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NEUROPROTECTIVE (+) 3S, 4S CANNABINOIDS WITH MODIFIED 5'-SIDE CHAIN

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Abstract: The synthesis and evaluation of two novel cannabinoids belonging to the (+) 3S, 4S nonpsychotropic series are described. These derivatives bind to the NMDA receptor but have lower affinities than dexanabinol (HU-211), the series benchmark. The novel compounds protect neurons against NMDA- induced toxicity in cortical cell cultures and have lower toxicity to host neurons than dexanabinol. Copyright © 1996 Elsevier Science Ltd

Introduction:

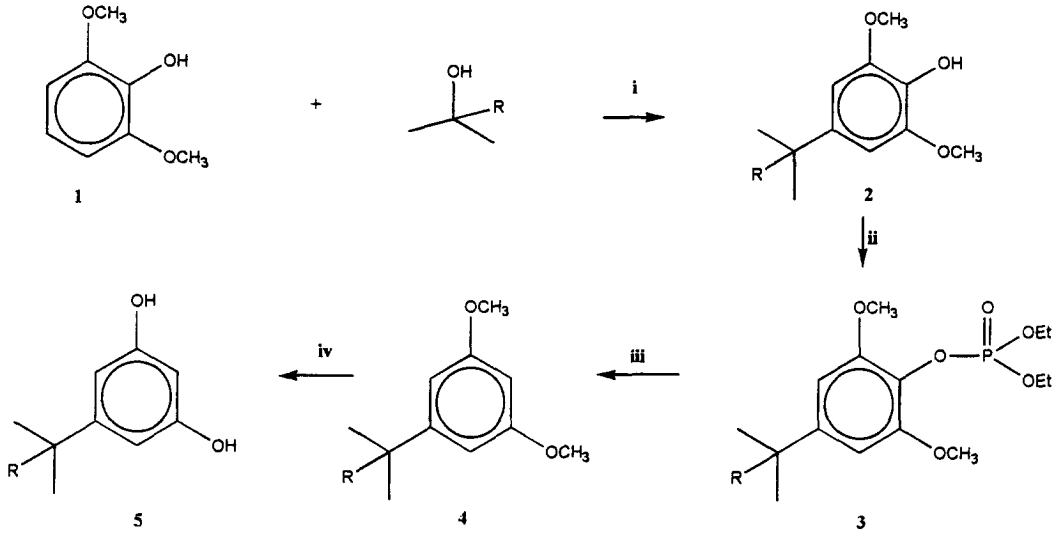
Cannabinoids are the components of *Cannabis sativa* and have been known from ancient times for their euphorigenic and psychomimetic properties. These compounds, as well as numerous synthetic analogs belonging to the (-) *3R, 4R* enantiomeric series, interact with specific receptors located in the central nervous system and the spleen. [1,2] Recently, it has been demonstrated that the activity of cannabinoids is stereospecific.[3] The (+) *3S, 4S* 5'-(1',1'-dimethylheptyl)-7-hydroxy-Δ^6-tetrahydrocannabinol (dexanabinol, HU-211, **14c**), first synthesized by Mechoulam et al.,[3] has only negligible affinity to the cannabinoid receptors and is devoid of any cannabimimetic activity, while its (-) *3R, 4R* isomer (HU-210) is one of the most potent cannabinoids.[3,4] Dexanabinol proved to be, on the other hand, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist[5] and an effective radical scavanger .[6] As various *in vitro* and *in vivo*-studies have demonstrated that dexanabinol has significant neuroprotective properties,[5, 7-10] its development as a therapeutic agent with potential use in the treatment of brain damage associated with stroke, cardiac arrest, and trauma,[11-12] has been initiated.

