Synthesis of Chiral 3-Methyl- and 3-Methyl-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline and Prevention of MPP^+-Induced Cytotoxicity

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Abstract: The chemical structure of selegiline, a commercially available drug for Parkinson's disease (PD), resembles that of 1,2,3,4-tetrahydroisoquinoline (TIQ), an endogenous parkinsonism-inducing compound. In the present study, we evaluated the direct cytotoxicity of (*R*)- and (*S*)-3-methyl-TIQ (3-MeTIQ) and (*R*)- and (*S*)-3-methyl-*N*-propargyl-TIQ (3-Me-*N*-propargyl-TIQ), as selegiline-mimetic TIQ derivatives, and their ability to prevent 1-methyl-4-phenylpyridinium iodide (MPP^+)-induced cell death. Synthesis of optically-pure 3-MeTIQs was achieved *via* the super acid-induced cyclization of chiral *N*-benzyl-*N*-[1-methyl-2-(phenylsulfinyl)ethyl]formamide using a Pummerer-type cyclization reaction as the key step in producing excellent yields. Subsequent *N*-propargylation of chiral 3-MeTIQs using propynylbromide gave the corresponding 3-Me-*N*-propargyl-TIQs. In our *in vitro* experiments, the direct cytotoxicity of chiral 3-MeTIQs and 3-Me-*N*-propargyl-TIQs was almost identical, with no relationship to optical chirality except for (*S*)-3-Me-*N*-propargyl-TIQ, which had significantly weaker direct cytotoxicity than the other 3-MeTIQ derivatives. However, the decreased viability of PC12 cells induced by treatment with MPP^+ was accelerated by the coexistence of 3-MeTIQs and inhibited by 3-Me-*N*-propargyl-TIQs without any participation of the stereochemistry at the 3-postion. These results suggest that the *N*-propargyl group is necessary for protection of cells against the toxicity of MPP^+. Furthermore, the stereochemistry of the 3-position appears to partially participate in the direct cytotoxicity of 3-Me-*N*-propargyl-TIQs.

Key Words: 1,2,3,4-tetrahydroisoquinoline, selegiline, structure-activity relationships, cytotoxicity, Parkinson's disease.

INTRODUCTION

The etiology of Parkinson's disease (PD) remains unknown, although nearly 200 years have passed since this disease was first described. In 1983, parkinsonism was shown to be induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [1]. Since then, various structurally MPTP-like endogenous amines have been identified as neurotoxins, and it is believed that 1,2,3,4-tetrahydroisoquinoline (TIQ) and its derivatives such as 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1-BnTIQ) and (*R*)-1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol) most likely participate in the pathogenesis of PD [2-5]. TIQ derivatives are also found in the brain of other animal species [4, 6, 7] and either TIQ or 1-BnTIQ can induce parkinsonism in rodents [4, 8]. Moreover, 1-BnTIQ is present in the cerebro-spinal fluid of PD patients at concentrations about 3 times higher than that normally present in healthy individuals [4]. Salsolinol is also present in both human and animal brains [9-11] and *N*-methylated (*R*)-salsolinol produces behavioral abnormalities with decreasing dopamine content, particularly in the substantia nigra of rat [12]. These findings suggest an intimate relationship between PD and the etiological potentiality of these TIQ derivatives.

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In contrast, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1-MeTIQ) is present in normal mouse brain, and has been shown to prevent TIQ- and 1-BnTIQ-induced behavioral abnormalities in rodents [4, 8]. We also previously showed that 1-MeTIQ possess stereoselective preventative effects against MPTP- and TIQ-induced parkinsonism-like symptoms in mice [13, 14]. Selegiline ((*R*)-*N*,α-dimethyl-*N*-2-propynyl phenethylamine, **1**), which has a similar structural to the TIQ derivatives, has been used in the treatment of PD as a putative neuroprotective agent [15]. Furthermore, selegiline has been reported to decrease endogenous 1-BnTIQ content in the mouse brain [16] and has neuroprotective properties against glutamate receptor-mediated toxicity in mesencephalic dopamine neurons with stereoselective activity [17]. Thus, we expect that analysis of the selegiline mimetic TIQ derivatives will lead to the development of new, more effective, therapeutic compounds for PD. In the present study, we attempted to estimate the preventative properties of 3-methyl-1,2,3,4-tetrahydroisoquinoline (3-MeTIQ, **2**) on 1-methyl-4-phenylpyridinium ion (MPP^+)-induced cytotoxicity *in vitro*. In addition, because the *N*-propargyl functional group has been shown to play an important role in neuroprotection [18], the effects of 3-methyl-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline (3-Me-*N*-propargyl-TIQ, **3**) were also investigated, as these compounds have a similar chemical structure to selegiline. We also evaluated the stereochemical influences of 3-Me- and 3-Me-*N*-propargyl-TIQ on the above *in vitro* properties. The structures of selegiline (**1**), 3-

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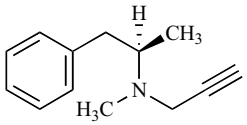
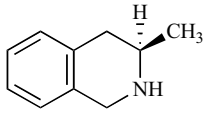
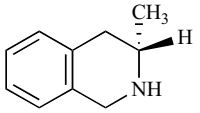
Me-TIQ (**2**) and 3-Me-*N*-propargyl-TIQ (**3**) are shown in Fig. **1**.



