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Synthesis and Resolution of Racemic Eliprodil and Evaluation of the Enantiomers of Eliprodil as NMDA Receptor Antagonists

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Abstract—A short synthesis of the NMDA receptor antagonist (*rac*)-Eliprodil (**9**) and its resolution into the enantiomers by chiral HPLC is described. The enantiomers (*R*)-**9** and (*S*)-**9** were found to exhibit markedly different affinities for NR2B subunit containing NMDA receptors. © 2000 Elsevier Science Ltd. All rights reserved.

(*rac*)-Eliprodil ((*rac*)-**9**) is known as a ligand that is selective for NMDA receptors containing the NR2B subunit. By acting as an antagonist at the ifenprodil or polyamine sensitive recognition site present on this subtype of NMDA receptors it exhibits neuroprotective properties as do other compounds with this mode of action. As a promising drug candidate (*rac*)-Eliprodil ((*rac*)-**9**) was evaluated for stroke,[1,2] until only recently the clinical trials with (*rac*)-Eliprodil ((*rac*)-**9**) were discontinued in phase III after side effects had emerged.[2] For the enantiomers of Eliprodil in a rat ischemia model it has been uncovered, that (*R*)-**9** shows a distinctly higher neuroprotective activity as compared to (*S*)-**9**.[3] Though the reason for the differences found for the enantiomers in their in vivo pharmacology might originate from different receptor affinities the in vitro potencies of the enantiomers of Eliprodil ((*R*)-**9** and (*S*)-**9**) as NMDA receptor antagonists have not been investigated up to now. Thus, it was the aim of the present study to uncover whether the enantiomers of Eliprodil ((*R*)-**9** and (*S*)-**9**) exhibit different binding profiles to NR2B subunit containing NMDA receptors.

In an ongoing study directed toward the development of more complex derivatives of Eliprodil with additional substituents at the piperidine nucleus we utilized a synthetic approach based on *N*-acylpyridinium ions to establish the piperidine core structure of these compounds. For simplicity reasons this method was also applied to the construction of **6**.[4] Furthermore it was envisaged to prepare the pure enantiomers from the racemic material by chiral chromatography, though an asymmetric synthesis is known.[3] This was expected to be more efficient, as a chromatographic procedure would provide both enantiomers in a single step and also would allow to easily check for the enantiomeric purity of the separated isomers.

In the key step for the preparation of **6** the *N*-acylpyridinium ion **3,** in situ generated from pyridine and acetyl chloride at −78 °C in THF, was trapped with the cuprate **4** to give the 4-substituted 1,4-dihydropyridine **5**. This reaction proceeded with high regioselectivity, no other regioisomer was detected.[5] Being aware, that 1,4-dihydropyridines are easily oxidized, compound **5** was subsequently hydrogenated over Pd/C and hydrolized with acetic acid/HCl without prior purification to give **6** in 44% overall yield. Finally, for the last two steps in the synthesis of (*rac*)-**9** the standard procedures displayed in the Scheme 1 were employed.

After some experimentation with various chiral columns it was found that (*rac*)-**9** may be efficiently separated[6] by employing a CHIRALPAK® AD HPLC column[7] to give the pure enantiomers (*R*)-**9** and (*S*)-**9**,[8] (ee for both enantiomers ⩾99%, checked by analytical HPLC).

The affinity of (*rac*)-**9**, (*R*)-**9** and (*S*)-**9** to the ifenprodil recognition site at NMDA receptors was evaluated in a [^{3}H]ifenprodil binding assay employing a synaptosomal fraction of porcine hippocampal brain membranes.[9] The displacement of [^{3}H]ifenprodil binding by the enantiomers was markedly different.

