Derivatives of Dexanabinol. II. Salts of Amino Acid Esters Containing Tertiary and Quaternary Heterocyclic Nitrogen with Increased Water-Solubility

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Purpose: Amino acid esters containing tertiary or quaternary nitrogen heterocycles were synthesized for dexanabinol (**1**) and evaluated as water-soluble prodrugs or congeners.

Methods: Syntheses were performed by conventional methods; stability studies in water, blood (rat, dog, human) and assay-media were performed by HPLC; NMDA receptor binding was determined by [^{3}H] MK-801 displacement; neuroprotection and neurotoxicity studies were performed in cortical cell cultures.

Results: 7-morpholino and N-methylpiperazino acetates and butyrates and the respective N-methylmorpholinium and piperazinium iodides as well as a 3′-N-methyl morpholino butyrate and the corresponding N-methyl quaternary type derivative were synthesized. All compounds were relatively soluble in water or 10% aqueous ethanol. The examined derivatives were stable in water and generally less stable in blood and assay media. Quaternary derivatives of dexanabinol were found to hydrolyze faster. All examined compounds inhibited NMDA receptor and protected neurons against NMDA induced toxicity. Neuroprotection (with one exception) is however attributed to the parent **1** released by hydrolysis during the assay.

Conclusions: Some of the examined derivatives could be further evaluated as prodrugs on congeners of **1.**

KEY WORDS: dexanabinol; prodrugs; congeners; neuroprotection.

INTRODUCTION

Dexanabinol (HU-211), the (+) 3S, 4S-5′-(1′,1′-dimethylheptyl)-7-hydroxy-Δ^6-tetrahydrocannabinol (**1**), a synthetic nonpsychotropic cannabinoid (1) is a noncompetitive N-methyl-D-aspartate (NMDA) receptor inhibitor (2) and a potent radical scavenger (3). Based on promising *in vitro* and *in vivo* results (4–7) the development of **1** as a therapeutic agent with potential use in the treatment of brain damage associated with stroke, cardiac arrest, ischemia and trauma has been initiated.

The nonpolar character of **1,** which enables it to cross the blood-brain barrier and gain access to the central nervous system is associated with poor solubility in water. For this reason it is difficult to develop aqueous formulations, suitable for intravenous administration of **1.** Water-soluble esters of **1** can be used as prodrugs (8), or active analogs depending on their hydrolytic and enzymatic stability and on their intrinsic activity. Various esters of **1** containing polar or permanent charge bearing moieties were synthesized and investigated (9). Detailed studies of the glycinate and N-substituted glycinate salts of **1** (10) indicated that 7-trimethyl- and triethylammonium acetates might be good, water-soluble prodrugs candidates since they are fairly stable in aqueous media and rapidly release **1** in human blood plasma. Synthesis and preliminary evaluation of another series of derivatives of **1,** the amino acid esters bearing nitrogen heterocycles (morpholine, N-methyl piperazine) or their quaternary derivatives are described herein. The new compounds are carboxylic esters at the allylic (C-7) or phenolic (C-3′) hydroxylic groups of **1.**

MATERIALS AND METHODS

Uncorrected melting points were determined on an Electrothermal melting point apparatus (Fisher Scientific). Elemental microcombustion analyses were performed by Atlantic Microlabs Inc., Atlanta, GA. Infrared (IR) spectra were determined on a Perkin-Elmer spectrometer in KBr pellets or liquid form. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on a Varian XL-300 spectrometer. Samples were dissolved in an appropriate deuterated solvent and chemical shifts are reported as parts per million (δ) relative to tetramethylsilane (0.00) which served as an internal standard. Coupling constants (J) are reported in Hertz. Thin layer chromatography was performed on EM reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with silica gel (60 mesh). All solvents and chemicals were reagent grade. Dexanabinol was obtained from Casali Institute of Applied Chemistry, Hebrew University of Jerusalem, Israel.

7-O-[2-(4-Morpholino)acetyl] dexanabinol (**3a**). To a solution of dexanabinol (**1**) (0.194 g, 0.50 mmol), 4-morpholinyl acetic acid hydrochloride (**2a**) (0.091 g, 0.50 mmol) (11) and N,N-dimethylamino-pyridine (DMAP) (1.2 mg) in dry methylene chloride (8 mL), was added dicyclohexylcarbodiimide (DCC) (0.103 g, 0.50 mmol) and the mixture was stirred at 20–25°C for 5 days. The precipitated dicyclohexylurea (DCU) was filtered and washed with methylene chloride (3 × 5 mL). The combined organic solutions were partitioned against 5% aqueous $NaHCO_3$ (25 mL), dried over anhydrous $MgSO_4$ and evaporated *in vacuo* (below 40°C). The resulting residue (0.319 g) was purified by column chromatography (30 g silica gel, eluent cyclohexane:diethylamine, 8:2) affording **3a** (0.154 g, yield 60%) as a light brown semi-solid. R_f (cyclohexane:diethylamine, 8:2 v/v) 0.44; Anal. Calcd for $C_{31}H_{47}NO_5$: C, 72.48; H, 9.22; N, 2.73. Found: C, 72.51; H, 9.25; N, 2.92.