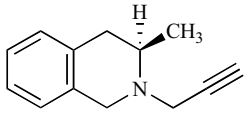
Selegiline (**1**)



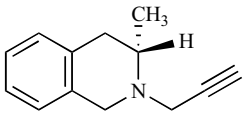
(*R*)-3-MeTIQ (**2a**)



(*S*)-3-MeTIQ (**2b**)



(*R*)-3-Me-*N*-propargyl-TIQ (**3a**)



(*S*)-3-Me-*N*-propargyl-TIQ (**3b**)

Fig. (1). Chemical structures of selegiline, and the 3-Me- and 3-Me-*N*-propargyl-TIQs.

MATERIALS AND METHODS

Chemistry

Synthesis of chiral 3-MeTIQs were achieved using the super acid promoted Pummerer-type cyclization reaction of chiral *N*-benzyl-*N*-[1-methyl-2-(phenylsulfinyl)ethyl]formamide (**12a**, **b**) as the key step.

(*R*)-1-Methyl-2-phenylsulfanylethylamine (**9a**) and its (*S*)-enantiomer (**9b**), which will comprise the chiral center at the 3-positon of the 3-MeTIQs, were synthesized from (*R*)- and (*S*)-alanine as follows. (*R*)-*N*-benzyloxycarbonyl-*O*-tosyl-2-aminopropanol (**7a**) was prepared by selective *N*-benzyloxycarbonylation of (*R*)-alaninol (**5a**) which was prepared from $LiAlH_4$ reduction of (*R*)-alanine (**4a**)**,** followed by tosylation of the resulting (*R*)-*N*-benzyloxycarbonyl-2-aminopropanol (**6a**), in 50% overall yield from **4a**. Substitution reaction of the tosylate (**7a**) with potassium thiophenolate gave **8a** and then alkaline hydrolysis of **8a** gave **9a** in excellent yield. The (*S*)-enantiomer (**9b**) was prepared from (*S*)-alanine in 26% overall yield in the same way.

Condensation reaction of **9a** with benzaldehyde in EtOH-acetic acid followed by $NaBH_4$ reduction of the resulting imines afforded amine (**10a**)**.** Formylation of amine (**10a**) by treatment with acetic-formic anhydride followed by oxidation of *N*-formyl sulfide (**11a**) with $NaIO_4$ produced the corresponding (*R*)-sulfoxide (**12a**) in high overall yields. (*S*)-sulfoxide (**12b**) was prepared from **9b** *via* the same process (Fig. **2**).

As previously described [19], sulfoxides lacking a methoxy group as the electron-donating group on the benzene ring do not cause cyclization under normal Pummerer-type cyclization reaction conditions (*i.e.,* treating the sulfoxide with trifluoroacetic anhydride (TFAA) in benzene). We already reported that the Pummerer type cyclization reaction of these sulfoxides was promoted using trifluoromethane sulfonic acid (TFSA), a super strong acid, as an additive acid [20].

A solution of **12a** in benzene was treated with a 5 molar equivalent of TFAA at room temperature for 30 min under

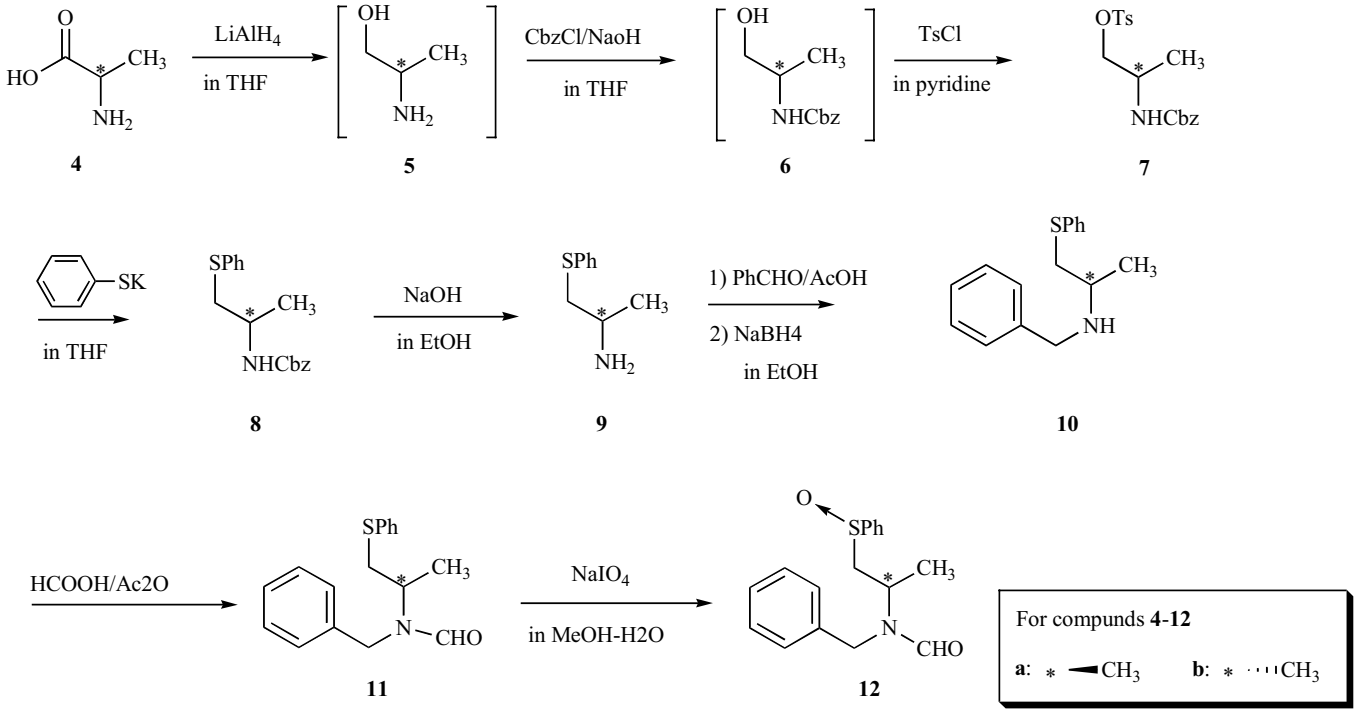


Fig. (2). Synthesis of chiral *N*-benzyl-*N*-[1-methyl-2-(phenylsulfinyl)ethyl]formamide **12a**, **b**.