Numerous evaluations have led to a well-established structure-activity relationship (SAR) for the natural, (+) *3R, 4R* cannabinoid series.[13, 14] Obviously these data do not apply to the (+) *3S, 4S* cannabinoids since they bind to different receptors. It is of interest therefore to investigate the influence of various structural modifications of dexanabinol on receptor binding and neuroprotective properties. The influence of the 5'-side chain has been considered in this study; the synthesis and preliminary evaluation of two analogs of dexanabinol (**14a** and **14b**) in which the 1',1'-dimethylheptyl side chain of **14c** was replaced by *tert*-butyl and 1',1'-dimethylbutyl chains, respectively, are presented herein.

Chemistry:

The synthesis of the target compounds is described in Schemes I-III and consist of the coupling of two key intermediates: an appropriate 5-substituted resorcinol (**5a,b**) and (+) 4-hydroxymyrtenol having the allylic hydroxyl functionality protected as the adamantane-1-carboxylate (**12**). The two resorcinols were synthesized from 2,6-dimethoxyphenol (**1**) following a procedure previously reported for analogous compounds[15] (Scheme I).

Scheme I



a. R = CH_3

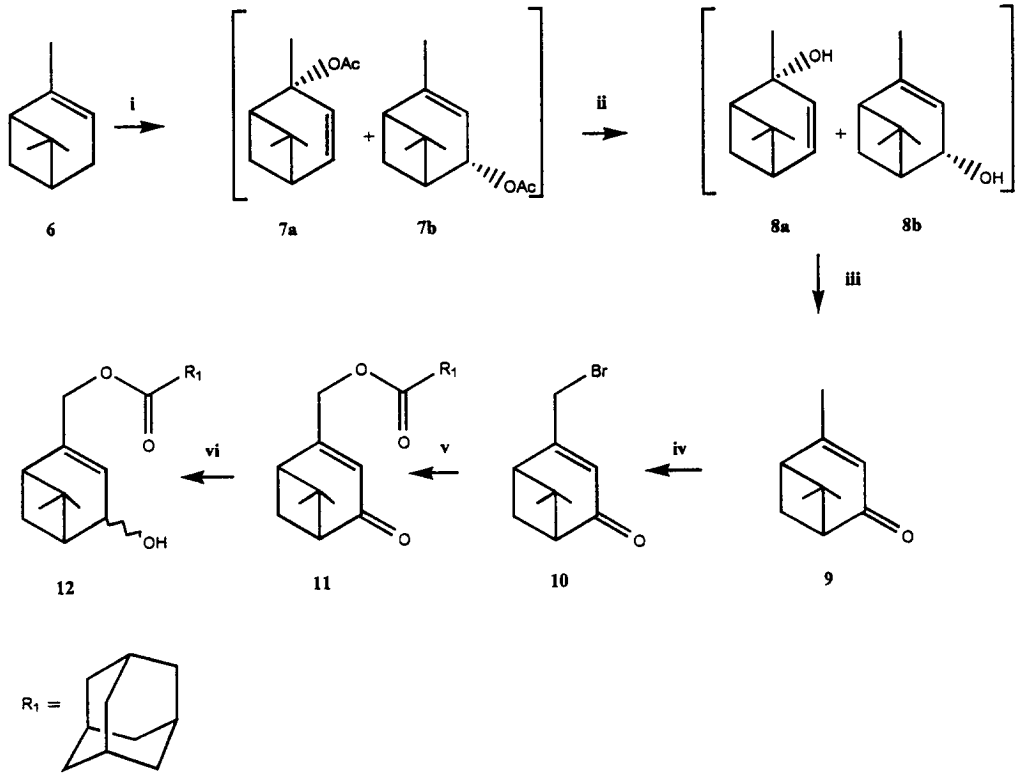
b. R = C_3H_7

Conditions: (i) CH_3SO_3H; (ii) $HPO(OC_2H_5)_2$, CCl_4, $N(C_2H_5)_3$; (iii) Li/NH_3; (iv) BBr_3, CH_2Cl_2; H_2O.