7-O-[2-(4-Morpholino)butyryl]-dexanabinol (**3b**). To a solution of **1** (0.194 g, 0.50 mmol), 4-morpholinyl butyric acid hydrochloride (**2b**) (12) (0.105 g, 0.50 mmol) and DMAP (1.2 mg) was added DCC (0.103 g, 0.50 mmol) and the mixture was stirred at 20–25°C for 16 h. The precipitated DCU was filtered off, and the product isolated as described for **3a.** The crude material (0.281 g) was purified by column chromatogra-

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phy (25 g, silica gel, eluent cyclohexane:diethylamine, 8:2 v/v) to afford **3b** (0.221 g, 82%) as a pale yellow oil. R_f (cyclohexane:diethylamine, 8:2, v/v) 0.42; IR (film, ν cm:$^{-1}$ 3358 (ν_{as} OH), 2927 (ν_{as} $CH_{2,3}$), 2852 (ν_s $CH_{2,3}$), 1736 (ν C=O, aliphatic ester), 1621 (ν C=C, olefin), 1576 (phenyl), 1450 (δ CH_3), 1266 (ν_{as} C-O-C), 1185 (ν_s C-O-C), 1118 (ν C-O), 967 (γ C-H aromatics), 859 (γ C-H, olefin); Anal. Calcd for $C_{31}H_{51}NO_5$: C, 73.15; H, 9.49; N, 2.59. Found: C, 72.92; H, 9.53; N, 2.62.

7-O-[4-(N-Methyl-1-piperazino)acetyl]-dexanabinol (**3c**). To a mixture of **1** (0.194 g, 0.50 mmol), (4-methyl-1-piperazinyl) acetic acid (**2c**) (13), (0.079 g, 0.50 mmol) and DMAP (1.2 mg) in dry methylene chloride (8 mL), was added DCC (0.103 g, 0.50 mmol) and the mixture was stirred at 20–25°C for 4 days. The precipitated DCC was filtered and rinsed with methylene chloride, and the organic solutions washed with 5% aqueous $NaHCO_3$ (25 mL), dried over anhydrous $MgSO_4$ and evaporated *in vacuo* (below 40°C). The residue (0.345 g) was purified by chromatography (30 g, silica gel, eluent: cyclohexane:diethylamine, 8:2 v/v) to provide 0.137 g (52%) of **3c** as a pale yellow thick oil. R_f (cyclohexane-diethylamine, 8:2, v/v): 0.37; Anal. Calcd for $C_{32}H_{50}N_2O_4$: C, 72.96; H, 9.57; N, 5.32. Found: C, 73.16; H, 9.85; N, 5.54.

7-O-[4-(4-Methyl-1-piperazino)butyryl]-dexanabinol (**3b**). To a mixture of **1** (0.194 g, 0.50 mmol), 4-(4-methyl-1-piperazinyl) butyric acid (**2d**) (14) (0.144g, 0.50 mmol), DMAP (1.2 mg) in dry methylene chloride (15 mL), was added DCC (0.103 g, 0.50 mmol) and the mixture was stirred at 20–25°C for 3 days. The DCC was filtered, rinsed with methylene chloride, the organic solution washed with 5% aqueous $NaHCO_3$ (2 × 25 mL), dried over $MgSO_4$ and evaporated *in vacuo* below 40°C. The residue (0.328 g) was purified by column chromatography (50 g, silica gel, eluent cyclohexane: diethylamine, 8:2 v/v), to provide 0.142 g (51%) of pure **3d** as a pale yellow gum-like material. R_f (cyclohexane:diethylamine, 8:2): 0.43; IR (film, ν, cm^{-1}): 3000 (very broad, ν OH), 2930 (ν $CH_{2,3}$), 1734 (ν C=O, aliphatic ester), 1620 (ν C=C olefin), 1575 (phenyl), 1461 (δ CH_3), 1371 (gem. dimethyl), 1283 and 1186 (ν C-O-C), 1087 (ν C-O, phenol), 968 (γ C-H aromatics), 816 (γ C-H olefin); Anal. Calcd for $C_{34}H_{54}N_2O_4$: C, 73.60; H, 9.81; N, 5.05. Found: C, 73.80; H, 10.12; N, 4.85.