an argon atmosphere, and then a 3 molar equivalent of TFSA was added to this solution. The reaction mixture was allowed to react at room temperature for a further 30 min. Separation of the crude product yielded two products: 2-formyl-4-phenylsulfanyl-3-MeTIQ (**13a**) and 2-formyl-4-phenyl-3-MeTIQ (**14a**).

In order to simplify the product analysis, the Pummerer products were purified after reductive elimination of the phenylsulfanyl group at the 4-positon of the TIQ ring system. The benzene solution of **12a** was treated with TFAA (5 mol eq) and TFSA (3 mol eq) followed by reduction with $NaBH_4$-$NiCl_2$ to yield (3*R*)-2-formyl-3-MeTIQ (**15a**) and **14a** in yields of 63% and 13%, respectively. This result demonstrated that the TFSA-promoted Pummerer reaction of **12a** induced the cyclization leading to the isoquinoline ring in a highly effective manner, although the 4-phenyl-TIQ derivative (**14a**) was an accompanying by-product.

The Pummerer cyclization reaction of **12b** and subsequent reductive elimination of the pehnylsulfanyl group gave (3*S*)-2-formyl-3-MeTIQ (**15b**) and 4-phenyl-3-MeTIQ (**14b**) in 79% and 9% yields, respectively.

Hydrolytic deprotection of the *N*-formyl group of **15a** and **15b** gave corresponding (*R*)- 3-MeTIQs (**2a**) and (*S*)-3-MeTIQs (**2b**), respectively. Moreover, TIQs (**2a**, **b**) were derived to *N*-propargyl-3-MeTIQs (**3a**, **b**), similar to the propargylamines such as selegiline (a potent neuroprotective agent), by the propargylation of the nitrogen atom at the 2-position *via* applying propargylbromide and cesium carbonate in acetonitrile as the solvent (Fig. **3**).

The optically-pure 3-MeTIQs (**2**) and 3-Me-*N*-propargyl-TIQs (**3**) synthesized here were then used in biochemical evaluation of their protective effects *in vitro*.

Chemical Identification

Unless otherwise stated, the following methods were used. Melting points were taken on a Yanagimoto SP-M1 hot-stage melting point apparatus and were uncorrected. Infrared spectra were measured using a HORIBA FT-710 Fourier transform infrared spectrophotometer, as films for oils and gums, disks for solids using KBr, and were in cm^{-1}. Nuclear magnetic resonance spectra were measured using a JEOL JNM-AL300 NMR spectrometer (^1H: 300 MHz, ^{13}C: 75 MHz) in chloroform-*d* with tetramethylsilane as the internal standard and were shown in δ value. High resolution electron-impact ionization mass spectrometry (HR-EIMS) and low resolution chemical ionization mass spectrometry (LR-CIMS) were measured on a JEOL JMS-D300 mass spectrometer at 70 eV (EIMS) or at 270 eV (CIMS: reactant gas: *iso*-butane) using a direct inlet probe. High resolution fast atom bomb ionization mass spectrometry (HR-FABMS) was performed on a JEOL HX-110A mass spectrometer using *m*-nitrobenzyl alcohol as the matrix and solvent, and was shown as *m/z*. Optical rotations were determined using a JASCO DIP-1000 digital polarimeter in MeOH. CD spectra were measured on a JASCO J-600 spectrometer in MeOH. The organic extracts from each reaction mixture were washed with brine, dried over $NaSO_4$, and concentrated *in vacuo* to dryness. The known compounds were characterized by MS, IR, and ^1H-NMR analysis.