Alkylation of **1** with 1,1-dimethylbutanol or *tert*-butanol (both, tertiary carbonium ion precursors) in methanesulfonic acid led to the 4-substituted 2,6-dimethoxyphenols (**2a,b**), with regioselective para substitution (yields: 90-95%). The phenolic hydroxyl groups were then removed in two steps, by converting the crude phenols in crystalline phosphate esters **3a,b** (yields: 74-75%), followed by their treatment with Li/NH_3 (yields: 85-90%). The resorcinols were then obtained by demethylation of the 1-substituted 3,5-dimethoxybenzene derivatives **4a,b** (yields: 60-65%). The 4-hydroxymyrtenyl -7-(adamantane-1-carboxylate) (**12**) was obtained according to the procedure described in Scheme II.

(+) α-Pinene (**6**) was oxidized to a mixture of acetates (**7a,b**) which were hydrolysed to give the alcohols **8a,b**; oxidation of the alcohols led to (+) verbenone (**9**) (yield related to **6**: 42%) that was then brominated to give 4-oxomyrtenyl-7-bromide (**10**). By reaction of **10** with sodium adamantane-1-carboxylate under phase transfer condition followed by reduction of the resulting 4-oxomyrtenyl adamantane-1-carboxylate (**11**), compound **12** was obtained (yield related to **9**: 25%).

Scheme II

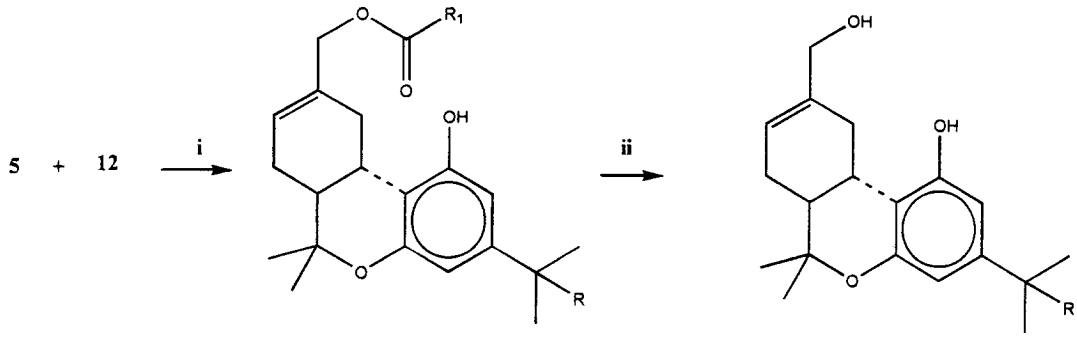


Conditions: **(i)** $Pb(OAc)_4$, C_6H_6; **(ii)** KOH, CH_3OH; **(iii)** $Na_2Cr_2O_7$, H_2O, H_2SO_4;
(iv) NBS, CCl_4; **(v)** sodium adamantane-1-carboxylate, Bu_4NHSO_4 , H_2O, CH_2Cl_2; **(vi)** $NaBH_4$, EtOH.

The coupling of **5** and **12** is presented in Scheme III (the mechanism and stereochemistry of the reaction were previously reported [16]). The ester **13** that results from coupling, followed by cyclization, is deprotected to give the final products (+) *3S, 4S* 5'-(*tert*-butyl)-7-hydroxy-Δ^6-tetrahydrocannabinol (**14a**) and 5'-(1',1'-dimethylbutyl)-7-hydroxy-Δ^6-tetrahydrocannabinol (**14b**) (yield reported to **12**: 35-38%,). The new derivatives were identified and fully characterized by using various techniques (^1H and ^{13}C NMR, UV, IR spectroscopy and elemental analysis); their

purities were determined by HPLC and GC. A high optical purity (ee > 99.8%) of **14a,b** (chiral HPLC) was obtained.