The IC$_{50}$-values exhibited by (*R*)-**9** (eutomer) and (*S*)-**9** (distomer) were 49.5 ± 0.7 nM and 545 ± 120 nM, respectively. This is in good accordance with the IC$_{50}$-value of 82.6 ± 10.0 nM obtained for (*rac*)-**9** (see Table 1 and Fig. 1) and a value of 111 nM given in the literature.[10]

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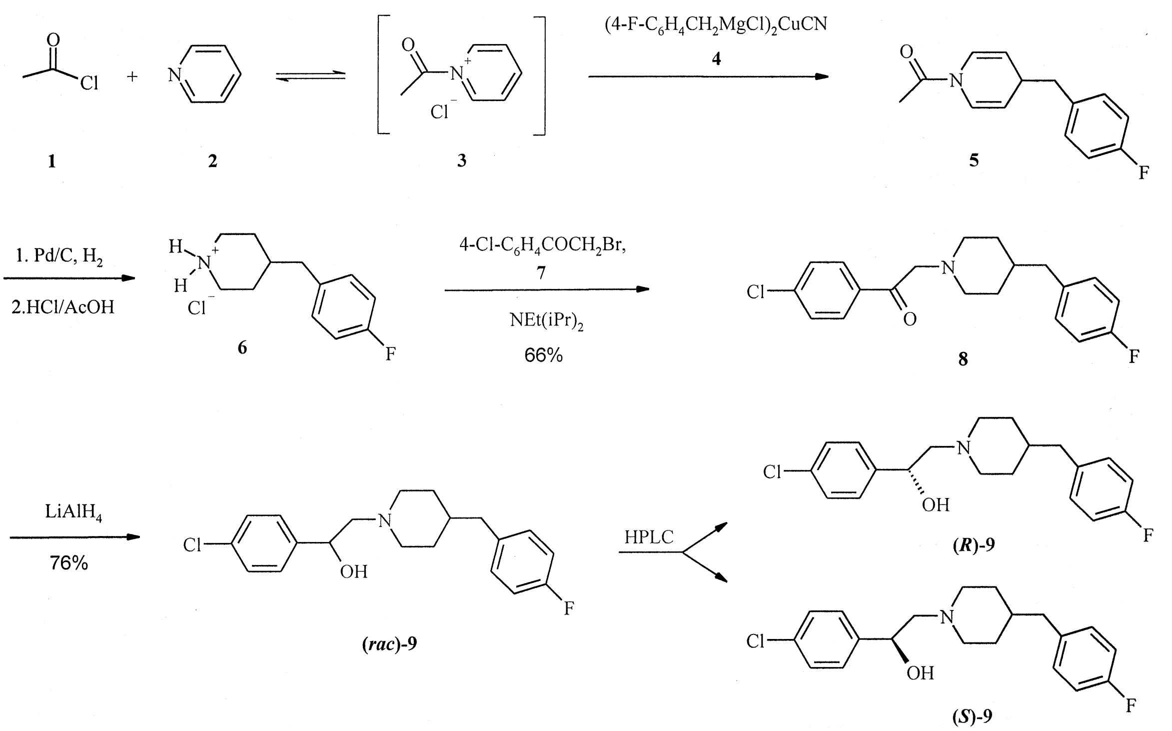

Scheme 1.

Table 1. Displacement of [^{3}H]ifenprodil binding and inhibition of [^{3}H]MK-801 binding

	IC$_{50}$ [^{3}H]Ifenprodil	IC$_{50}$ [^{3}H]MK-801	
		High-affinity fraction	Low-affinity fraction
(*R*)-9	49.5±0.7 nM	213±76 nM	23.7±7.8 μM
(*S*)-9	545±120 nM	5.10±0.77 μM[a]	
(*rac*)-9	82.6±10.0 nM	277±103 nM	11.3±2.7 μM

[a]Not assigned.

NR2B subunit selective NMDA receptor antagonists such as ifenprodil or (*rac*)-**9** have been shown to inhibit [^{3}H]MK-801 or [^{3}H]TCP binding to brain membranes under non-equilibrium conditions in a biphasic way, with a high-affinity phase reflecting allosteric inhibition at NR2B subunit containing NMDA receptors and a low-affinity phase reflecting inhibition at NMDA receptors lacking NR2B subunits.[10–12] A correlation between the displacement of [^{3}H]ifenprodil binding and the high-affinity phase of inhibition of [^{3}H]MK-801 or [^{3}H]TCP binding to brain membranes attributed to NR2B subunit containing NMDA receptors has been demonstrated for this type of NMDA receptor antagonists.[10,11] Therefore it appeared of interest to study the inhibition of [^{3}H]MK-801 binding effected by the enantiomers (*R*)-**9** and (*S*)-**9** as well. These experiments were performed using the synaptosomal fraction of porcine hippocampal brain membranes mentioned above, however, this time in the presence of 100 μM L-glutamate and 30 μM glycine.[9,11]