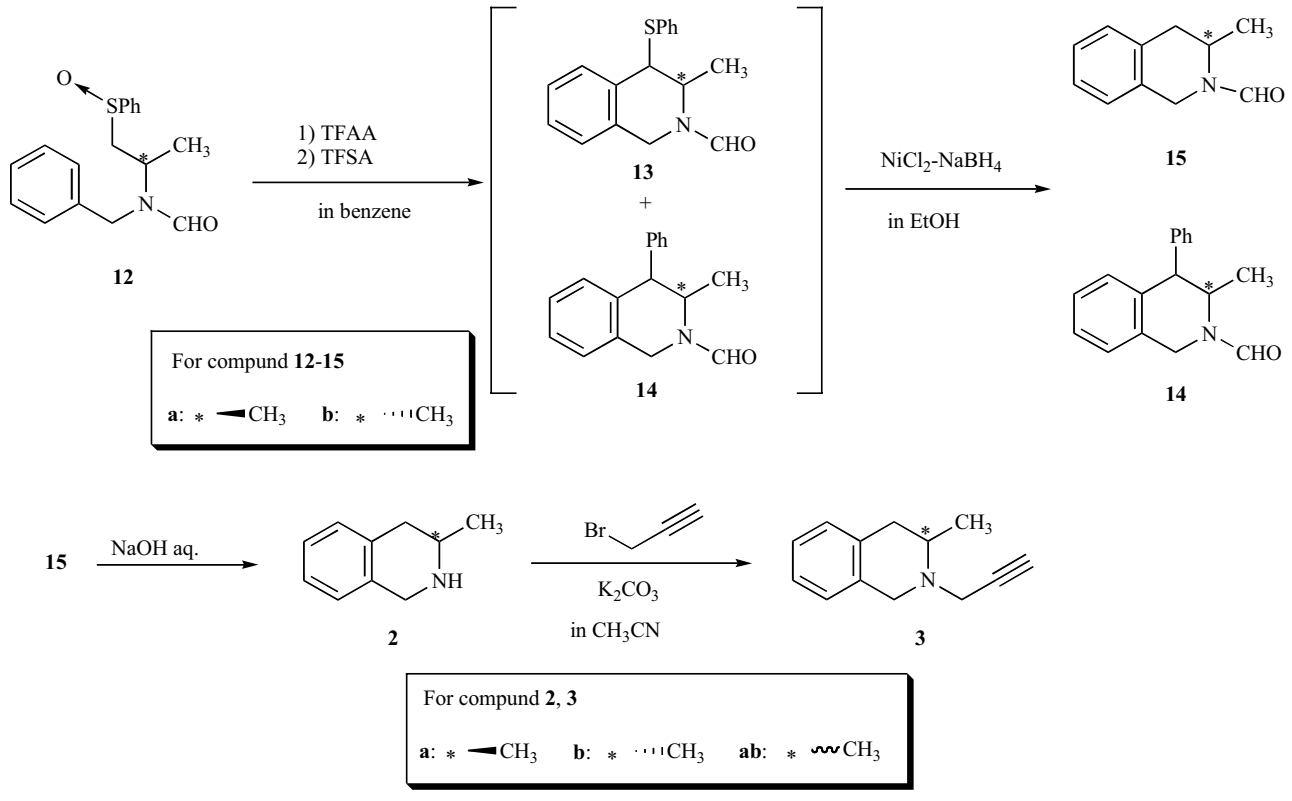


Fig. (3). Synthesis of chiral 3-MeTIQs using the super acid promoted Pummerer-type cyclization reaction.

Synthesis of (*R*)- and (*S*)-*N*-benzyloxycarbonyl-*O*-tosyl-2-aminopropanol (7) from (*R*)- and (*S*)-alanine (4)

To the suspension of $LiAlH_4$ (2.56 g, 67.4 mmol) in THF (60 ml), (*R*)-alanine (**4a**, 3.0 g, 33.7 mmol) was added in small portions under ice cooling and the mixture refluxed for 6 hr. After decomposition of excess $LiAlH_4$ with 8% NaOHaq, the precipitates were removed by filtration, and the filtrate was diluted with THF (100 ml) and 8% NaOHaq (19 ml). To this solution, benzyloxycarbonylchloride (6.03 g, 31.6 mmol) was added under ice cooling and stirring for 10 min. After acidification of the reaction mixture with 10% HClaq, the reaction mixture was extracted with $CHCl_3$. After removal of the solvent *in vacuo*, the residue was dissolved in dry pyridine (55 ml) and then *p*-toluenesulfonyl chloride (6.03 g, 31.6 mmol) was added and the mixture was stirred for 14 hr at 5°C. The reaction mixture was then diluted with ether and washed with 10% HClaq. After removal of the solvent, the residue was purified by column chromatography with AcOEt : hexane (4:1)-$CHCl_3$-MeOH to give **7a** (6.1 g, 16.9 mmol, 50%).

Similarly**, 7b** (32.3 g, 32%) was obtained from (*S*)-alanine (25.0 g).

Compounds **7a** and **7b** were identical to the compounds produced in our previous study [21].

Compounds **7**-**12** were prepared as previously described [21].

Pummerer Reaction of (*R*)-*N*-benzyl-*N*-(1-methyl-2-phenylsulfinyl)formamide (12a)

TFAA (3.49 g, 7.93 mmol) was added to a solution of **12a** (1.00 g, 3.32 mmol) in benzene (50 ml) at room temperature, and the mixture was stirred under an argon atmosphere for 30 min. TFSA (0.996 g, 7.93 mmol) was then added and the reaction mixture was further stirred at the same temperature for 30 min. To this reaction mixture, 5% $NaHCO_3$aq (50 ml) was added and the mixture extracted with $CHCl_3$. The residue was purified by column chromatography to give **13a** and **14a**, but yields of **13a** and **14a** were not obtained.

(R)-2-Formyl-3-methyl-4-phenylsulfanyl-1,2,3,4-tetrahydroisoquinoline (13a)

Yellow oil. IR: 1670. ^{1}H-NMR: 1.11, 1.20, 1.28, 1.38 (total 3H, each d, *J*=7.0 Hz, 6.8 Hz, 6.8 Hz, 6.8 Hz, -Me), 3.9-5.3 (4H, m, C_1-H, C_3-H, C_4-H), 7.1-7.6 (9H, m, Phx2), 8.08, 8.20, 8.35 (total 1H, each s, -CHO). LR-EIMS *m/z*: 283 (M^+), 173 (base peak). HR-EIMS (M^+): Calcd for $C_{17}H_{17}NOS$: 283.1031, found: 283.1049. $[\alpha]_D$ = -6.7 (*c*=1.00%, $CHCl_3$).

