Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2119–2124

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NOVEL CONFORMATIONALLY RESTRICTED TETRACYCLIC ANALOGS OF Δ^8-TETRAHYDROCANNABINOL

Atmaram D. Khanolkar[a], Dai Lu[a], Pusheng Fan[a], Xiaoyu Tian[a] and Alexandros Makriyannis[a,b,c]*

Departments of [a]Pharmaceutical Sciences and [b]Molecular and Cell Biology and [c]Institute of Materials Science

University of Connecticut, Storrs, CT 06269, U.S.A.

Received 3 March 1999; accepted 25 May 1999

Abstract: Novel analogs of (-)-Δ^8-tetrahydrocannabinol (Δ^8-THC) in which the conformation of the side chain was restricted by incorporating the first one or two carbons into a six membered ring fused with the aromatic phenolic A ring were synthesized. The affinities of the novel ligands for CB1 and CB2 indicated that the "southbound" chain conformer retained the highest affinity for both receptors.

Structure–activity relationship studies of classical cannabinoids have established that the alkyl chain at the C3 position is an essential pharmacophore for cannabimimetic activity. Naturally occurring cannabinoids such as Δ^9-THC and cannabinol possess an *n*-pentyl side chain at this position. However, it is also known that a 1',1'-dimethylheptyl or 1',2'-dimethylheptyl side chain is optimal for activity.[1] Our interest in exploring the role of side chain conformation on the activity of classical cannabinoids led us to design and synthesize analogs in which the side chain is conformationally constrained. In earlier work we restricted the rotation around the C1´-C2´ bond in otherwise flexible *n*-heptyl chain by introducing unsaturation (C=C, C≡C) either in 11β-hydroxyhexahydrocannabinol[2] or Δ^8-tetrahydrocannabinol.[3] These analogs were found to have high affinity for the cannabinoid receptors. Here we report the synthesis and cannabinoid receptor binding of novel tetracyclic analogs of Δ^8-THC in which the alkyl side chain is conformationally more defined by adding a fourth ring in the tricyclic cannabinoid skeleton fused to the aromatic phenolic A ring. Analogs **1** and **2** incorporate a cyclohexane ring fused to the C2-C3 position carrying either an *n*-pentyl or *n*-hexyl chain (C_5 or C_6) at a suitable position so as to produce analogs in which the *n*-heptyl chain is conformationally restricted around the C1´-C2´ and/or C3-C1´ bond (Fig. 1). In a third analog **8**, the six-membered ring is fused to the C3-C4 bond of the A ring.

Synthesis. Tetralone **4** was used as a common intermediate for the synthesis of analogs **1**, **2** and **8**. It was prepared,[4] as shown in Scheme 1, via initial Friedel–Crafts reaction of 1,2,3-trimethoxybenzene and *mono*-methyl succinate followed by alkaline hydrolysis of the product ester to give keto-carboxylic acid **3**. Catalytic hydrogenolysis[5] of **3** at atmospheric pressure afforded the corresponding 4-arylbutyric acid, which was cyclized intramolecularly using polyphosphopric acid[6] to afford tetralone **4**. Addition of *n*-hexyl magnesium bromide to **4** followed by dehydration of the resulting tertiary carbinol gave alkene **5** which was hydrogenated using 10% Pd-C as catalyst to **6**. Subsequently, the C6 methoxy group was selectively removed by treatment with