3'-O-[2-(4-Morpholino)butyryl]-dexanabinol (**7**). To a mixture of 7-trifluoroacetyl dexanabinol (**6**) (obtained from **1** and trifluoroacetic acid) (15) (0.241 g, 0.50 mmol), 4-morpholinyl butyric acid hydrochloride (0.105 g, 0.50 mmol) and DMAP (1.2 mg) in dry methylene chloride (8 mL), was added DCC (0.103 g, 0.50 mmol) and stirred at 20–25°C for 16 h. The DCU was filtered, the organics were washed with 5% aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* below 40°C. The residue (0.330 g) was purified by chromatography (25 g silica gel, eluent toluene: diethylamine, 9:1 v/v), to give 0.134 g (71%) of pure **7** as a pale yellow thick oil. R_f (toluene:diethylamine, 9:1) 0.45; IR (film, ν, cm^{-1}): 3408 (ν OH), 2929 (ν_{as} $CH_{2,3}$), 2856 ($\nu_s CH_{2,3}$),1757 (ν C=O, phenolic ester), 1624 (ν C=C olefin), 1563 (phenyl), 1459 (δ CH_3), 1370 (gem. dimethyl), 1119 (ν C-O), 864(γ C-H olefin); Anal. Calcd for $C_{33}H_{51}NO_5$: C, 73.15;H, 9.49; N, 2.59. Found: C, 72.93; H, 9.50; N, 2.60.

General Procedure for the Preparation of Hydrobromide Salts (**4, 8**). To a solution of free base (**3, 7**) in dry methylene chloride, one or two equivalents of an HBr solution in dry methylene chloride was added slowly at 0–5°C. The solvent was evaporated *in vacuo,* the residue was dissolved in 500 volumes of deionized water (vortex for 5 min) then the solution was filtered through a fritted glass filter (porosity M) and freeze dried. The mono hydrobromides are white or off-white hygroscopic solids.

General Procedure for the Preparation of Quaternary Salts (**5, 9**). To a solution of free base (**3, 7**) in dry acetone, 10 equivalents of iodomethane was added. The solution was kept at 20–25°C for 2 days, then evaporated *in vacuo* below 40°C. The residue was slurried with dry ethyl ether or n-hexane, filtered and dried *in vacuo.* The resulting quaternary salts (**5a–c** and **9**) are light yellow or off-white solids.

Stability, Radioligand, and Activity-Neurotoxicity Studies

All studies were conducted according to previously described procedures (10).

RESULTS AND DISCUSSION

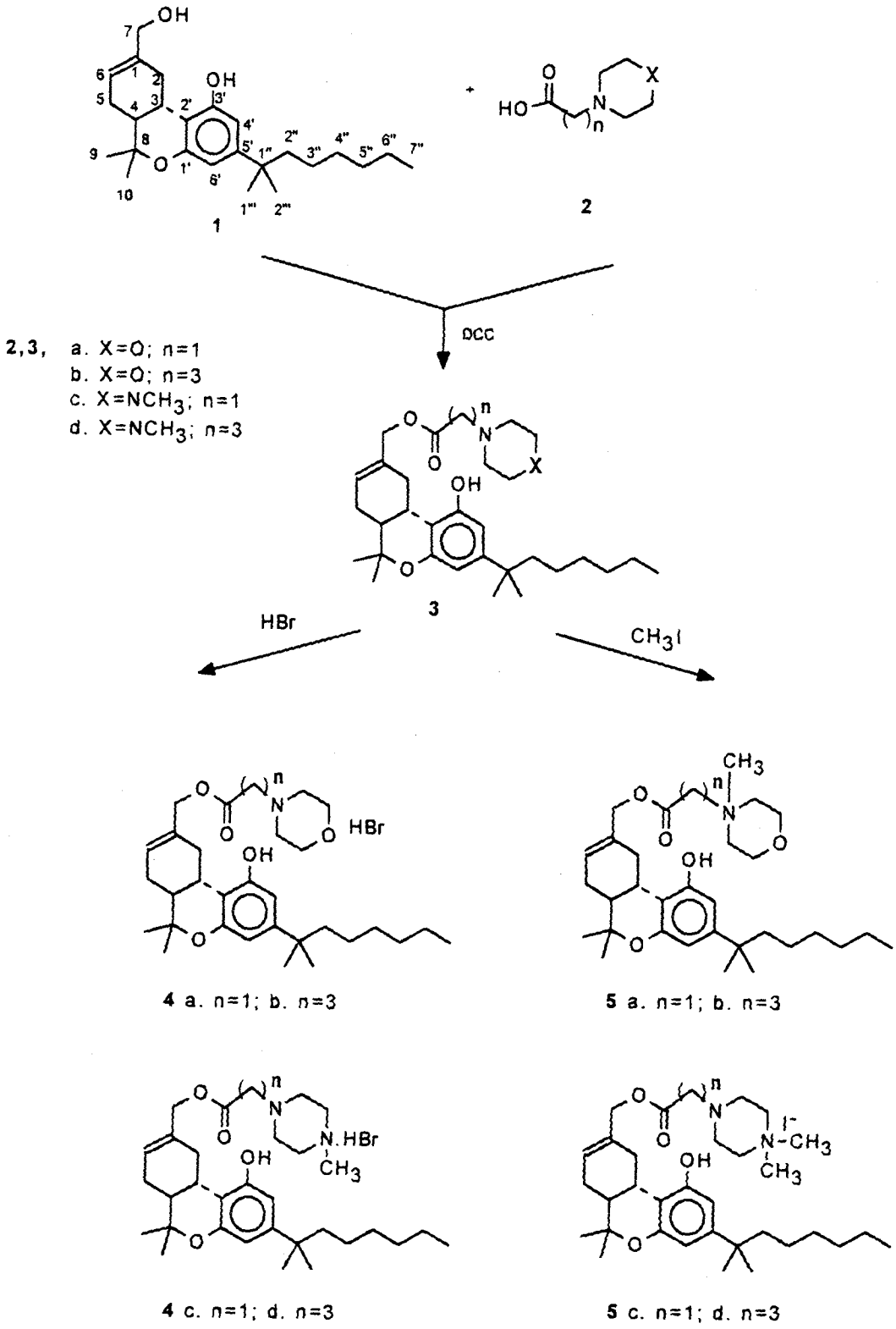
Chemistry. Esters of **1** were synthesized by acylation of the allylic alcohol (C-7) or the phenol (C-3′) with heterocyclic moiety containing acids. The acylation occurs preferentially at C-7 although small amounts of phenolic esters generally accompanied the major product. The latter contaminants were removed by column chromatography. The synthesis of the phenolic (C-3′) esters was accomplished by acylation of the 7-O protected dexanabinol.