(R)-2-Formyl-3-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (14a)

Colorless needles. mp. 110-112°C. IR: 1654. ^{1}H-NMR: 1.39 (3H, d, *J*=6.8 Hz, -Me), 3.91 (1H, dq, *J*=1.5 Hz, 6.8 Hz, C_3-H), 4.04 (1H, s, C_4-H), 4.25 (1H, d, *J*=17.8 Hz, C_1-H), 5.30 (1H, d, *J*=17.8 Hz, C_1-H), 6.9-7.5 (9H, m, Phx2), 7.65 (1H, s, -CHO). LR-EIMS *m/z*: 251 (M^+), 91 (base peak). HR-EIMS (M^+): Calcd for $C_{17}H_{17}NO$: 251.1310, found: 251.1325. $[\alpha]_D$ = +86.7 (*c*=1.00%, $CHCl_3$).

Similarly, the Pummerer reaction of *N*-benzyl-*N*-(1-methyl-2-phenylsulfinyl)formamide (**12b**) gave **13b** and **14b**, but yields of **13b** and **14b** were not obtained.

(S)-2-Formyl-3-methyl-4-phenylsulfanyl-1,2,3,4-tetrahydroisoquinoline (13b)

Colorless gum. IR: 1671. ^{1}H-NMR: 1.11, 1.20, 1.28, 1.38 (total 3H, each d, *J*=7.0 Hz, 6.8 Hz, 7.0 Hz, 6.8 Hz, -Me), 3.9-5.3 (4H, m, C_1-H, C_3-H, C_4-H), 7.2-7.7 (9H, m, Phx2), 8.08, 8.20, 8.35 (total 1H, each s, -CHO). LR-EIMS *m/z*: 283 (M^+), 173 (base peak). HR-EIMS (M^+): Calcd for $C_{17}H_{17}NOS$: 283.1031, found: 283.1058. $[\alpha]_D$ = +12.2 (*c*=1.00%, $CHCl_3$).

(S)-2-Formyl-3-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (14b)

Colorless needles. mp. 111-113°C. IR: 1654. ^{1}H-NMR: 1.39 (3H, d, *J*=6.9 Hz, -Me), 3.92 (1H, dq, *J*=1.5 Hz, 6.9 Hz, C_3-H), 4.04 (1H, s, C_4-H), 4.25 (1H, d, *J*=17.9 Hz, C_1-H), 5.31 (1H, d, *J*=17.9 Hz, C_1-H), 6.9-7.3 (9H, m, Phx2), 7.66 (1H, s, -CHO). LR-EIMS *m/z*: 251 (M^+, base peak). HR-EIMS (M^+): Calcd for $C_{17}H_{17}NO$: 251.1311, found: 251.1348. $[\alpha]_D$ = -84.2 (*c*=1.00%, $CHCl_3$).

Pummerer reaction of (*R*)-*N*-benzyl-*N*-(1-methyl-2-phenylsulfinyl)formamide (12a) and Reductive Desulfurization of Pummerer Products

TFAA (26.1g, 124.4 mmol) was added to a solution of **12a** (7.5 g, 24.9 mmol) in benzene (340 ml) at room temperature, and the mixture stirred under an argon atmosphere for 30 min. TFSA (11.2 g, 74.7 mmol) was added to the reaction mixture, and the mixture was further stirred at the same temperature for 30 min. To this reaction mixture, 5% $NaHCO_3$aq (200 ml) was added and the mixture was extracted with $CHCl_3$. The residue was dissolved in EtOH (380 ml) and $NiCl_2 \cdot 6H_2O$ (41.5 g, 174.4 mmol) was added to this solution under ice cooling. $NaBH_4$ (19.9 g, 523.3 mmol) was added in small portions to the stirred mixture at 0°C, and the mixture was stirred at room temperature for 30 min. Ice water was added to the reaction mixture then the precipitate was removed by filtration. The filtrate was concentrated *in vacuo* and the residue then extracted with $CHCl_3$. The residue was purified by column chromatography with AcOEt: hexane (1:1) to give **15a** (2.8 g, 63%) and **14a** (798 mg, 13%).

(R)-2-Formyl-3-methyl-1,2,3,4-tetrahydroisoquinoline (15a)

Colorless oil. IR: 1668. ^{1}H-NMR: A : B = 2 : 1. A : 1.26 (3H, d, *J*=6.8 Hz, -Me), 2.70 (1H, dd, *J*=2.4 Hz, 15.8 Hz, C_4-H), 3.14 (1H, dd, *J*=5.5 Hz, 15.8 Hz, C_4-H), 4.15 (1H, ddq, *J*=2.4 Hz, 5.5 Hz, 6.8 Hz, C_3-H), 4.29 (1H, d, *J*=17.6 Hz, C_1-H), 5.04 (1H, d, *J*=17.6 Hz, C_1-H), 7.1-7.3 (4H, m, Ph-H), 8.25 (1H, s, -CHO). B : 1.22 (3H, d, *J*=6.8 Hz, -Me), 2.60 (1H, dd, *J*=2.9 Hz, 16.2 Hz, C_4-H), 3.11 (1H, dd, *J*=6.2 Hz, 16.2 Hz, C_4-H), 4.85 (1H, ddq, *J*=2.9 Hz, 6.2 Hz, 6.8 Hz, C_3-H), 4.45 (1H, d, *J*=16.2 Hz, C_1-H), 4.51 (1H, d, *J*=16.2 Hz, C_1-H), 7.1-7.3 (4H, m, Ph-H), 8.20 (1H, s, -CHO). A and B were rotational isomers of each other with regard to the formyl group. LR-EIMS *m/z*: 175 (M^+, base peak). HR-EIMS (M^+): Calcd for $C_{11}H_{13}NO$: 175.0994, found: 175.0962. $[\alpha]_D$ = +59.1 (*c*=1.00%, $CHCl_3$).