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00355-8

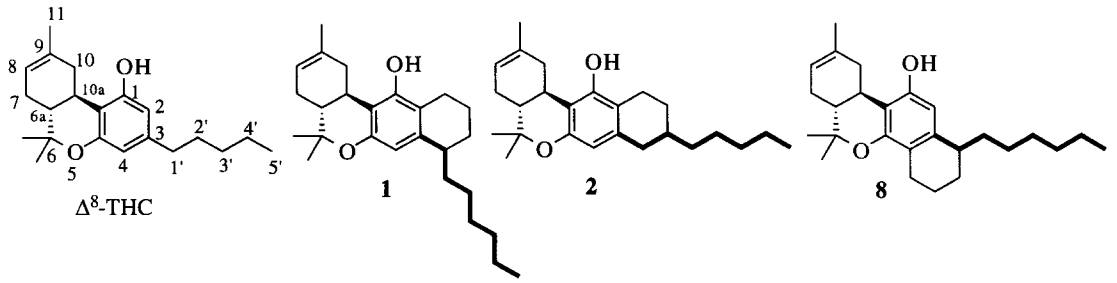
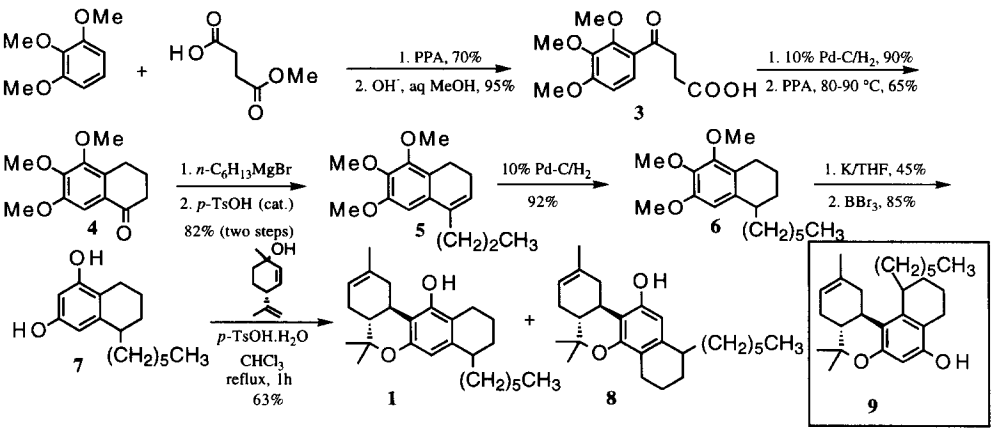


Figure 1. Conformationally restricted Δ^8-THC analogs.

potassium metal in THF according to the procedure described by Azzena et al.[7,8] and the resulting dimethyl ether demethylated using boron tribromide. The resulting resorcinol **7** was, then, coupled with *cis/trans-p*-menthadienol in the presence of *p*-toluenesulfonic acid monohydrate as catalyst to give the tetracyclic analog **1** as the major product (~85%) along with a small amount (10–15%) of a regioisomer **8** in which the cyclohexane ring is fused to C3-C4 position of the phenol ring. A third possible product *abn*-Δ^8-THC analog **9** was not isolated from the product mixture.

Scheme 1

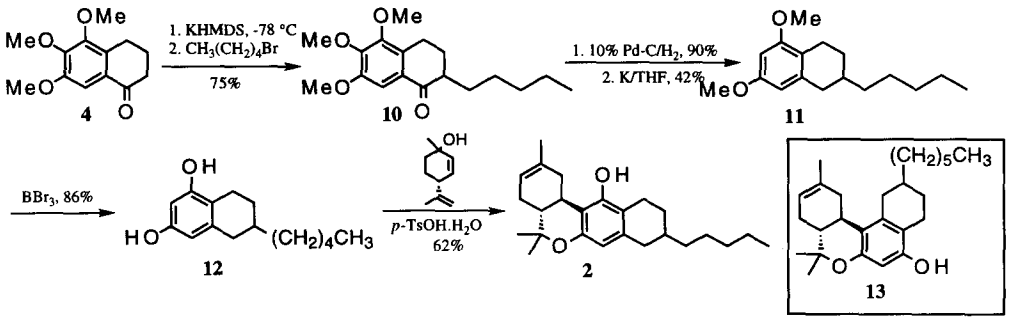


Synthesis of analog **2** from tetralone **4** is outlined in Scheme 2. Alkylation of the potassium enolate of **4**, generated by treatment with potassium bis(trimethylsilyl)amide, with 1-bromopentane gave ketone **10**. Subsequent reduction of **10** and C6 demethoxylation followed by treatment of **11** with boron tribromide gave the desired resorcinol **12**. Coupling of **12** with *cis/trans-p*-menthadienol, as described above, gave the tetracyclic analog **2**. No regioisomer was formed during this coupling reaction as judged from TLC and the proton NMR spectrum of the crude product. Also, another possible isomer, *abn*-Δ^8-THC analog **13**, was not isolated from the product mixture. It should be noted that analogs **1**, **2**, and **8** were obtained as diastereomeric mixtures which were not separated as individual isomers.