The 4-morpholinyl acetic and butyric acids (**2a** and **2b** respectively) as well as the 4-(4-methylpiperazinyl) acetic and butyric acids (**2c** and **2d**, respectively), employed as acylating agents, were obtained using reported procedures (11–14).

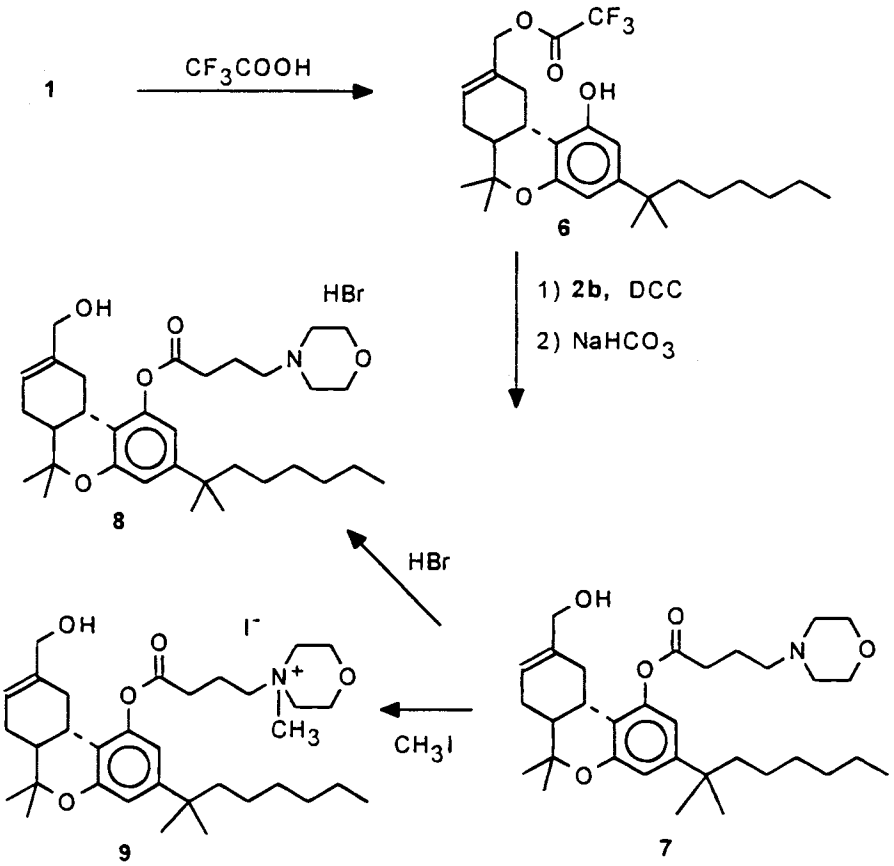
The acylation of **1** with **2 a–d** was performed in methylene chloride using 1,3-dicyclohexylcarbodiimide (DCC) as dehydrating agent and dimethylaminopyridine (DMAP) as catalyst, followed by column chromatography of the resulting esters (**3a–d**). Hydrobromide salts were then prepared by treatment of the free base esters with HBr in methylene chloride. Quaternary N-methylmorpholinium and piperazinium iodides (**5a–d**) were obtained by reacting **3a–d** with methyl iodide in acetone (Scheme 1).

A convenient protection procedure of the allylic C-7 hydroxylic group of **1** proved to be *via* the trifluroacetate **6**, easily obtained by reacting **1** in chloroform with trifluoroacetic acid (15). The acylation with **2b** of the phenolic group of **6** was accomplished in the conditions used for the allylic alcohol. The allylic alcohol was easily deprotected by simply washing the methylene chloride solution of the product with 5% aqueous sodium bicarbonate. The resulting ester **7** (Scheme 2) was purified by column chromatography. The hydrobromide salt **8** and the quaternary derivative **9** were obtained as described before.