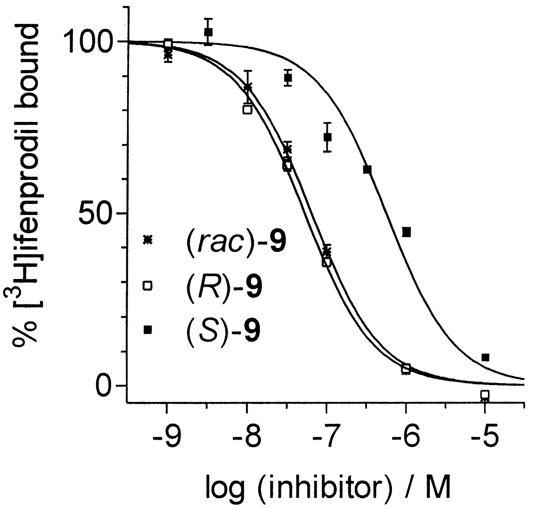

Figure 1. Displacement of [^{3}H]ifenprodil binding (means ± SEM of triplicates from one representative experiment out of three) to porcine hippocampal brain membranes.[9]

(*R*)-**9** Inhibited [^{3}H]MK-801 binding under these conditions in a biphasic manner just as (*rac*)-**9** with IC$_{50}$-values for the high-affinity fraction of inhibition of [^{3}H]MK-801 binding being 213 ± 76 nM ((*R*)-**9**) and 277 ± 103 nM ((*rac*)-**9**), respectively. These values correspond well to the potencies obtained for the displacement of [^{3}H]ifenprodil binding. For the low-affinity fraction of inhibition of [^{3}H]MK-801 binding IC$_{50}$-values of 23.7 ± 7.8 μM ((*R*)-**9**) and 11.3 ± 2.7 μM ((*rac*)-**9**), respectively, were calculated (see Table 1 and Fig. 2).

In contrast to the biphasic inhibition of [^{3}H]MK-801 binding caused by (*R*)-**9** and (*rac*)-**9**, for the enantiomer (*S*)-**9** only a monophasic inhibition of [^{3}H]MK-801 binding with an IC$_{50}$ of 5.10 ± 0.77 μM) ($n_H = 1.05$) was found (see Table 1 and Fig. 2). It may be assumed, that the affinities of (*S*)-**9** for the sites attributed to allosteric inhibition at NR2B subunit containing NMDA receptors and

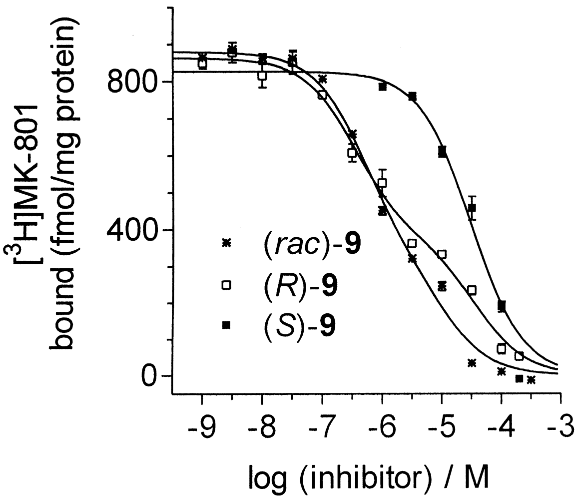

Figure 2. Inhibition of [^{3}H]MK-801 binding (means ± SEM of triplicates from one representative experiment out of three) to porcine hippocampal brain membranes in the presence of 100 µM L-glutamate and 30 µM glycine after incubation for 15 min (non-equlibrium conditions).[9]

to inhibition at NR2B subunit lacking NMDA receptors, respectively, are close together leading to a monophasic curve. Alternatively the affinity of (*S*)-**9** for the site attributed to allosteric inhibition at NR2B subunit containing receptors may be so low that the effect mediated at this site could not be detected in the [^{3}H]MK-801 binding assay. This could be due to a higher affinity of (*S*)-**9** for the radioligand binding site as compared to the site attributed to allosteric inhibition at NR2B subunit containing receptors.