Similarly, the Pummerer reaction of *N*-benzyl-*N*-(1-methyl-2-phenylsulfinyl)formamide (**12b**) (7.5 g, 24.9 mmol)

and reductive desulfurization of the Pummerer products gave **15b** (3.4 g, 79%) and **14b** (588 mg, 9%)

(R)-2-Formyl-3-methyl-1,2,3,4-tetrahydroisoquinoline (15b)

Colorless oil. IR: 1670. ^{1}H-NMR: A : B = 2 : 1. A : 1.26 (3H, d, *J*=6.8 Hz, -Me), 2.70 (1H, dd, *J*=2.4 Hz, 15.9 Hz, C_4-H), 3.14 (1H, dd, *J*=5.5 Hz, 15.9 Hz, C_4-H), 4.15 (1H, ddq, *J*=2.4 Hz, 5.5 Hz, 6.8 Hz, C_3-H), 4.29 (1H, d, *J*=17.6 Hz, C_1-H), 5.04 (1H, d, *J*=17.6 Hz, C_1-H), 7.1-7.2 (4H, m, Ph-H), 8.25 (1H, s, -CHO). B : 1.22 (3H, d, *J*=6.8 Hz, -Me), 2.60 (1H, dd, *J*=2.9 Hz, 16.0 Hz, C_4-H), 3.11 (1H, dd, *J*=6.1 Hz, 16.0 Hz, C_4-H), 4.85 (1H, ddq, *J*=2.9 Hz, 6.1 Hz, 6.8 Hz, C_3-H), 4.45 (1H, d, *J*=16.4 Hz, C_1-H), 4.51 (1H, d, *J*=16.4 Hz, C_1-H), 7.1-7.2 (4H, m, Ph-H), 8.21 (1H, s, -CHO). A and B were rotational isomers of each other with regard to the formyl group. LR-EIMS *m/z*: 175 (M^+, base peak). HR-EIMS (M^+): Calcd for $C_{11}H_{13}NO$: 175.0995, found: 175.0995. $[\alpha]_D$ = -55.5 (*c*=1.00%, $CHCl_3$).

Hydrolysis of 2-Formyl-3-MeTIQs (15)

A solution of **15a** (4.9 g, 28 mmol) in EtOH (300 ml) and 20% NaOHaq (300 ml) was refluxed for 16 hr. The reaction mixture was diluted with water, and extracted with $CHCl_3$. The residue was purified by column chromatography over aluminum oxide with AcOEt to give **2a** (3.0 g, 72%) as a pale yellow oil.

Similarly, the isomer (**15b**, 6.4 g, 36.6 mmol) gave **2b** (4.5 g, 85%).

(-)-(R)-3-Methyl-1,2,3,4-tetrahydroisoquinoline (2a)

Yellow oil. IR: 2923, 744. ^{1}H-NMR: 1.22 (3H, d, *J*=6.4 Hz, -Me), 2.47 (1H, dd, *J*=10.7 Hz, 16.4 Hz, C_4-H), 2.75 (1H, dd, *J*=4.0 Hz, 16.4 Hz, C_4-H), 2.99 (1H, ddq, *J*=4.0 Hz, 6.4 Hz, 10.7 Hz, C_3-H), 3.99 (1H, d, *J*=15.9 Hz, C_1-H), 4.08 (1H, d, *J*=15.9 Hz, C_1-H), 7.0-7.1 (4H, m, Ph-H). ^{13}C-NMR : 22.3(q), 37.1(t), 48.4(t), 49.0(d), 125.5(d), 125.8(dx2), 128.9(d), 134.7(s), 135.2(s). LR-EIMS *m/z*: 147 (M^+), 104 (base peak). HR-EIMS (M^+): Calcd for $C_{10}H_{13}N$: 147.1045, found: 147.1020. $[\alpha]_D$ = -113.6 (*c*=1.00%, $CHCl_3$). CD (HCl salt, *c*=1.19 x 10^{-3} M, in MeOH) $[\theta]^{25}$(nm): -170(268), -146(263).

14a・HCl: Colorless needles. mp: 186-189°C.

(+)-(S)-3-Methyl-1,2,3,4-tetrahydroisoquinoline (2b)

Brown oil. IR: 2958, 744. ^{1}H-NMR: 1.21 (3H, d, *J*=6.4 Hz, -Me), 2.46 (1H, dd, *J*=10.7 Hz, 16.4 Hz, C_4-H), 2.74 (1H, dd, *J*=4.0 Hz, 16.4 Hz, C_4-H), 2.97 (1H, ddq, *J*=4.0 Hz, 6.4 Hz, 10.7 Hz, C_3-H), 3.98 (1H, d, *J*=16.0 Hz, C_1-H), 4.07 (1H, d, *J*=16.0 Hz, C_1-H), 7.0-7.1 (4H, m, Ph-H). ^{13}C-NMR : 22.5(q), 37.2(t), 48.6(t), 49.2(d), 125.6(d), 125.9(dx2), 129.0(d), 134.9(s), 135.3(s). LR-EIMS *m/z*: 147 (M^+), 104 (base peak). HR-EIMS (M^+): Calcd for $C_{10}H_{13}N$: 147.1046, found: 147.1016. $[\alpha]_D$ = +120.6 (*c*=1.00%, $CHCl_3$). CD (HCl salt, *c*=1.14 x 10^{-3} M, in MeOH) $[\theta]^{25}$(nm): 146(268), 158(261).

