Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2853–2855

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Neuroprotective or Neurotoxic Activity of 1-Methyl-1,2,3,4-tetrahydroisoquinoline and Related Compounds

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Received 18 April 2003; accepted 4 June 2003

Abstract—1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) **1** and various 5- or 6,7-substituted analogues were synthesized and assayed for neurotoxicity towards SH-SY5Y cells. Among mono-substituted derivatives of **1**, hydroxyl substitution decreased the toxicity, while methoxyl substitution increased it. Disubstituted derivatives of **1**, **5a** and **5b**, showed the opposite tendency. Hydroxy-1MeTIQ derivatives were tested for neuroprotective activity, and **3b** and **4b** exhibited greater efficacy than **1**. We suggest that hydroxy-1MeTIQ derivatives, especially **4b**, may have potential for the treatment of Parkinson's disease.

Introduction

Since the discovery that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) could induce parkinsonism in humans,[1] 1,2,3,4-tetrahydroisoquinoline (TIQ) derivatives have been considered as candidate endogenous causative factors of idiopathic Parkinson's disease (PD), because of their structural similarity to MPTP. We have reported that tetrahydroisoquinoline derivatives, such as TIQ, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) **1**,[2] 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ),[3] and 1-(3′,4′-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (3′,4′DHBnTIQ),[4] exist in the brain of several mammalian species. While the content of 1BnTIQ tends to be increased in cerebrospinal fluid (CSF) of PD patients,[3] **1** is significantly decreased in the parkinsonian brain and MPTP-injected mouse brain.[7,8] Bradykinesia, one of the symptoms of PD, was induced by injection of TIQ, 1BnTIQ, or 3′,4′DHBnTIQ in mouse,[2,3] so these TIQs are considered to be parkinsonism-inducing substances. MPTP and TIQs have inhibitory activities towards complex I of the mitochondrial respiratory chain and tyrosine hydroxylase.[5,6] MPTP- or TIQ-induced bradykinesia was prevented by pretreatment with **1**,[8] which also suppresses the inhibition of mitochondrial complex I by 1-methyl-4-phenylpyridinium (MPP^+), an active metabolite of MPTP.[9] Thus, **1** is a possible endogenous PD-preventing substance. However, the mechanism of the parkinsonism-preventing activity of **1** is still unknown. We synthesized derivatives of **1** and evaluated their neurotoxicity by using SH-SY5Y cells. We also tested the protective activity of **1** and hydroxyl-substituted **1** against toxicity due to **5b**.

Chemistry

Compounds **1**, **4a** and **4b** were synthesized via reported methods.[8,10] The other compounds (except **5a** and **5b**) were synthesized by means of minor modifications of the same methods. General synthetic methods for **1** derivatives are summarized in Scheme 1. Structure determinations of all synthesized compounds were done by ^{1}H NMR using NOE analysis.[13] Among these compounds, **2b** (synthesized from *o*-methoxy-2-phenylethylamine) is a novel compound. Compound **5b** was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Pharmacological evaluation

All the compounds were tested for neurotoxicity towards human neuroblastoma SH-SY5Y cells by MTT dye conversion assay.[11] The cells were plated on 96-well culture plates at a density of 2×10^4 cells per 100 μL per well before a 24-h assay. Test compounds, adjusted to four times of the final concentration with culture medium, were added to the cultures at 50 μL and adjusted

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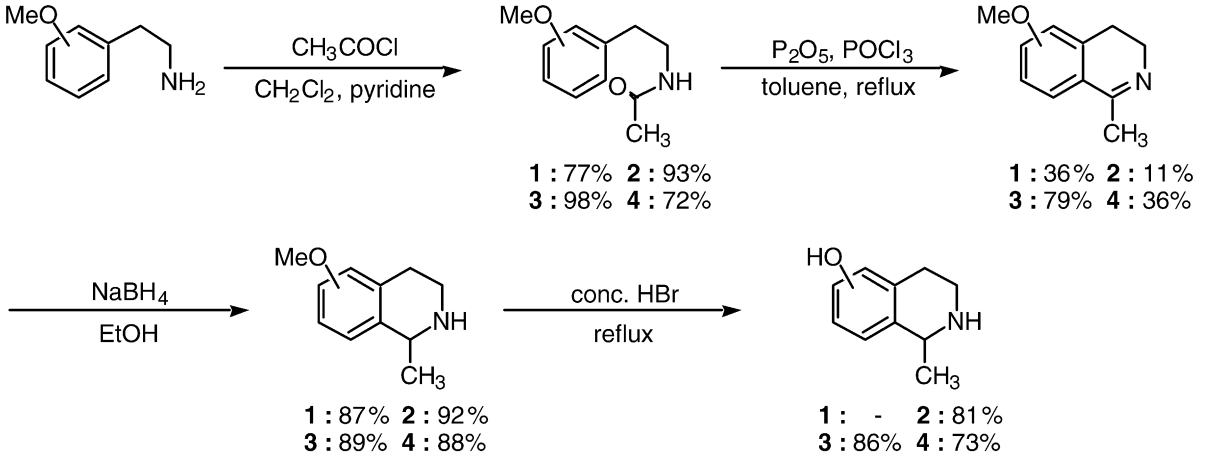
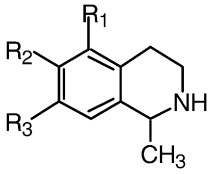
doi:10.1016/S0960-894X(03)00583-3