Structural assignments. The structures of analogs **1**, **2**, and **8** were assigned based on their proton and

2-D DQFNOESY NMR spectra.[9,10] The latter are shown in Figure 2. A strong NOE between the phenolic OH (δ 4.45, exchangeable) and the C2 aromatic proton (δ 6.20) was observed only for analog **8** which indicates that in this compound the cyclohexane ring is attached to the C3-C4 carbons of the phenol. For analogs **1** and **2**, NOEs were observed between the phenolic OH and the benzylic protons (δ ~2.5 ppm) on the cyclohexane ring. No such cross peaks were observed for analog **8**. Additionally, the proton NMR spectra of **1**, **2**, and **8** showed peaks characteristic of tetrahydrocannabinols such as three singlets at δ 1.69, 1.37, and 1.07 for three methyl groups at C9, 6α, and 6β positions, respectively, and a triplet at δ 0.88 for the terminal methyl group of the *n*-alkyl side chain. All three analogs also showed a singlet due to an olefinic proton (C8) at about δ 5.4 as well as a characteristic multiplet at ~δ 3.2 due to the 10α proton. Based on the NMR spectra (Fig. 3), structures such as **9** and **13** were excluded.

Scheme 2



Biological evaluation of analogs. To date two cannabinoid receptors have been identified. Devane et al[11] first demonstrated the existence of a cannabinoid receptor in the brain (CB1) using tritiated CP-55,940 while a second receptor CB2 that specifically binds cannabinoid ligands was identified in macrophages and the marginal zone of spleen by Munro et al.[12] Both receptors have been sequenced, cloned, and expressed in various tissues. The presence of a cannabinoid receptor in macrophages is suggestive of a possible role of cannabinoids in immunomodulation. Binding affinities of analogs **1**, **2**, and **8** for cannabinoid receptors were evaluated using rat forebrain synaptosomal membranes and mouse spleen membranes as a source of CB1 and CB2, respectively, following methods reported in our earlier publications.[13,14] K_i values were determined using a standard filtration assay for the displacement of specifically bound tritiated CP-55,940.

Results and discussion. Receptor binding affinities of conformationally restricted analogs **1**, **2**, and **8**, presented in Table 1, were compared with those of (-)-*n*-heptyl-Δ^8-THC, the prototypical ligand with a totally unrestrained seven-membered side chain.[15] The data clearly indicate that incorporation of the first one or two carbons of the side chain into a six-membered ring leads to a significant reduction in affinities for both CB1 and CB2. This may be attributed either to the conformational restriction imposed on the early part of the chain or, alternatively, to the presence of additional carbons of the six-membered ring which may interfere with the binding of the ligand at the active site. In this regard, it has been reported that (-)-Δ^8-THC analogs with substituents such as CH_3, C_2H_5, Cl, I *ortho* to the phenolic hydroxyl retain substantial biological activity while

para substitution results in loss of activty.[16] This is congruent with our results since analogs **1** and **2** in which the cyclohexane ring is attached to the phenol at 2-position have higher receptor affinities than **8**, their 4-substituted counterpart. Analog **1** in which the side chain is fixed at a distance of 1-1.5 A° from the phenol ring

