ 0.91 (t, *J* = 6 Hz, 3H), 1.09 (s, 3H), 1.38 (s, 3H), 1.71 (br s, 3H), 2.37 (t, *J* = 6.6 Hz, 2H), 2.58–3.07 (m, 2H), 5.43 (br d, *J* = 5 Hz, 1H), 6.52–6.70 (m, 2H); TLC R_f 0.5 (1:19 EtOAc-hexanes); GLC >84%; HRMS (CI) calcd. For $C_{23}H_{30}FO$ (MH+) 341.2281, found 341.2247, Δ = + 3.4 mmu; (c) compound **5**. δ 0.89 (t, *J* = 6 Hz, 3H), 1.12 (s, 3H), 1.39 (s, 3H), 1.72 (br s, 3H), 2.33 (q, *J* = 6 Hz, 2H), 2.6–3.1 (m, 2H), 5.44 (br d, *J* = 4 Hz, 1H), 5.62 (dt, *J* = 12, 7 Hz, 1H), 6.23 (br d, *J* = 12 Hz, 1H), 6.52(dd, *J* = 12.2, 1.6 Hz, 1H), 6.54 (br s, 1H); TLC R_f 0.2 (5 x hexanes); GLC 90%; HRMS (CI) calcd. For $C_{23}H_{32}FO$ (MH^+) 343.2437, found 343.2444 Δ = −0.7 mmu; (d) compound **6**. δ 0.88 (t, *J* = 6 Hz, 3H), 1.11 (s, 3H), 1.28 (br s, 8H), 1.38 (s, 3H), 1.71 (br s, 3H), 2.49 (t, *J* = 7 Hz, 2H), 2.6–3.1 (m, 2H), 5.43 (br d, *J* = 4 Hz, 1H), 6.41 (dd, *J* = 11.9, 1.8 Hz, 1H), 6.44 (br s, 1H); TLC R_f 0.3 (5 x hexanes); GLC 87%; (HRMS) (CI) calcd. For $C_{23}H_{34}FO$ (MH^+) 345.2594, found 345.2603, Δ = −0.9 mmu.

reason which prompted us to carry out this study was the finding in our laboratory[5, 6] that substituting a fluorine atom for the 2'-hydroxyl(O-585) in anandamide (AEA) increased its CB1 binding affinity 10-fold. A similar increase was found in the 2-methyl-2'-F-AEA (O-689) compared to 2-methyl-AEA (O-680). Earlier studies by Charalambous et al.,[7] and Martin et al.,[8, 9] had shown that substitution of fluorine for hydrogen on C-5' (or C-5") of the pentyl side chain had relatively little effect on the pharmacological activity of the THCs (tetrahydrocannabinols) or CBD (cannabidiol). This was attributed to the fact that both fluorine and hydrogen atoms occupy comparable volumes although their electrostatic properties are different. Tius and co-workers[10] reported on C-9 difluoro and monofluoro-THC analogs and found them to have marginal anti-inflammatory activity. They also introduced fluorine probes in the aromatic ring of nabilone and found diminished affinity[11a] for the CB1 receptor thus confirming the hypothesis that the phenolic hydroxyl group is involved in a hydrogen bonding interaction with the receptor. A (−)-5'-^{18}F-Δ^8-THC analog was studied[12] for brain distribution in a primate, using positron emission tomography (PET) technique, but the results were inconclusive due to low binding affinity of the ligand.

