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Pharmacological Evaluation of Halogenated Δ^8-THC Analogs

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CHARALAMBOUS, A., S. LIN, G. MARCINIAK, A. BANIJAMALI, F. L. FRIEND, D. R. COMPTON, B. R. MARTIN AND A. MAKRIYANNIS. *Pharmacological evaluation of halogenated Δ^8-THC analogs.* PHARMACOL BIOCHEM BEHAV **40**(3) 509–512, 1991.—(−)-5′-Bromo-Δ^8-THC, (−)-5′-trifluoromethyl-Δ^8-THC, (−)-5′-iodo-Δ^8-THC, (−)-5′-fluoro-Δ^8-THC, (−)-11-fluoro-Δ^8-THC and (−)-2-iodo-Δ^8-THC were synthesized and evaluated in male ICR mice for their effects on sedation, temperature, catalepsy and antinociception following intravenous injection. The analogs were also tested for relative affinities for cannabinoid binding sites derived from rat cortex membranes, using [^{3}H] CP-55,940 as the tritiated ligand. The results showed that the 5′-bromo, 5′-iodo and 5′-trifluoromethyl analogs were 2–40 times more potent than (−)-Δ^8-THC in all biological tests, while the 5′-fluoro and 11-fluoro derivatives were less active. With the 2-iodo analog, a 12-fold separation was observed between antinociception and sedation, pointing to the importance of the side chain orientation in determining cannabinoid activity and to the possible involvement of more than one cannabinoid receptor site. The pharmacological data closely paralleled the data obtained from the binding assay.

(−)-5′-Bromo-Δ^8-THC (−)-5′-Iodo-Δ^8-THC (−)-5′-Trifluoromethyl-Δ^8-THC (−)-5′-Fluoro-Δ^8-THC
(−)-11-Fluoro-Δ^8-THC (−)-2-Iodo-Δ^8-THC Locomotor activity Rectal temperature Ring immobility
Antinociception

SINCE the early 1940s, when the first cannabinoid analogs were synthesized (1), cannabinoid research has maintained sustained progress resulting in the synthesis of a large number of analogs with a varied degree of resemblance to the natural substances.

Although accurate correlation between the structure and function for the several hundred cannabinoid analogs is complicated by uncertainties about the enantiomeric purity of the different analogs (12) and by the large variability of the testing procedures (12), some general features associated with pharmacological activity can be identified from the existing literature (18). Briefly, essential cannabinoid features for biological activity appear to be the aromatic ring, which when substituted with alkyl or electronegative groups usually results in loss of activity (5), an unsubstituted phenolic hydroxyl group (5, 8, 14), the C_5 aliphatic side chain which when increased to 7 carbons and/or substituted with small alkyl groups at position 1′ and 2′ contributes to increased activity (9) and a trans B/C ring junction (5). Finally, hydroxyl substitutions in the C ring can also result in enhancement of activity (7). However, there is very little information available concerning the effect of halogen substitution. Even though 5′-bromo and 5′-iodo (−)-Δ^8-THCs have been synthesized (16,17) they have not been extensively evaluated for their biological activities.

In the present study, halogenated analogs of (−)-Δ^8-THC were synthesized and evaluated for biological activity. We chose (−)-Δ^8-THC as our parent compound because this tetrahydrocannabinol isomer is chemically more stable and almost equipotent with (−)-Δ^9-THC (18). More specifically, we introduced various halogens at the end of the alkyl chain as well as at positions 2 of the aromatic ring and 11 of the C ring. These derivatives enabled us to obtain information concerning the sensitivity of the different molecular positions towards halogen substitution. Furthermore, by varying the halogen at the end of the side chain we were able to correlate halogen size and electronegativity with their effects on biological activity.

We were also interested in the affinities of these analogs for the cannabinoid receptor and in the possible correlation of ED_{50} values obtained from biological tests with the IC_{50} values from the binding experiments.

In this publication we briefly describe the synthesis of (−)-5′-bromo-Δ^8-THC, (−)-5′-iodo-Δ^8-THC, (−)-5′-trifluoromethyl-Δ^8-THC, (−)-5′-fluoro-Δ^8-THC, (−)-11-fluoro-Δ^8-THC and (−)-2-iodo-Δ^8-THC as well as the results obtained from the biological evaluation and binding experiments.