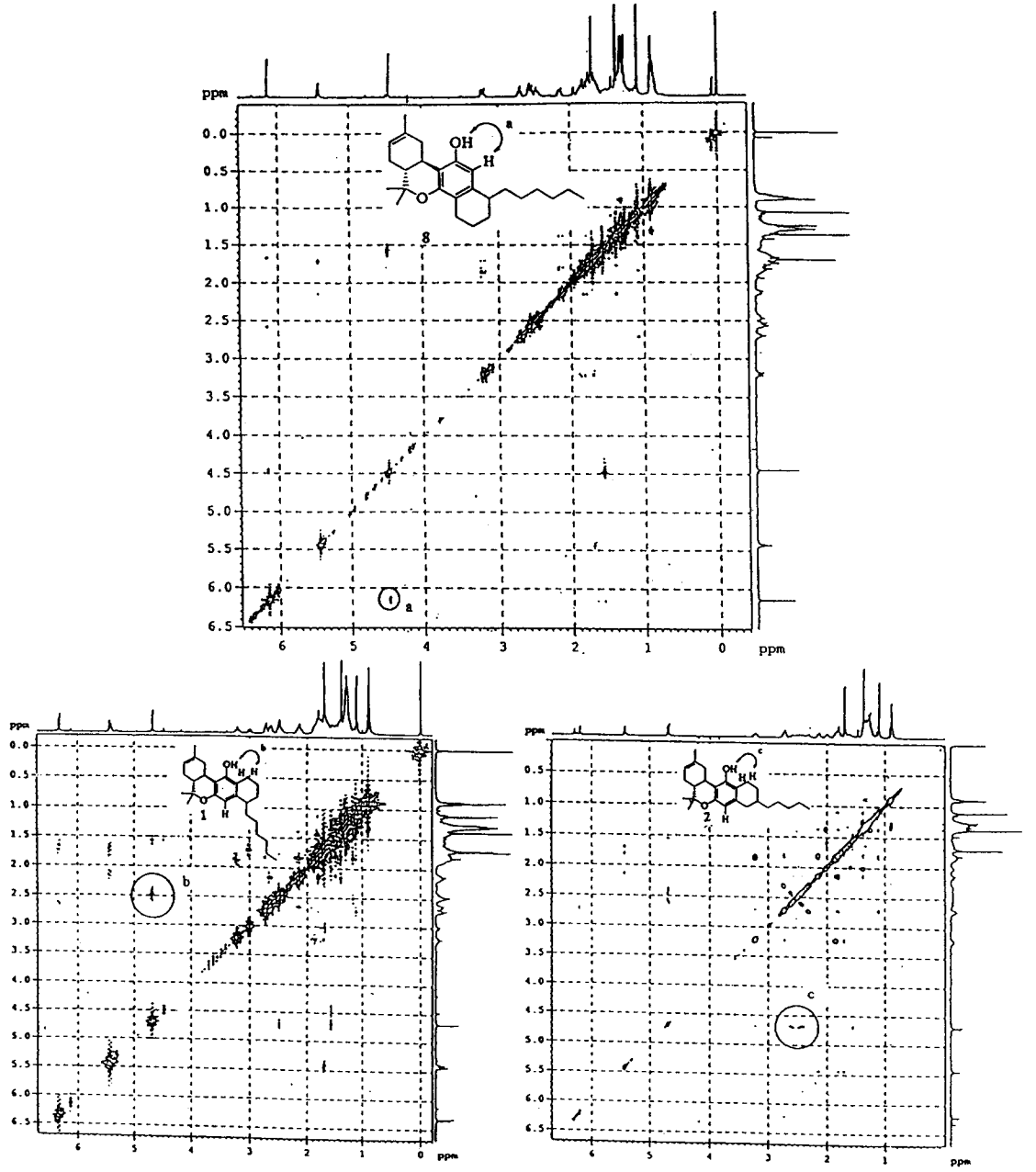


Figure 2. 2-D DQF NOESY[9] spectra of conformationally restricted Δ^8-THC analogs.

and has its side chain pointing downwards has an 18-fold higher CB1 affinity than **2** in which the side chain is fixed at a distance of at least 3-3.5 A° with a lateral orientation. Analog **1** also exhibits 3-fold higher affinity for the CB2 receptor than **2**. These results suggest that cannabinoid receptor affinities decrease significantly when the side chain is forced into a lateral orientation and further away from the phenol ring. Recently an analog of (-)-Δ^8-THC in which a pentyl chain (instead of heptyl as in **2**) is similarly restricted, was also found to have weak affinity for CB1 (K_i 703 nM).[17] Analog **8** in which the orientation of the side chain is constrained laterally eastward exhibited the lowest affinity for both receptors. This can be attributed to its undesirable sideways chain orientation and to the steric constraints of the C3-C4 fused ring with the receptor binding site.