With this background, we synthesized 1-Fluoro-1-deoxy-Δ^8-THC analogs and examined their pharmacological activity. We prepared the alkane/ene/yne series of 1-fluoro-THCs (**4**, **5** and **6**) with side-chains seven carbons in length, which previous SAR studies indicated to be near optimum length for cannabinoid activity. The synthesis[13a] is shown in Scheme 1. Commercially available 1-fluoro-3,5-dimethoxybenzene (**1**) was demethylated with boron tribromide (2.5 equivalents, CH_2Cl_2, −78°C for 10 min, then warmed to room temperature for 1 h) to afford 5-fluororesorcinol which was condensed (TsOH catalytic amount, benzene, reflux Dean-Stark trap, 2 h) with cis-p-menth-2-en-1,8-diol to afford a mixture from which two THC isomers were isolated (silica gel chromatography) in approximately equal amounts (~10% each). These isomers differed in the relative substitution pattern on the aromatic THC ring, one isomer being the desired 1-fluoro-3-hydroxy-Δ^8-THC (**2**), the other being the undesired 3-fluoro-1-hydroxy-Δ^8-THC (**3**). The structures were assigned on the basis of shifts found for the aromatic protons (H-2 and H-4) when their NMR's were taken in $CDCl_3$ and C_6D_6, as reported by Arnone, A. et al.[13b] The desired isomer was then activated as a triflate before palladium (0)-catalyzed coupling[14] to 1-heptyne to afford 1-fluoro-3-(1-heptynyl)-Δ^8-THC (**4**). This in turn was reduced to both the cis-alkene (**5**, 1 atm H_2, Lindlar's catalyst, 1 drop of quinoline, alcohol)[15] and the alkane (**6**, 1 atm H_2, 5% Pd-C, alcohol) in both cases without reducing the Δ^8-double bond. The target compounds were all characterized[16] on the basis of their ^{1}HNMR, High resolution mass spectrum, TLC and GLC analyses. In vitro binding assays for CB1 and CB2 receptors were determined and their in vivo activity was examined in the tetrad tests.[17] For tail-flick (TF) test, we also tested them by intrathecal (i.t.) route[18] in order to investigate if there were any potential differences in receptor interaction or activation based on different routes of administration. The results are shown in Table 1, and for comparison purposes we have also included the activity of the parent 1-hydroxy-THCs, (O-964) for compound **4**, and (O-1317) for compound **5** respectively. It is quite clear from Table 1 that substitution of a fluorine at C-1 in THCs has a significant detrimental effect on the CB1 binding affinity. The alkane analog **6** has 38-fold less binding affinity than Δ^9-THC whereas the alkene analog **5** has 7-fold less than Δ^9-THC and 331-fold less than its parent analog (O-1317). The yne analog **4** is 245-fold and 277-fold less to both Δ^9-THC and the parent analog (O-964). Similar findings were reflected in the tetrad tests and the TF (i.t.) tests. Based on these results and the previous literature studies related to the effect of fluorine substitution in the C-5', C-9 and C-11 positions, it can be concluded that substitution by a fluorine, especially at C-1 position, has a detrimental effect on CB1 binding which is supported by in vivo testing. This is in contrast to our findings in the anandamide analogs (see above).

These findings have several implications in connection with the SAR of THCs particularly in connection with the role of the C-1 hydroxyl group of THC with the CB1 receptor and the

determination of the common pharmacophore in the THC/AEA molecular modeling overlaying studies. With regard to the former, previous studies[19] have attempted to determine the nature of the hydrogen-bonding by preparing and testing specific analogs of Δ^9- and Δ^8-THCs. However, the interpretation of the data obtained is controversial. So far the studies which support the hypothesis that the phenolic hydroxyl group is involved in a hydrogen-bonding interaction with the CB1 receptor is by Tius et al.,[11a] and Song and Bonner.[11b] The latter authors prepared a mutant CB1 receptor in which lysine 192 was replaced by an alanine and examined the binding affinity of various CB1 agonists. Based on this study they arrived at the same conclusions as Tius et al.[11a] Our results suggest that the hydroxyl group of THC is functioning as a hydrogen-bond donor in its interaction with the CB1 receptor and not solely as a hydrogen-bond acceptor.[11c,d] In our molecular modeling studies[4] of overlaying THC with AEA, we had used the pharmacophore fit involving the pyran oxygen of Δ^9-THC with the carbonyl oxygen of AEA thereby leaving the phenolic hydroxyl of THC as the counterpart to the terminal hydroxyl of anandamide. Using this alignment, reasonably good COMFA correlations were obtained for THC and AEA analogs. However, because of our findings in the AEA series, we had expected enhanced binding affinity for the C-1-fluoro-substituted-THCs. Our results do not support the pharmacophore fit we used and lends support to the Tong model[20] which uses the superposition of the hydroxyl of the AEA to the cyclohexyl at C-9 of 9-nor-9β-OH-hexahydrocannabinol (HHC). The difference in the CB1 binding affinity[9] between 11-F-Δ^8-THC and its parent 11-OH-Δ^8-THC was marginal, Ki = 107 nM versus 55 nM respectively. It is interesting to note that the alkene analog **5** bound to CB1 receptors significantly better than the alkane and alkyne analogs. A similar pattern was observed in the 11-hydroxy-THC series by Makriyannis and co-workers.[21] The alkene analog also showed some CB2 selectivity in binding (7-fold) as found in the 1-deoxy- Δ^8-THC-DMH series.[22, 23] Moreover, the loss of CB2 affinity in analog **6** was unexpected given the observation that 1-deoxy-THC analogs retain CB2 affinity.[22] These findings suggest that electrostatic properties at C-1 are crucial for CB2 receptor affinity.