CHEMISTRY

For the synthesis of (−)-5′-bromo-Δ^8-THC (Scheme 1) we followed a modification of the method described by Pitt (16),

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TABLE 1

PHARMACOLOGICAL ACTIVITY OF HALOGENATED (−)-Δ^8-THC ANALOGS

Analog	Mouse ED_{50} and MPE_{50} (mg/kg) Values			
	Sedation*[1] (locom. activity)	Hypothermia*[1] (Δ temperature)	Catalepsy*[1] (ring imm.)	Analgesia†[1] (tailflick assay)
(−)-Δ^8-THC	0.9	15.5	5.2	1.5
	(79%)	(−5.9°C)	(58%)	(100%)
(−)-11-F-Δ^8-THC	3.0	6.9	7.0	5.0
	(44%)	(−9.2°C)	(71%)	(100%)
(−)-5′-F-Δ^8-THC	3.9	10.2	9.2	2.1
	(92%)	(−9.2°C)	(71%)	(100%)
(−)-5′-CF_3-Δ^8-THC	0.3	1.0	2.4	0.3
	(86%)	(−3.8°C)	(61%)	(100%)
(−)-5′-Br-Δ^8-THC	0.3	0.4	0.2	0.2
	(74%)	(−4.8°C)	(43%)	(100%)
(−)-5′-I-Δ^8-THC	0.1	0.8	0.6	0.4
	(83%)	(−4.2°C)	(55%)	(100%)
(−)-2-I-Δ^8-THC	3.5	8.8	1.9	0.3
	(48%)	(−7.6°C)	(49%)	(100%)

[1]Values in parentheses represent the maximal inhibitory effect produced.
*Sedation hypothermia and catalepsy values expressed as ED_{50}.
†Analgesia values expressed as MPE_{50}.

which mainly consists of condensing 5′-bromo olivetol with (+)-cis/trans-p-mentha-2,8-dien-1-ol in the presence of p-toluenesulfonic acid. The (−)-5′-trifluoromethyl-Δ^8-THC derivative (Scheme 2) was obtained following the same methodology; thus we first synthesized 5′-trifluoromethyl olivetol and then condensed it with (+)-cis/trans-p-mentha-2,8-dien-1-ol. (−)-5′-Fluoro-Δ^8-THC (Scheme 3) was obtained by converting (−)-5′-hydroxy-Δ^8-THC (16) to its triflate diester through reaction with triflic anhydride, followed by nucleophilic substitution with potassium fluoride. Removal of the phenolic triflate ester with lithium aluminum hydride, resulted in the desired compound. The (−)-11-fluoro-Δ^8-THC analog (Scheme 4) was obtained after producing the 11-bromo (−)-Δ^8-THC acetate derivative (20) followed by subsequent reaction with n-tetrabutylammonium fluoride and hydrolysis of the acetate function with 1N hydrochloric acid. For the (−)-5′-iodo-Δ^8-THC analog (Scheme 5), we followed the method described in the literature (17) which consists of nucleophilic substitution of 5′-bromo (−)-Δ^8-THC with sodium iodide in 2-butanone. Finally, for the (−)-2-iodo-Δ^8-THC (Scheme 6) we followed a method described for obtaining halogenated resorcinols (19); thus, by reacting (−)-Δ^8-THC with sodium iodide and m-chloroperbenzoic acid in the presence of 18-crown-6 we obtained the desired product. Conversion of (−)-Δ^8-THC, synthesized from olivetol and (+)-cis/trans-p-mentha-2,8-dien-1-ol, to the Mosher's ester and analysis using ^{1}H NMR (unpublished data) revealed that the enantiomeric purity of the above molecules is greater than 99.5%. The details on the syntheses will be published elsewhere.