14b・HCl: Colorless needles. mp: 194-196°C (sublimation).

Synthesis of 3-Methyl-2-propynyl-1,2,3,4-tetrahydroisoquinoline (3)

A solution of propargylbromide (1.88 g, 15.8 mmol) in acetonitrile (10 ml) was added to the solution of **2a** (1.93 g, 13.2 mmol) and K_2CO_3 (2.18 g, 15.8 mmol) in acetonitrile (20 ml) under an argon atmosphere and then the resulting mixture was refluxed for 80 min. After cooling, the insoluble residue was removed by filtration and the filtrate then concentrated *in vacuo*. The resulting mixture was extracted with chloroform. The organic layer was concentrated *in vacuo* and then purified by column chromatography on silica gel with benzene-acetone (50:1) to give **3a** (1.04 g, 43%).

Similarly, the isomer (**2b**, 500 mg, 3.4 mmol) gave **3b** (404 mg, 64%).

(-)-(R)-3-Methyl-2-propynyl-1,2,3,4-tetrahydroisoquinoline (3a)

Brown oil. IR: 3291, 2794, 754. ^{1}H-NMR: 1.21 (3H, d, *J*=6.3 Hz, -Me), 2.22 (1H, t, *J*=2.4 Hz, C_3'-H), 2.67 (1H, dd, *J*=9.5 Hz, 16.3 Hz, C_4-H), 2.8-3.0 (2H, m, C_3-H, C_4-H), 3.45 (1H, dd, *J*=2.4 Hz, 17.4 Hz, C_1'-H), 3.74 (1H, dd, *J*=2.4 Hz, 17.4 Hz, C_1'-H), 3.77 (1H, d, *J*=15.2 Hz, C_1-H), 3.96 (1H, d, *J*=15.2 Hz, C_1-H), 7.0-7.1 (4H, m, Ph-H). ^{13}C-NMR : 18.4(q), 37.4(t), 42.2(t), 51.5(d), 53.9(t), 73.1(d), 78.2(s), 125.6(d), 126.0(d), 126.1(d), 128.3(d), 134.0(s), 134.4(s). LR-CIMS *m/z*: 186 (MH^+, base peak). $[\alpha]_D$ = -87.1 (*c*=1.00%, $CHCl_3$). CD (HCl salt, *c*=0.98 x 10^{-3} M, in MeOH) $[\theta]^{25}$(nm): 54(271), 46(265).

3a・HCl: Colorless needles. mp: 149-151°C.

(+)-(S)-3-Methyl-2-2-propynyl-1,2,3,4-tetrahydroisoquinoline (3b)

Yellow oil. IR: 3291, 2917, 746. ^{1}H-NMR: 1.23 (3H, d, *J*=6.3 Hz, -Me), 2.23 (1H, t, *J*=2.4 Hz, C_3'-H), 2.69 (1H, dd, *J*=9.6 Hz, 16.4 Hz, C_4-H), 2.8-3.0 (2H, m, C_3-H, C_4-H), 3.48 (1H, dd, *J*=2.4 Hz, 17.3 Hz, C_1'-H), 3.76 (1H, dd, *J*=2.4 Hz, 17.3 Hz, C_1'-H), 3.79 (1H, d, *J*=15.1 Hz, C_1-H), 3.98 (1H, d, *J*=15.1 Hz, C_1-H), 7.0-7.1 (4H, m, Ph-H). ^{13}C-NMR : 18.4(q), 37.4(t), 42.2(t), 51.5(d), 53.9(t), 73.1(d), 78.3(s), 125.6(d), 126.1(d), 126.1(d), 128.3(d), 134.0(s), 134.5(s). LR-CIMS *m/z*: 186 (MH^+, base peak). $[\alpha]_D$ = +84.5 (*c*=1.00%, $CHCl_3$). CD (HCl salt, *c*=0.95 x 10^{-3} M, in MeOH) $[\theta]^{25}$(nm): -97(272), -77(264).

3b・HCl; Colorless needles. mp: 145-147°C (sublimation).

Cell Culture

PC12 cells were obtained from the American Tissue Type Culture Collection (reference no. CRL 1721). Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO (USA), and fetal calf serum (FCS) was purchased from Roche Diagnostics (Swiss). PC12 cells were routinely maintained in DMEM containing 10% heat-inactivated FCS, 0.5 unit penicillin/ml and 0.05 mg streptomycin/ml under an atmosphere containing 5% CO_2 at 37°C The medium was changed every 2-3 days.

Evaluation of Direct Cytotoxicity of 3-Me-TIQ (2) and 3-Me-*N*-propargyl-TIQ (3)

Cells in the logarithmic growth phase were used for the experiments. Viable cell ratios were determined using the colorimetric assay of formazan produced by the reduction of water-soluble tetrazolium salt, similar to the MTT assay. Cells were suspended in serum contained DMEM at a con-

centration of 3×10^5 cells/ml and then transferred to 96-well plates in a volume of 100 µl/well. After 24 hr incubation, supernatants were removed and various concentrations of racemic or chiral 3-Me-TIQ (**2**), 3-Me-*N*-propargyl-TIQ (**3**) solution (prepared using serum-free DMEM) or selegiline (**1**) solution were added at a volume of 100 µl/well. After 48 hr incubation with these compounds, Cell Counting Kit-8 solution (10 µl/well, Dojindo, Kumamoto, Japan) was added to each well and the plates incubated for a further 4 hr at 37°C. The absorbance at 490 nm was measured using a microplate reader (Bio-Red, Model 550), and cell viability was evaluated in terms of A_{490} and presented as a percentage of the untreated control cells, defined as 100% viability.