Compound Stability in Water and Susceptibility to Hydrolysis in Rat, Dog, and Human Plasma. The synthesized hydrobromides (**4, 8**) and quaternary ammonium salts (**5, 9**) were


1
2
DCC
2,3, a. X=O; n=1
b. X=O; n=3
c. X=NCH3; n=1
d. X=NCH3; n=3
3
HBr
CH3I
4 a. n=1; b. n=3
5 a. n=1; b. n=3
4 c. n=1; d. n=3
5 c. n=1; d. n=3

Scheme 1



Scheme 2

soluble in water or 10% aqueous ethanol in the range of 4.9 to 7.3 mg/mL.

The stability of selected compounds (**4b,4d,8**) was examined in water (aqua pura) (pH 5.6–5.7) and plasma (rat, dog, human) by measuring % of **1** formed *via* hydrolysis by HPLC. Three time points (0, 1 and 24 h) were considered in this study the purpose of which was to determine the potential use of the new derivatives as prodrugs or analogs. Stabilities were also determined in buffers and media used for receptor binding and activity tests. The results, summarized in Table 1, indicate that the investigated esters were stable in water for as long as 24 h. The most stable was **4d** which released less than 3% of **1** in 24 h. Derivative **4b** and the corresponding phenolic ester (**8**) were somewhat less stable but still more than 90% of the compounds were found unchanged after 24 h of incubation. The stability studies performed in plasma indicated, as expected, that esterase activity was species-specific (10). In each case, the fastest hydrolysis occurred in rat plasma. Compound **4b** hydrolyzed only 23% and 32% after 1 and 24 h respectively, while **4d** and especially **8** appeared to be better substrates for rat blood esterases, hydrolyzing completely after 24 h. No significant difference was observed however in the stability of these compounds in dog and human plasma. The stability increased in both dog and human as compared to the rat plasma. Both allylic esters (**4d** and particularly **4b**) released only small amounts of **1** after 24 h of incubation. More importantly, in human plasma, hydrolysis was less than 10%. The phenolic ester **8** was again the least stable, hydrolyzing ~60% in dog and 55% in human plasma.

Table 1. Stability of Dexanabinol Esters Containing Nitrogen Heterocycles

		Dexanabinol Recovered (%)[a]			
Comp.	Time (h)	Water	Rat Plasma	Dog Plasma	Human Plasma
4b	0	0.00	0.00	0.00	0.00
	1	7.97	23.02	6.09	5.81
	24	7.87	32.06	9.37	8.06
4d	0	0.00	0.00	0.00	0.00
	1	2.74	43.60	2.86	2.11
	24	2.98	101.74	12.68	8.70
8	0	0.00	0.00	0.00	0.00
	1	5.58	87.00	8.79	8.32
	24	11.43	100.00	59.06	54.99

[a]Mean recovery ± max 10% SD.

Receptor Binding Studies. The inhibition of [^{3}H] MK-801, a standard noncompetitive NMDA antagonist, was determined for most of the synthesized compounds. The radioligand displacement procedure was performed using embryonic rat forebrain membranes. Preliminary hydrolysis experiments were performed for various esters in the assay media with or without the presence of rat forebrain membranes. Esters at concentrations of 10 or 100 μM were incubated for 2 h, the duration

of activity tests, in this media. No significant (less than 5%) hydrolysis was registered in these conditions for any of the examined compounds. This implies that concentrations of dexanabinol after the incubation were too low to inhibit [^{3}H] MK-801 binding and hence the receptor binding characteristics observed are properly assigned to the esters themselves and not to the potential hydrolysis product **1**. The results collected in Table 2 indicate that all of the novel compounds inhibit [^{3}H] MK-801 binding. Derivatives **4c**, **5b** and **9**, which demonstrate some diversity in structures, proved to be the most active inhibitors (IC_{50}, 2–3.8 μM as compared to 7.5 μM for dexanabinol). Compound **4d** had the highest IC_{50} (19 μM). All of the compounds are NMDA receptor inhibitors (> 90% inhibition) at 100 μM concentration.

Activity: Neurotoxicity Studies. Addition of NMDA (100–1000 μM) to primary cortical neuronal cultures results in 50–70% cell death within 24 h. Dexanabinol (2–10 μM) has been shown to reduce or totally prevent cell death associated with this type of intoxication (3). The neuroprotective effects of selected novel derivatives have been investigated herein in a similar model.