The results of the evaluation of both enantiomers of (*rac*)-**9** at NMDA receptors from porcine hippocampal brain membranes suggest, that (*R*)-**9** shows a substantially higher affinity for NR2B subunit containing versus NR2B subunit lacking NMDA receptors. Although the affinity found for (*S*)-**9** cannot be assigned to one of the two binding sites that have been discussed above, it is obvious that the potency of (*S*)-**9** at the NR2B subunit containing NMDA receptors must be far lower. Assuming, that the neuroprotective effects of (*rac*)-**9** are mediated at NMDA receptors containing NR2B subunits, our results are in line with an investigation, which demonstrated, that (*R*)-**9** shows a distinctly higher neuroprotective activity in a rat ischemia model as compared to (*S*)-**9**.[3] At a first view this seems to be in contrast to a conclusion of the authors of the aforementioned study according to which a discrepancy between their in vivo data and the results of an in vitro study exists.[13] Though the later study describes that (*R*)-**9** and (*S*)-**9** are almost equally potent, this refers to σ and not to NMDA receptors.

Experimental and Analytical Data

4-(4-Fluorobenzyl)-piperidine hydrochloride[4] (6). 984 mg (8 mmol) of **1** and 632 mg (8 mmol) of **2** in 14 mL of THF were stirred for 1 h at −78 °C. To the resulting suspension **4** generated from 786 mg (8.78 mmol) of CuCN and 25.8 mL (17.54 mmol) of 4-F-$C_6H_4CH_2MgCl$ (0.68 M) in 23 mL of THF was added. After 18 h first 18 mL of phosphate buffer (1M, pH 7) and after warming to rt 10 mL of a saturated NH_4Cl-solution was added. The aqueous layer was extracted with H_2CCl_2 (4×) and the combined organic phases were dried (Na_2SO_4) and concd iv. The residue was dissolved in 180 mL of ethyl acetate and hydrogenated for 24 h at 1 atm in the presence of 970 mg of Pd/C (10%). Filtration and evaporation of the solvent iv yielded a colorless solid, which was dissolved in 12 mL of acetic acid and 6 mL of concd HCl and heated to 120 °C for 4 h in a sealed tube. Then, at rt H_2O was added and the mixture was washed with Et_2O. Finally the aqueous layer was brought to pH 12–14 by the addition of KOH and extracted with Et_2O. The combined organic layers were dried ($MgSO_4$), treated with HCl_{gas} and concd iv to give a residue which upon recrystallisation from Et_2O/EtOH yielded **6** as colorless crystals, 809 mg (44%); mp 103–104 °C; IR (KBr) $\tilde{\nu} = 2922, 1599, 1583, 1511\ cm^{-1}$; ^1H NMR ($CDCl_3$) $\delta = 1.50$–1.75 (m, 3H, CH_2CHCH_2), 1.82 (d_{br}, $J = 11.0$ Hz, 2H, CH_2CHCH_2), 2.57 (d, $J = 6.2$ Hz, 2H, CH_2Ph), 2.79 (qu_{br}, $J \sim 12$ Hz, 2H, NCH_{2ax}), 3.46 (d_{br}, $J \sim 12$ Hz, 2H, NCH_{2eq}), 6.94–7.00 (m, 2H, $H_{aromat.}$), 7.05–7.10 (m, 2H, $H_{aromat.}$), 9.36 (s_{br}, 1H, $^+NH_2$), 9.65 (s_{br}, 1H, $^+NH_2$); MS (CI, CH_4): *m*/*z* (%): 194 (100)[$M + H^+ - HCl$]. Anal. calcd for $C_{12}H_{17}NFCl$: C 62.74; H 7.46; N 6.10. Found: C 62.78; H 7.49; N 6.03.