Table 1. Cannabinoid Receptor Binding Affinities of Tetracyclic Δ^8-THCs.[a]

Analog	CB1 K_i, nM	CB2 K_i, nM
n-Heptyl-Δ^8-THC	0.43 (0.35,0.55)	0.39 (0.26,0.55)
1	22.3 (19.8,25.2)	58.6 (53.6,64.3)
2	402.4 (366.8,441.6)	161.5 (145.4,179.4)
8	542.1 (524.1,560.1)	455.6 (390.7,531.2)

[a] 95% confidence limits are indicated in the parenthesis

Acknowledgment: This work was supported by Grants DA-3801, DA-152, DA-7215 (A.M.) and DA05703 (A.D.K.) from the National Institute on Drug Abuse.

References and notes

1. Adams, R.; Harfenist, M.; Lowe, S. *J. Am. Chem. Soc.* **1949**, *71*, 1624.
2. Busch-Petersen, J.; Hill, W. A. G.; Fan, P.; Khanolkar, A. D.; Xie, X. Q.; Tius, M. A.; Makriyannis, A.*J. Med. Chem.* **1996**, *39*, 3790.
3. Papahatjis, D. P.; Kourouli, T.; Abadji, V.; Makriyannis, A. *J. Med. Chem.* **1998**, *41*, 1195.
4. Cameron, D. W.; Feutrill, G. I.; Pietersz, G. A. *Aust. J. Chem.* **1982**, *35*, 1481.
5. Rama Rao, A. V.; Chandra, B.; Borate, H. B. *Tetrahedron Lett.* **1982**, *38*, 3555.
6. Gardner, P. D. *J. Am. Chem. Soc.* **1954**, *76*, 4550.
7. Azzena, U.; Denurra, T.; Fenude, E.; Melloni, G.; Rassu, G. *Synthesis* **1989**, 28.
8. Azzena, U.; Denurra, T.; Melloni, G.; Rassu, G. *J. Chem. Soc. Chem. Commun.* **1987**, 1549.
9. Bax, A.; Lerner, L. *Science* **1986**, *232*, 960.
10. All intermediates and the final compounds were characterized by ^{1}H NMR spectra recorded on a Bruker 270 MHz instrument. The DQFNOESY spectra of final compounds **1**, **2**, and **8** were acquired at 500 MHz on a Bruker AVANCE DMX500 spectrometer equipped with a Bruker 5 mm TXI probe at room

temperature. Deuterated chloroform was used as the solvent and TMS as the internal standard. Samples were degassed by the freeze-thaw method, and sealed in high quality 5 mm NMR tubes. NOESY spectra were recorded with a spectral width of 5000 Hz spectral width and 1024 data points in the F2 dimension for 512 transients. Mixing times of 100, 200, and 600 ms with a pulse delay of 3s were employed. Mixing time of 600 ms was found to be optimal.

11. Devane, W.A.; Dysarz, F.A., III; Johnson, R.M.; Melvin, L.S.; Howlett, A.C. *Mol. Pharmacol.* **1988**, *34*, 605.
12. Munro, S.; Thomas, K. L.; Abu-Shaar, M. *Nature* **1993**, *365*, 61.
13. Khanolkar, A. D.; Abadji, V.; Lin, S.; Hill, W. A. G.; Taha, G.; Abouzid, K.; Meng, Z.; Fan, P.; Makriyannis, A. *J. Med. Chem.* **1996**, *39*, 4515.
14. Charalambous, A.; Guo, Y.; Houston, D. B.; Howlett, A. C.; Compton, D. R; Martin, B. R.; Makriyannis, A. *J. Med. Chem.* **1992**, *35*, 3076.
15. *n*-Heptyl-Δ^8-THC was prepared by condensation of 5(*n*-heptyl)resorcinol and *p*-menthadienol in the presence of *p*-toluenesulfonic acid and characterized by proton NMR and GC-MS.
16. Edery, H.; Grunfeld, Y.; Porath, G.; Ben-Zvi, Z.; Shani, A.; Mechoulam, R. *Arzneim.-Forsch.* **1972**, *22*, 1995.
17. Huffman, J. W.; Yu, S. *Bioorg. Med. Chem.* **1999**, *6*, 2281.