	R_1	R_2	R_3
1	H	H	H
2a	OMe	H	H
2b	OH	H	H
3a	H	OMe	H
3b	H	OH	H
4a	H	H	OMe
4b	H	H	OH
5a	H	OMe	OMe
5b	H	OH	OH

Scheme 1. Synthetic scheme for 1-methyl-1,2,3,4-tetrahydroisoquinoline derivatives.

final volume to 200 μL. Cultivation was continued at 37 °C for 24 h. MTT assay was performed, and the absorbance at 570 nm was measured with a microplate reader. Cell viability was evaluated in terms of A_{570} and is presented as a percentage of the untreated control. The TC_{50} values represent the concentration which results in a 50% cell viability.

Results and Discussion

The pharmacological results are shown in Table 1. The introduction of one hydroxyl group into **1** (**2b** and **3b**) did not affect the toxicity. Compound **4b**, which is expected to be a biosynthetic product of tyrosine, showed somewhat lower toxicity. The introduction of one methoxyl group increased the toxicity, especially in the case of **4a**. Nevertheless, TC_{50} values remained at the millimolar level. Substitution at the 7-position seemed to afford the highest toxicity. Disubstitution with methoxyl or hydroxyl groups (**5a** or **5b**) resulted in an opposite tendency; **5b** showed very strong toxicity, and **5a** showed weaker toxicity. Since dimethoxyl and methoxyl substitutions resulted in similar levels of toxicity, the very strong toxicity of dihydroxyl substitution may be related to the catechol structure.

It is well known that **5b** is neurotoxic,[12] whereas **1** is a parkinsonism-preventing substance. Therefore, we tested the neuroprotective activities of **1**, **2b**, **3b** and **4b** against toxicity due to **5b**.[13] As shown in Figure 1, **1** showed a slight, but not significant, protective effect against the toxicity of 20 μM **5b**. Compounds **3b**, **4b** showed greater (statistically significant) protective effects at 20 μM concentration against the toxicity of 20 and 40 μM **5b**. Since this concentration is 150–200th of the TC_{50} values, the influence of differences of toxicity may be negligible. Hydroxylated 1MeTIQs are likely to be formed as metabolites of **1** by cytochrome P450. This metabolic transformation reduces the toxicity, reinforcing the protective effect. Furthermore, **4b** could be an endogenous biosynthetic derivative of tyrosine. We also discovered that **4b** has no toxicity towards rat mesencephalon primary cultured cells even at extremely high concentration (data not shown). We consider that hydroxy-1MeTIQ derivatives, especially **4b**, may have potential for the treatment of Parkinson's disease.

Table 1. Neurotoxicity[a] against SH-SY5Y cells

Compd	TC_{50} (mM)
1	3.59
2a	2.45
3a	2.79
4a	1.39
5a	2.73
2b	3.38
3b	3.63
4b	4.15
5b	0.034

[a]These values were calculated from dose–response curves as sample amount required to cause 50% cell death.