Cortical cells derived from Sprague-Dawley rats were cultured and prepared as previously described (3). Cells were exposed to dexanabinol esters (or to an appropriate vehicle) with or without NMDA (1 μM) and glycine (300 μM). Cell mortality was quantitatively assessed by measurement of the lactate dehydrogenase (LDH) levels in the extracellular medium 20 h after the incubation was started. The stability of dexanabinol esters (10 μM) in aqueous solutions containing 0.2% of ethanol and 0.25% of bovine serum albumin (BSA) (final concentration) in the incubation medium with or without the presence of cell cultures was determined after 20 h (duration of experiment). The results (in the presence of cell cultures) are included in Table 3. Compounds **5a** and **9** showed high degree of hydrolysis releasing ~80% of dexanabinol. These compounds hydrolyzed to the same extent in the absence of cell cultures (**5a**, ~80%, 9, 46%). Other members of the series proved to be more stable, releasing less than 20% of **1** after 20 h of incubation (with or without cell cultures). The most stable compound was **5b** which did not hydrolyze in vehicle and released only ~5% of **1** in the presence of cell cultures. The activity observed in the case of **5a** and **9** (77% protection and 100% protection in both cases at 5 μM and 10 μM concentration, respectively) is probably due to dexanabinol released by hydrolysis; on the other hand, compound **5b** appears to have intrinsic activity (85% and 100% protection against NMDA toxicity at 5μM and 10 μM concentration) since a concentration of only 0.5 μM of **1** (associated with hydrolysis) cannot explain the high protection level attained. For the other members of the series the results are not as conclusive; the activity may be attributed to the ester, to the released dexanabinol or to both. At concentrations of 10 μM (not included in Table 3) all the examined esters manifested significant protection (100%) against NMDA-mediated cell death. However at this concentration most of the compounds also manifested some degree of cytotoxicity. By reducing the concentration to 5 μM, the toxicity of these compounds was considerably reduced (not tabulated) but the protection effect was also reduced, in several cases being less than 100%.

Table 2. Receptor Binding Properties of Dexanabinol Esters Containing Nitrogen Heterocycles

Compound	Inhibition of [^{3}H] MK-801 binding[a,b]	
	IC_{50}, μM	% inhibition at 100 μM
1	7.5	95
4b	6.8	92
4c	2.0	97
4d	19.0	94
5a	8.0	91
5b	2.0	94
5c	3.8	95
8	6.0	90
9	3.0	95

[a]All compounds stable (<5% hydrolysis to 1) in buffer and buffer + forebrain membranes for 2 h (duration of experiments).
[b]Average values of 2 experiments.

Table 3. Activity of Dexanabinol Esters Containing Nitrogen Heterocycles

Compound	Hydrolysis (% of 1 released) after 20h incubation (mean recovery ± 10% SD)	Mean protection against NMDA toxicity at 5μM (range for 2–4 experiments)
4c	11.5	86 (78–94)
4d	14.4	69 (21–118)
5a	80.0	77 (60–94)
5b	4.5	95 (89–101)
5c	20.4	83 (64–101)
9	79.0	77 (65–89)

CONCLUSION

The morpholino- and N-methylpiperazino acetyl or butyryl esters of dexanabinol increased the aqueous solubility of the drug. All of the compounds prepared proved to be inhibitors of MK-801 binding to the NMDA receptor and manifested high protection against NMDA induced toxicity in cell culture. However, some of the compounds did exert varying degrees of neurocytotoxicity at the active doses. While receptor binding are intrinsic properties of the new derivatives, the neuroprotection can be probably attributed to the dexanabinol released by hydrolysis during the experiments and only in one case, i.e., the 7-O-[2-(N-methyl-4-morpholinium)-butyryl dexanabinol (**5b**), to the ester. This compound will be further examined as a possible active analog. Compounds such as **5a** and **9** which are less stable hydrolytically, will be investigated as possible prodrugs.

ACKNOWLEDGMENTS

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APPENDIX

NMR Data for the Novel Derivatives

(3a). ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, t, $J_{7'',6''}$ = 6.9 Hz, C7''-H_3), 0.95–1.29 (m, including 2 singlets at 1.11 [C10-H_3]

and 1.20 [C1‴-H_3 + C2‴-H_3]), 1.39 (3H, s, C9-H_3), 1.45–1.53 (2H, m), 1.77–1.95 (3H, m, C2-Hβ + C4-H + C5-Hα), 2.18–2.29 (1H, m, C5-Hβ), 2.57–2.65 (4H, m, N-CH_2 morpholine), 2.65–2.75 (m, C3-H), 3.26 (2H, s, CO-CH_2-N), 3.42 (1H, dd, $J_{2\alpha,2\beta}$ = 16.1 Hz, $J_{3,2\alpha}$ = 4.5 Hz, C2-Hα), 3.73–3.80 (4H, m, O-CH_2, morpholine), 4.57 (2H, AB qa, C7-H_2), 5.77–5.82 (1H, m, C6-H), 6.23 (1H, d, $J_{4',6'}$ = 1.8 Hz, C4′-H), 6.36 (1H, d, C6′-H); ^{13}C NMR 14.05 (C7″), 18.43 (C10), 22.65 (C6″), 24.61 (C5″), 27.55 (C9), 27.73 (C5), 28.67 and 28.73 (C1‴ + C2‴), 30.02 (C4″), *31.26 (C2), 31.77 (C3″), *31.93 (C3), 37.31 (C1″), 44.49 (C2″), 44.71 (C4), 53.14 (N-CH_2, morpholine), 59.64 (CO-CH_2-N), 66.80 (O-CH_2, morpholine), 68.57 (C7), 76.33 (C8), 105.45 (C4′), 107.55 (C6′), 109.64 (C2′), 124.76 (C6), 133.50 (C1), 150.10 (C5′), 154.39 and 154.92 (C1′ and C3′), 170.05 (CO);