Scheme III



a. $R = CH_3$ R_1 = Adamant-1-yl

b. $R = C_3H_7$

c. $R = C_6H_{13}$

Conditions: **(i)** $BF_3.(C_2H_5)_2O$, CH_2Cl_2; **(ii)** $LiAlH_4$, $(C_2H_5)_2O$

Receptor Binding Properties:

Dexanabinol is a noncompetitive NMDA receptor antagonist that completely inhibits the binding of [^{3}H] (+) 5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate ([^{3}H] MK- 801) and [H]-N-[1-(2-thienyl)cyclohexyl]piperidine (TCP), two well known NMDA receptor antagonists, to rat forebrain membranes. The IC_{50} of dexanabinol is about 10 μM in this paradigm, but competition does not occur after full equilibration of tissues with labelled ligands, suggesting that dexanabinol binds at a site close to, but not identical with, the MK- 801 site in the NMDA receptor-linked channel. The NMDA receptor binding of **14a** and **14b** was examined using similar methods, by determining their ability to displace [^{3}H] MK- 801 in rat forebrain membranes. Briefly, brains were removed from Sprague-Dawley rats within 5 min after decapitation and membranes were prepared according to a previously described procedure.[8] Binding of [^{3}H] MK-801 to membranes was conducted in the presence of 10 μM glutamate and 30 μM glycine. Membranes were suspended in 50 mM tris-acetate, pH 7.4 buffer and incubated with 10 nM [^{3}H] MK- 801 either alone or in the presence of **14a** or **14b** (100 μM). Non-specific binding was determined

in the presence of 100 μM of TCP. After 2 h of incubation at 25 °C, the radioligand binding process was terminated by rapid filtration through Whatman grade GF/B filters (Aldrich) presoaked in 0.1% polyethyleneimine and washing the filters with 15 mL cold buffer. Filters were counted in scintillation fluid using a liquid scintillation counter. Data were analyzed using the iterative non-linear squares curve fitting program LIGAND[17].

The results summarized in Table 1 indicate that both **14a** and **14b** inhibit the NMDA receptor but have a higher IC_{50} as compared to dexanabinol. It is believed that the receptor binding of the novel derivatives is similar in type and site with that of dexanabinol.

Table 1. Receptor Binding and Activities of 14a and 14b

Compound	Inhibition of [^{3}H] MK-801 binding[a]		Protection of NMDA toxicity	Neuronal cell toxicity
	IC_{50} (μM)	% Inhibition at 100 μM	% Protection at 10 (or 5) μM[b]	% Morbidity[a]
14a	30	83	10 μM: 24-130	10 μM: 15
				20 μM: 36
14b	30	80	5 μM: 36-74	10 μM: 0
				20 μM: 0
14c	10	90	10 μM: 63-108	10 μM: 25

[a] Average values of 2-3 determinations

[b] Range ; the lower values resulted in the XTT assays, while the higher ones in the LDH assays.

***In Vitro* Neuroprotective Properties and Neurotoxicity of 15a and 15b:**

It was demonstrated that dexanabinol (**14c**) protects neurons against NMDA induced toxicity. *In vitro* incubation of cortical neuron cell cultures extracted from fetal rat brains grown over a feeder layer of glial cells with 100 to 1000 μM of NMDA resulted in 50-60% cell death within 24 h. When dexanabinol was co-incubated at concentrations of 1-10 μM, with neurons exposed to NMDA, cell death was reduced or totally prevented in a dose-dependent manner. This effect was similar in appearance and magnitude to the effect of MK-801. The protection of NMDA induced neural toxicity of **14a** and **14b**, determined by using the same procedure[8, 18] gave results shown in Table 1. Both lactate dehydrogenase (LDH) and 2,3-bis[2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilelide inner salt (XTT)-based assays were employed in these determinations. The LDH test and culture morphology indicated

that cells were intact, while XTT results indicated the extent of cell mortality. Both compounds attenuated NMDA toxicity at concentrations of 10 and (in case of **14b**) even 5 μM. Importantly, the intrinsic toxicity of these compounds was very low even compared to dexanabinol, which has an inocuous *in vivo* profile. The 5'-*tert*-butyl derivative **14b** was completely devoid of neuronal toxicity at concentrations of 10 and 20 μM; the toxicity of **14a** was also lower than that of dexanabinol.