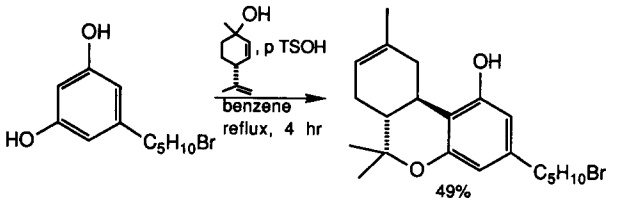
RESULTS AND DISCUSSION

The cannabinoid analogs were evaluated in male ICR mice for their effects on sedation (changes in locomotor activity) and catalepsy (induction of ring immobility) as measures of drug-induced behavioral effects (13). They were also evaluated for effects on body temperature and for antinociceptive activity (mouse tail-flick assay) as previously described (13). Table 1 depicts the tests results as ED_{50}s or MPE_{50}s in mg/kg and under these values and in parentheses, the maximum effect produced.

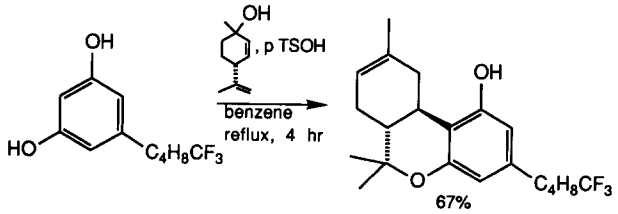
The biological results showed that the 5′-bromo, 5′-iodo, and 5′-trifluoromethyl analogs were 2–40 times more potent than (−)-Δ^8-THC in all pharmacological measures including antinociception. When examining the order of potency, it can be seen that the bromo analog is more potent than the iodo, which in turn is more potent than the fluoro derivative. This order is unrelated to that of halogen electronegativity as calculated by either Allred-Rochow, Pauling or Mulliken (3), according to which fluorine is the most electronegative and iodine is the least electronegative atom. A better correlation may be obtained with the molecular refractivity parameter (MR), which reflects the steric or bulk effects of the substituent. The MR value is 8.88 for Br (2) and 13.94 for I (2) respectively. Knowing that the MR value for the ethyl group (10.30) (2) is closer to that of Br and that the length of the side alkyl chain which is optimal for biological activity is 7 carbons ($-C_5H_{11}$ + ethyl group) (9), it is tempting to relate the highest activity of the 5′-bromo derivative mostly to the bulk of the halogen substituent.

In the case of the 11-fluoro analog, we isosterically substituted an H at the 11 position with an F atom; as can be seen in Table 1, the derivative was less potent than the parent compound in all tests except in its ability to decrease body temperature where it was shown to be more active. The lower potency of this compound may be linked to the high electronegativity of its fluorine atom.

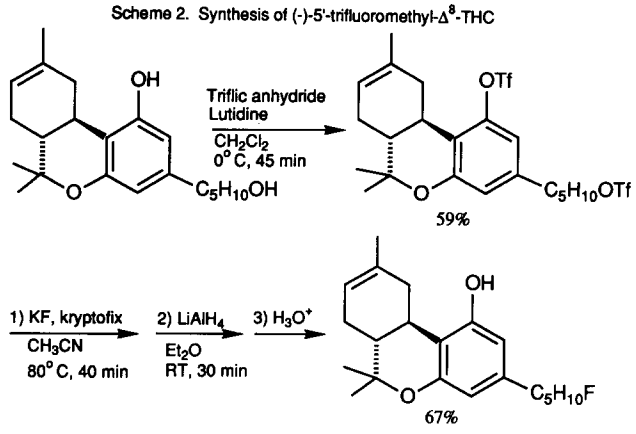
The halo cannabinoid analogs were also evaluated for their abilities to compete with the synthetic cannabinoid [^{3}H] CP-55,940 for occupancy of cannabinoid binding sites in membrane preparations from rat brain cortex (4). Table 2 shows the IC_{50} values obtained from the assay. As can be seen, the order of binding affinity is the same as that of the potencies observed in vivo indicating that pharmacokinetics and metabolism do not play a significant role in determining the rank order of potencies for the individual analogs. 5′-Br and 5′-I (−)-Δ^8-THCs showed the highest affinities for the cannabinoid receptor while interestingly, (−)-11-F-Δ^8-THC even though less potent in vivo than (−)-Δ^8-THC still shows affinity for the binding sites compara-