(3b). ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, t, $J_{7'',6''}$ = 7.0 Hz, C7″-H_3), 0.95–1.3 (m, including 2 singlets at 1.11 [C10-H_3] and 1.20 [C1‴-H_3 + C2‴-H_3]}, 1.39 (3H, s, C9-H_3), 1.45–1.55 (2H, m), 1.78–1.96 (3H, m, C-Hβ + C4-H + C5-Hα), 2.30–2.75 (5H, m, N-CH_2, morpholine, C3-H), 3.44 (1H, dd, $J_{2\alpha,2\beta}$ = 16.5 Hz, $J_{3,2\alpha}$ = 3.6 Hz, C2-Hα), 3.65–3.79 (4H, m, O-CH_2, morpholine), 4.54 (2H, AB qa, C7-H_2), 5.74–5.81 (1H, m, C6-H), 6.26 (d, $J_{4',6'}$ = 1.7 Hz, C4′-H), 6.37 (d, C6′-H); ^{13}C NMR: 14.07 (C7″), 18.34(C10), 21.76 (CO-CH_2-CH_2), 22.65 (C6″), 24.60 (C5″), 27.51 (C9), 27.73 (C5), 28.71 (C1‴ + C2‴), 30.01 (C4″), *31.34 (C2), *31.43 (C3), 31.76 (C3″), 32.35 (CO-CH_2), 37.28 (C1″), #44.51 (C2″), #44.75 (C4), 53.50 (N-CH_2, morpholine), 58.06 (N-CH_2), 66.69 (O-CH_2, morpholine), 68.12 (C7), 76.32 (C8), 106.00 (C4′), 107.50 (C6′), 110.04 (C2′), 124.35 (C6), 133.93 (C1), 150.01 (C5′), 154.29 and 157.07 (C1′ and C3′), 173.22 (CO);

(3c). ^{1}H NMR ($CDCl_3$): δ 0.85 (3H, t, $J_{7'',6''}$ = 7,4 Hz, C7″-H_3), 1.0–1.3 (m, including 2 singlets at 1.13 [C10-H_3] and 1.21 [C1‴-H_3 + C2‴-H_3]), 1.40 (3H, s, C9-H_3), 1.45–1.54 (2H, m), 1.75–1.97 (3H, m, C2-H_β + C4-H + C5-H_α), 2.18–2.29 (1H, m, C5-H_β), 2.39 (3H, s, N-CH_3), 2.42–2.87 (m, C3-H + N-CH2 piperazine), 3.34 (2H, AB qa, $J_{A,B}$ = 16.1 Hz, CO-CH_2-N), 3.68 (1H, dd, $J_{2\alpha,2\beta}$ = 16.7 Hz, $J_{3,2\alpha}$ = 3.9 Hz, C2-Hα), 4.56 and 4.72 (1H, 1H, d, d, $J_{A,B}$ = 12.4 Hz, C7-H_2), 5.74–5.80 (1H, m, C6-H), 6.17 (1H, d, $J_{4',6'}$ = Hz, C4′-H), 6.34 (1H, d, C6′-H); ^{13}C NMR δ: 14.07 (C7″), 18.42 (C10), 22.65 (C6″), 24.63 (C5″), 27.54 (C9), 27.84 (C5), 28.75 and 28,82 (C1‴ and C2‴), 30.04 (C4″), *31.38 (C2), 31.79 (C3″), *31.97 (C3), 37.28 (C1″), 44.56 (C2″), 44.83 (C4), 45.72 (N-CH_3), 51.24 and 55.00 (piperazine), 59.46 (CO-CH_2-N), 68.60 (C7), 76.38 (C8), 105.97 (C4′), 106.98 (C6′), 110.33 (C2′), 124.18 (C6), 133.99 (C1), 149.87 (C5′), 154.29 and 155.44 (C1′ and C3′), 170.38 (CO).

(3d). ^{1}H NMR ($CDCl_3$): δ 0.84 (t, $J_{7'',6''}$ = 6.6 Hz, C7″-H_3), 0.95–1.3 (m, including 2 singlets at 1.11 [C10-H_3] and 1.21 [C1‴-H_3 + C2‴-H_3]), 1.39 (s, C9-H_3), 1.45–1.55 (m), 1.8–2.0 (m, C2-Hβ + C4-H + C5-Hα), 2.2–2.75 (m, including a singlet at 2,34 [N-CH_3]), 3.58 (dd, $J_{2\alpha,2\beta}$ = 16.8 Hz, $J_{3,2\alpha}$ = 3.8 Hz, C2-Hα), 4.54 (AB qa, $J_{A,B}$ = 12 Hz, C7-H_2), 5.79 (d, J = 4.8 Hz, C6-H), 6.22 (d, $J_{4',6'}$ = 1.8 Hz, C4′-H), 6.34 (C6′-H); ^{13}C NMR: δ 13.96 (C7″), 18.37 (C10), 21.81 (CO-CH_2-CH_2), 22.59 (C6″), 24.61 (C5″), 27.54 (C9), 27.82 (C5), 28.71 and 28.78 (C1‴ and C2‴), 30.00 (C4″), *31.49 (C2), *31.69 (C3), 31.73 (C3″), 32.94 (CO-CH_2), 37.29 (C1″), 44.54 (C2″), 44.89 (C4), 45.84 (N-CH_3), 52.09 and 54.92 (piperazine), 57.46 (N-CH_2), 68.36 (C7), 76.19 (C8), 105.90 (C4′), 107.22 (C6′), 110.07 (C2′), 124.75 (C6), 134.15 (C1), 134.15 (C1), 149.88 (C5′), 154.41 and 154.34 (C1′ and C3′), 173.13 (CO).