This study presents some interesting conclusions: (a) substitution of 1-hydroxyl group in Δ^8-THC by a fluorine results in a significant decrease in its interaction with the CB1 receptor, (b) the 1-hydroxyl in Δ^8-THC is functioning as a hydrogen-bond donor in its interaction with the CB1 receptor, (c) the results support the molecular modeling overlay studies proposed in the Tong model, (d) some CB2 selectivity (7-fold) is observed in the alkene analog **5**, and (e) it seems unlikely that any advantage will be gained by substituting fluorine in the template of classical cannabinoids.

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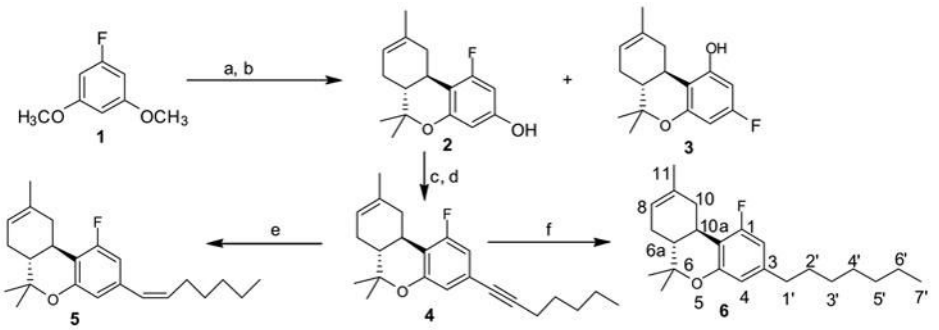
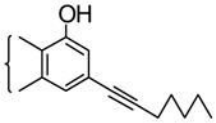
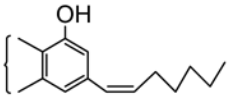


(a) BBr_3, CH_2Cl_2, -78 °C to 25 °C, 1 h; (b) p-menthenediol, TsOH, benzene, reflux, 2 h; (c) Tf_2O, py, CH_2Cl_2, 0 °C, 1 h; (d) 1-heptyne, $Pd(Ph_3)_4$, piperidine, 80 °C, 2 h; (e) Lindlar's catalyst (quinoline, 1 drop), H_2, ethanol, 25 °C; (f) Pd/C, H_2, ethanol, 25 °C.

Scheme 1.

Table 1

Binding affinity and Tetrad Tests of 1-Fluoro-1-deoxy-THCs

Compound	structure	CB1 (nM)	CB2 (nM)	Tetrad Tests (ED_{50}, mg/kg)			TF (i.t.)
				SA	TF	RT	µg/mouse
Δ^9-THC		40.7 ± 1.7 [a]	36.4 ± 10 [b]	1.0	1.4	1.4	29 (24–36)[c]
4		>10,000	>4550	8% @ 30 mg	4% @ 30 mg	−2.2° @ 30 mg	156.9 (114.1–216)
(O-964)		36 ± 0.8	-	3.68	3.24	2.96	-
5		285 ± 34	40 ± 8	0.51	5.8	4.1	99.5 (62.5–158)
(O-1317)		0.86 ± 0.09	-	0.09	0.09	0.13	-
6		1557 ± 203	1508	31.1	21.5	6.9	36% @ 100µ g

[*] Behavioral Evaluation (tetrad tests); SA (spontaneous activity), TF (tail-flick), RT (rectal temperature), RI (ring immobility) were carried out in mice. The ED_{50} data is given in mg/kg. For details see references 17 and 8. RI test was not carried out f or any of the compounds and is therefore not given.

[a] see reference 9.

[b] see reference 6.

[c] The results are presented as ED_{50} (95% confidence limits in parenthesis); see reference 18.