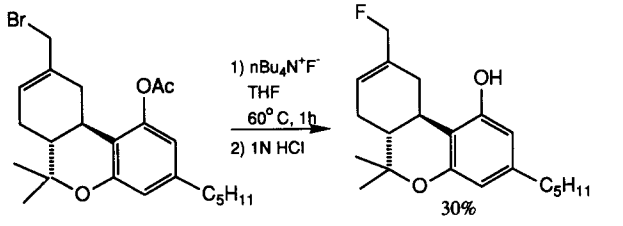
Scheme 1. Synthesis of (-)-5'-bromo-Δ^8-THC



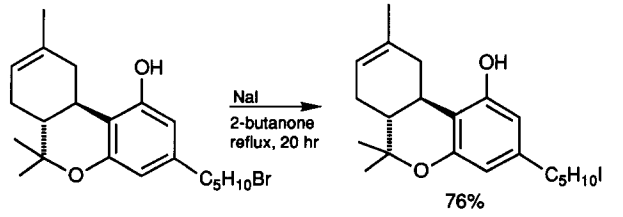
Scheme 2. Synthesis of (-)-5'-trifluoromethyl-Δ^8-THC



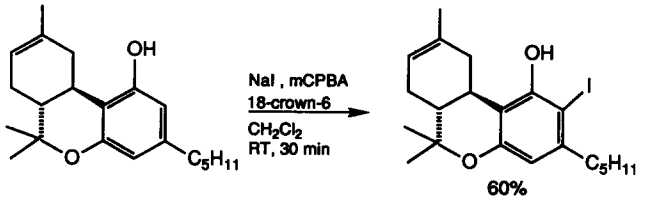
Scheme 3. Synthesis of (-)-5'-fluoro-Δ^8-THC



Scheme 4. Synthesis of (-)-11-fluoro-Δ^8-THC



Scheme 5. Synthesis of (-)-5'-iodo-Δ^8-THC



Scheme 6. Synthesis of (-)-2-iodo-Δ^8-THC

TABLE 2

EVALUATION OF ANALOGS IN THE [^{3}H] CP-55,940 BINDING ASSAY

Cannabinoid	IC_{50} nM*
(−)-Δ^8-THC	219.0 ± 37
(−)-11-F-Δ^8-THC	146.0 ± 29
(−)-5'-F-Δ^8-THC	81.0 ± 3.5
(−)-5'-CF_3-Δ^8-THC	34.9 ± 8.1
(−)-5'-Br-Δ^8-THC	10.8 ± 1.8
(−)-5-I-Δ^8-THC	11.0 ± 3.4
(−)-2-I-Δ^8-THC	126.0 ± 22

*Means ± S.E.M from at least three experiments.

ble to that of the parent compound. This data suggests that the observed biological effects of Table 1 are the results of interactions between the drug and the cannabinoid receptor.

Lastly, we discuss the results obtained with the (−)-2-iodo-Δ^8-THC derivative. Compared to (−)-Δ^8-THC, the molecule possesses considerable biological potency in lowering body temperature, in inducing catalepsy and especially in producing antinociception, while it is less active in producing sedation (Table 1). It is of great interest that it is the only compound of the series that shows a separation between antinociception and sedation (12-fold), which are effects of pharmacological relevance to humans. On the other hand, its binding affinity for the cannabinoid receptor is almost the same as that of the parent compound (Table 2).

Taking the above data into consideration, it is not unreasonable to postulate the existence of more than one cannabinoid receptor site, one for antinociception and another associated with the behavioral properties of the cannabinoids. Regarding the separation between antinociceptive and other pharmacological properties evaluated in this study, we can speculate that for maximum antinociception, a conformation might be necessary in which the alkyl side chain because of steric hindrance from the 2-iodo substituent, is pushed away from the phenolic hydroxyl group and towards the southern direction (12) of the molecule. This hypothesis is congruent with previous work from our laboratory (10, 11, 15), where we have studied the topography of cannabinoids in model membranes. Using deuterium solid-state NMR and x-ray diffraction we showed that the longitudinal axis of the cannabinoid tricyclic core assumes an orientation perpendicular to the bilayer chains while the cannabinoid alkyl side chain orients itself perpendicular to the tricyclic core.

In conclusion, in this work we synthesized a series of halogenated cannabinoids and evaluated them for their biological activities and their relative binding affinities for cannabinoid receptor site(s). We found (−)-2-iodo-Δ^8-THC to be an interesting probe because it shows the greatest separation between antinociceptive and sedative cannabinoid properties.

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