1-(4-Chlorophenyl)-2-[4-(4-fluorobenzyl)-piperidin-1-yl]-ethanon[4] (8). To a solution of 800 mg (3.48 mmol) of **6** in 15 mL methanol and 45 mL acetone, 1.2 mL (7.31 mmol) of NEt^iPr_2 and 894 mg (3.83 mmol) of 2-bromo-4′-chloroacetophenone was added. After 2 h 2N HCl was added until pH 1–2 was reached and extracted with Et_2O. With KOH the aqueous layer was brought to pH 12–14 and again extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and evaporated to dryness. The resulting residue was purified by flash chromatography (heptane:ethyl acetate 6:4). Colorless crystals (66%); mp 66 °C; IR (KBr) $\tilde{\nu} = 2937, 2918, 1691, 1589, 1508, 1212\ cm^{-1}$; ^1H NMR ($CDCl_3$) $\delta = 1.38$ (qd, $J \sim 12/3.6$ Hz, 2H, NCH_2CH_{2ax}), 1.44–1.55 (m, 1H, NCH_2CH_2CH), 1.61 (d_{br}, $J \sim 12$ Hz, 2H, NCH_2CH_{2eq}), 2.07 (td, $J \sim 12/2.2$ Hz, 2H, NCH_{2ax}), 2.50 (d, $J = 7.0$ Hz, 2H, $PhCH_2$), 2.94 (d_{br}, $J \sim 12$ Hz, 2H, NCH_{2eq}), 3.71 (s, 2H, NCH_2CO), 6.92–6.98 (m, 2H, $H_{aromat.}$), 7.05–7.11 (m, 2H, $H_{aromat.}$), 7.40–7.44 (m, 2H, $H_{aromat.}$), 7.95–8.00 (m, 2H, $H_{aromat.}$); MS (CI, CH_4): *m*/*z* (%): 346 (100)[$M + H^+$], 206 (18). Anal. calcd for $C_{20}H_{21}NOClF$: C 69.46; H 6.12; N 4.05. Found: C 69.40; H 6.19; N 4.04.

(*R*,*S*)-(4-Fluorophenyl)-2-[4-(4-fluorobenzyl)-piperidin-1-yl]-ethanol[4] (*rac*-9). To a stirred solution of 45 mg (0.13 mmol) of **8** in 1.5 mL Et_2O 142 µL (0.14 mmol) of $LiAlH_4$ in THF (1M) was added at 0 °C. After 3 h the reaction was quenched with H_2O. Addition of H_2CCl_2 and 2N NaOH gave a clear aqueous layer, which was extracted with H_2CCl_2. The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The resulting solid was purified by flash chromatography (heptane/ethyl acetate 55:45 + 1% $NEtMe_2$). Colorless crystals (76%); mp 129–130 °C; IR (KBr) $\tilde{\nu} = 2919, 2803, 2769, 2747, 1600, 1510, 1488, 1447, 1220\ cm^{-1}$; ^1H NMR

($CDCl_3$) $\delta = 1.21–1.38$ (m, 2H, NCH_2CH_2), 1.46–1.58 (m, 1H, NCH_2CH_2CH), 1.60–1.70 (m, 2H, NCH_2CH_2), 1.97 (td, $J = 11.5/2.6$ Hz, 1H, NCH_{2ax}), 2.25 (td, $J = 11.5/2.6$ Hz, 1H, NCH_{2ax}), 2.35 (dd, $J = 12.6/10.6$ Hz, 1H, CH_2CHOH), 2.45 (dd, $J = 12.6/3.8$ Hz, 1H, CH_2CHOH), 2.52 (d, $J = 7.3$ Hz, 2H, $PhCH_2$), 2.76 (d_{br}, $J = 11.5$ Hz, 1H, NCH_{2eq}), 3.11 (d_{br}, $J = 11.5$ Hz, 1H, NCH_{2eq}), 4.17 (s_{br}, 1H, OH), 4.67 (dd, $J = 10.6/3.8$ Hz, 1H, $CHOH$), 6.94–6.99 (m, 2H, $H_{aromat.}$), 7.06–7.12 (m, 2H, $H_{aromat.}$), 7.30 (s, 4H, $H_{aromat.}$); MS (CI, CH_4): m/z (%): 348 (100)$[M + H^+]$, 206 (40). Anal. calcd for $C_{20}H_{23}NOClF$: C 69.06; H 6.66; N 4.03. Found: C 69.12; H 6.69; N 3.94.

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