In summary, the two novel modified side chain homologs of dexanabinol, **14a** and **14b**, synthesized by novel pathways, were found to bind to the NMDA receptor and demonstrated *in vitro* activity/toxicity profils comparable to **14c**. Further investigation of these compounds, including their *in vivo* neuroprotective properties is of interest.

References:

(1) (a) Devane, W.A.; Dysarz, F.A,III.; Johnson, M.R.; Melvin, L.S.; Howlett, A.C. *Mol.Pharmacol.* **1988**, *36*, 605. (b) Devane, W.A.; Breuer, A.; Sheskin, T.; Jarbe, T.U.C.; Eisen, M.S.; Mechoulam, R. *J .Med. Chem.* **1992,** *35*, 2065. For a review on the cannabinoid receptor see Howlett, A.C.; Bidaut-Russell, M.; Devane, W.A.; Melvin, L.S.; Johnson, M.R.; Herkenham, M. *Trends Neurosci.* **1990,** *13*, 420.

(2) Munro, S.; Thomas, K. L.; Abu-Shaar, M. *Nature (London)* **1993**, *365*, 61.

(3) Mechoulam, R.; Lander, N.; Breuer, A.; Zahalka, J. *Tetrahedron: Assymetry* **1990,** *1*, 315.

(4) Mechoulam, R.; Devane, W.A.; Glaser, R. In *Marijuana/cannabinoids: neurobiology and neurophysiology.* Murphy, L; Bartke, A., Ed.; CRC: Boca Raton, 1992; pp 1-33.

(5) Feigenbaum, J. J.; Bergmann, F.; Richmond, S. A.; Mechoulam, R.; Nadler, V.; Kloog, Y.; Sokolovscy, M. *Proc. Nat. Acad. Sc. U.S.A.* **1989,** *86*, 9584.

(6) Eshhar, N.; Striem, S.; Kohen, R.; Tirosh, O.; Biegon, A. *Eur .J. Pharmacol.* **1995,** *283*, 19.

(7) Zeltser, R.; Seltzer, Z.; Eisen, A.; Feigenbaum, J. J.; Mechoulam, R. *Pain* **1991,** *47*, 95.

(8) Eshhar, N.; Striem, S.; Biegon, A. *Neuro Repor,* **1993**, *5*, 237.

(9) Nadler, V.; Mechoulam, R.; Sokolovsky, N. *Brain Res.* **1993**, *622*, 79.

(10) Nadler, V.; Mechoulam, R.; Sokolovsky, N. *Neurosci. Let.* **1993**, *162*, 43.

(11) Shohami, E.; Novikov, M.; Mechoulam, R. *J .Neurotrauma* **1993**, *10*, 109.

(12) Bar-Joseph, A.; Berkovitch, Y.; Adamchik, J.; Biegon, A. *Molecular and Chemical Neuropathology* **1994**, *23*, 125.

(13) Razdan, R.K. *Pharm.Rev.* **1986**, *38*, 75.

(14) Mechoulam, R.; Feigenbaum, J. J. *Progr .med .Chem.* **1987**, *24*, 159 .

(15) Dominianni, S. J.; Ryan, C. W.; DeArmitt, C. W. *J. Org. Chem.* **1977**, *42*, 344.

(16) Mechoulam, R.; Breuer, A.; Jarbe, U. C.; Hiltunen, A. J.; Glaser, R. *J. Med. Chem.* **1990**, 33, 1037.

(17) Munson, P. J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.

(18) Pop, E.; Liu, Z. Z.; Brewster, M. E.; Barenholz, Y.; Korablyov, V.; Mechoulam, R.; Nadler, V.; Biegon, A. *Pharm. Res.* **1996**, *13*, 62.

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