(5a). ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, t, $J_{7'',6''}$ = 6.8 Hz, C7″-H_3), 1.11 (3H, s, C10-H_3), 1.18 (6H, s, C1‴-H_3 + C2‴-H_3), 1.38 (3H, s, C9-H_3), 3.78 (3H, s, N^+CH_3), 3.90–4.30 (10H, m, 3× [N^+CH_2] + CH_2-O-CH_2), 4.67 (2H, AB qa, $J_{A,B}$ = 11.5 Hz, C7-H_2), 5.8 (1H, C6-H), 6.35 and 6.50 (2 × 1H, C-4′-H + C6′H).

(5b). ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, t, $J_{7'',6''}$ = 6.6 Hz, C7″-H_3), 1.10 (3H, s, C10-H_3), 1.18 (6H, C1‴-H_3 + C2‴-H_3), 1.38 (3H, s, C9-H_3), 3.53 (3H, s, N^+CH_3), 3.60–4.20 (10H, m, 3 × [N^+CH_2] + CH_2-O-CH_2}, 4.61 (2H, AB qa, $J_{A,B}$ = 12 Hz, C7-H_2), 5.70 (1H, C6-H), 6.33 and 6.67 (2 × [1H, d, $J_{4',6'}$ = 1.6 Hz], C4′-H + C6′-H).

(5c). ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, t, $J_{7'',6''}$ = 6.6 Hz, C7″-H_3), 1.10 (3H, s, C10-H_3), 1.19 (6H, s, C1‴-H_3 + C2‴-H_3), 1.38 (3H, s, C9-H_3), 3.15 (4H, 2 × [NCH_2]), 3.51 (6H, s, 2 × [N^+CH_3]), 3.72 (4H, 4 × [N^+CH_2]), 3.90–4.10 2H, OC-CH_2), 4.59 (2H, s, C7-H_2), 5.80 (1H, C6-H), 6.33 and 6.84 (2 × [1H, d, $J_{4',6'}$ = 1.6 Hz], C4′-H + C6′-H).

(7). ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, t, $J_{7'',6''}$ = 6.7 Hz, C7″-H_3), 1.0–1.35 (m, including 2 singlets at 1.12 [C10-H_3] and 1.23 [C1‴-H_3 + C2‴-H_3]), 1.39 (3H, s, C9-H_3), 1.47–1.58 (2H, m), 1.75–2.6 (m), 2.79–2.82 (1H, m, C3-H), 3.20 (1H, dd, $J_{2\alpha,2\beta}$ = 17Hz, C2-Hα), 3.73 (4H, br s, O-CH_2 morpholine), 4.01 (2H, AB qa, $J_{A,B}$ = 12 Hz, C7-H_2), 5.63 (1H, d, J = 4 Hz, C6-H), 6.51 (1H, d, $J_{4',6'}$ = 1.9 Hz, C4′-H), 6.69 (d, C6′-H); ^{13}C NMR: 14.06 (C7″), 18.42 (C10), 21.48 (CO-CH_2-CH_2), 22.61 (C6″), 24.48 (C5″), 27.29 (C9), 27.78 (C5), 28.50 and 28.57 (C1″ 'and C2‴), 29.91 (C4″), *31.51 (C3), 31.69 (C3″), *32.01 (C2), 32.59 (CO-CH_2), 37.45 (C1″), 44.41 (C2″), 45.42 (C4), 53.52 (N-CH_2, morpholine), 57.96 (N-CH_2), 66.48 (C7), 66.54 (O-CH_2, morpholino), 76.74 (C8), *112.16 (C4′), *113.02 (C6′), 115.50 (C2′), 121.59 (C6), 139.10 (C1), 149.64 (C3′), 150.25 (C5′), 154.07 (C1′), 171.34 (CO).

(9). ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, t, $J_{7'',6''}$ = 6.7 Hz, C7″-H_3), 1.11 (3H, s, C10-H_3), 1.22 (6H, s, C1‴-H_3 + C2‴-H_3), 1.39 (3H, s, C9-H_3), 3.51 (3H, s, N^+CH_3), 3.40–4.20 (12H, m, C7-H_2 + 3 × [N^+CH_2] + CH_2-O-CH_2), 5.68 (1H, C6-H), 6.51 and 6.70 (2 × [1H, d, $J_{4',6'}$ = 1.9 Hz], C4′-H + C6′-H).

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