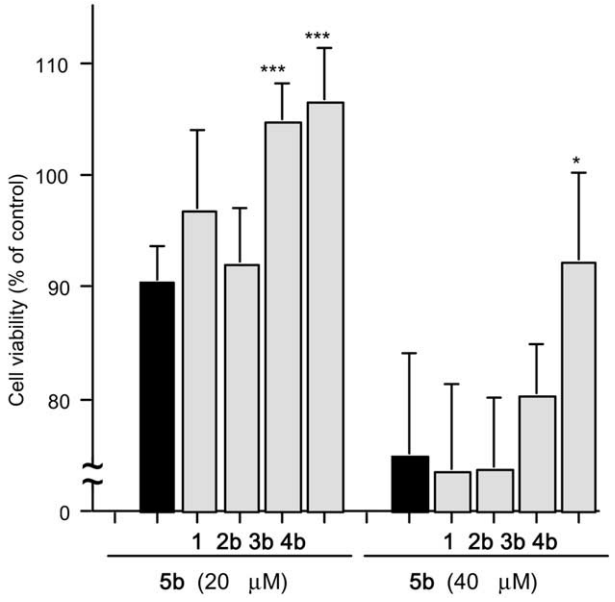


Figure 1. Neuroprotective effect of 1MeTIQ and its derivatives against salsolinol toxicity. Cultured SH-SY5Y cells were exposed to salsolinol (20 and 40 μM) and 1MeTIQs (20 μM) concurrently for 24 h at. Cell viability was determined by MTT assay. Black columns represent the cell viability of **5b** itself. Each value represents the mean + SD of three (control $n=9$) experiments. $p<0.05$ (*), $p<0.001$ (***) versus 1MeTIQs nontreated control (black column) by Student's *t*-test.

Acknowledgements

The authors wish to thank Prof. Hiroshi Hara, Department of Pharmacy, Tokyo Science University, Japan, for providing **5a**.

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12. Storch, A.; Kaftan, A.; Burkhardt, K.; Schwarz, J. *Brain Res.* **2000**, *855*, 67.
13. Compounds were characterized by ^{1}H NMR. **2a**: ($CDCl_3$) δ 1.83 (d, 3H, $-CH_3$, $J=6.8$ Hz), δ 3.01–3.16 (m, 2H, 4-H), δ 3.30–3.56 (m, 2H, 3-H), δ 3.82 (s, 3H, $-OCH_3$), δ 4.59 (q, 1H, 1-H, $J=6.8$ Hz), δ 6.73–6.77 (m, 2H, 6-H, 8-H), δ 7.21 (t, 1H, 7-H, $J=8.0$ Hz). **2b**: (DMSO-d_6) δ 1.55 (d, 3H, $-CH_3$, $J=76.8$ Hz), δ 2.75–2.87 (m, 2H, 4-H), δ 3.41–3.48 (m, 2H, 3-H), δ 4.51 (q, 1H, 1-H, $J=6.8$ Hz), δ 6.72–6.78 (m, 2H, 6-H, 8-H), δ 7.09 (t, 1H, 7-H, $J=8.0$ Hz), δ 9.69 (s, 1H, –OH). **3a**: (CD_3OD) δ 1.67 (d, 3H, $-CH_3$, $J=6.8$ Hz), δ 3.02–3.19 (m, 2H, 3-H), δ 3.37–3.58 (m, 2H, 4-H), δ 3.78 (s, 3H, $-OCH_3$), δ 4.55 (q, 1H, 1-H, $J=6.8$ Hz), δ 6.79 (d, 1H, 5-H, $J=2.4$ Hz), δ 6.87 (dd, 1H, 7-H, $J=2.4$ Hz, 8.8 Hz), δ 7.21 (d, 1H, 8-H, $J=8.8$ Hz). **3b**: (DMSO-d_6) δ 1.54 (d, 3H, $-CH_3$, $J=7.2$ Hz), δ 2.85–3.02 (m, 2H, 4-H), δ 3.24–3.43 (m, 2H, 3-H), δ 4.45 (q, 1H, 1-H, $J=7.2$ Hz), δ 6.59 (d, 1H, 5-H, $J=2.4$ Hz), δ 6.68 (dd, 1H, 7-H, $J=2.4$ Hz, 8.4 Hz), δ 7.09 (d, 1H, 8-H, $J=8.4$ Hz), δ 9.44 (s, 1H, –OH). **4a**: (CD_3OD) δ 1H, 1-H, $J=6.8$ Hz), δ 6.72–7.06 (m, 3H, ArH). **4b**: (DMSO-d_6) δ 1.55 (d, 3H, $-CH_3$, $J=1.60$ (dd, 3H, $-CH_3$, $J=2.0$ Hz, 6.8 Hz), δ 2.91–3.02 (m, 2H, 4-H), δ 3.26–3.47 (m, 2H, 3-H), δ 3.68 (s, 3H, $-OCH_3$), δ 4.49 (q 6.8 Hz), δ 2.82–2.97 (m, 2H, 4-H), δ 3.24–3.44 (m, 2H, 3-H), δ 4.49 (q, 1H, 1-H, $J=6.8$ Hz), δ 6.65 (d, 1H, 5-H, $J=2.4$ Hz), δ 6.70 (dd, 1H, 7-H, $J=2.4$ Hz, 8.4 Hz), δ 7.01 (d, 1H, 8-H, $J=8.4$ Hz), δ 9.34 (s, 1H, –OH).