Inhibitory Effects of 3-Me-TIQ (2) and 3-Me-*N*-propargyl-TIQ (3) on MPP^+-Induced Cell Death

Cell viability in the presence of 2.0 mM MPP^+ and 3-MeTIQ derivatives or selegiline (**1**) was estimated as mentioned above. The cell-death preventive effects of the 3-MeTIQ derivatives were calculated as the percentage of MPP^+-induced cell death, which was defined as 100 percent.

Data Analysis

Data are expressed as mean ± standard error (S.E.) for each group. At first, each datum was analyzed by Bartlett's test for homogeneity of variance. After using one-way analysis of variance (ANOVA), the significance of difference was analyzed by Dunnett's multiple comparison test. In direct cytotoxicity assays of 3-MeTIQs, the significance of differences between racemic and each chiral TIQ derivative was analyzed using Tukey-Kramer's multiple comparison test. A value of $P<0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

The cell viability upon treatment with the 3-MeTIQs (**2**), which are regioisomers of neuroprotective (*R*)-1-MeTIQ with regard to the position of the methyl group on the TIQ ring system, was almost identical among the 3-MeTIQs (**2**), with no relationship to the optical chirality of the methyl group at the 3-position observed. No reduction in cell viability was observed below a 3-MeTIQ (**2**) concentration of 1.0 mM. The ratio of dead cells gradually increased as the concentration increased over 1.0 mM, and at the highest concentration (2.0 mM) of each compound approximately 40% of cells died (Fig. **4A**).

(*R,S*)-3-Me-*N*-propargyl-TIQ (**3ab**) and (*R*)-3-Me-*N*-propargyl-TIQ (**3a**) , in which a propargyl group has been introduced to the nitrogen atom of the 3-MeTIQs (**2**) and which have structural similarity to selegiline, also showed similar cytotoxic effects against the viability of PC12 cells. These compounds decreased cell viability dose-dependently, and approximately 50% cells died at the highest concentration (2.0 mM) of both compounds. However, the cytotoxic effect of (*S*)-3-Me-*N*-propargyl-TIQ (**3b**) was significantly weaker than the compounds mentioned above. The ratio of cell death at the highest concentration was about 30% in the present study. The cytotoxicity of selegiline (**1**) was similar to (*S*)-3-Me-*N*-propargyl-TIQ (**3b**) until 1.5 mM, and moreover it showed same way to (*R,S*)-3-Me-*N*-propargyl-TIQ (**3ab**) and (*R*)-3-Me-*N*-propargyl-TIQ (**3a**) at more high concentration (Fig. **4B**).

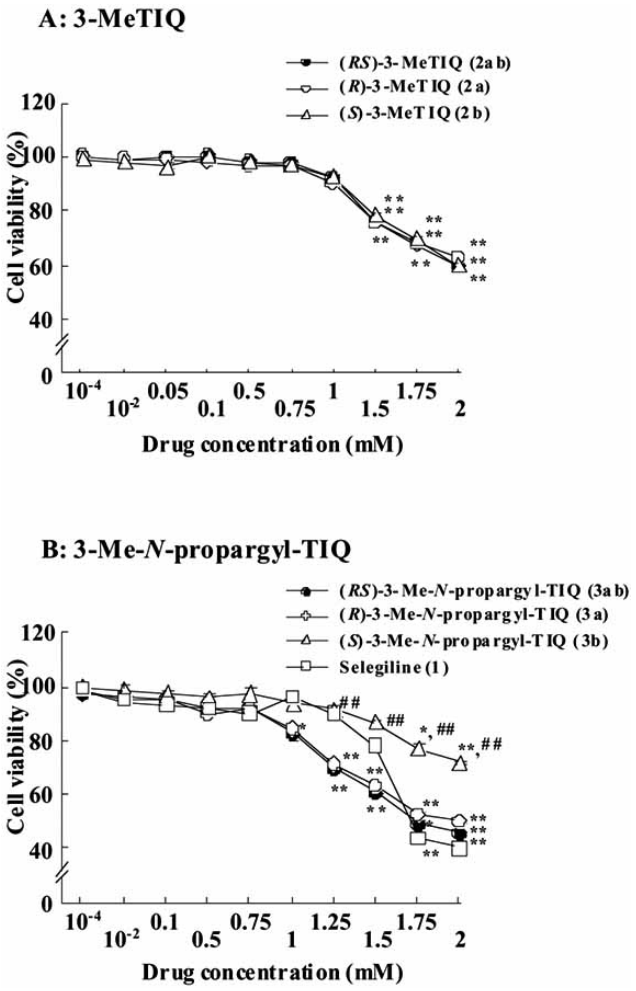


Fig. (4). Directly cytotoxic effect of racemic and chiral 3-MeTIQ, 3-Me-*N*-propargyl-TIQs and selegiline on cultured PC12 cells. The cells were exposed to each compound for 48 hr and viability then expressed as a percentage of the viability of the untreated control cells (this viability was defined as 100% viable). Data express mean ± standard error (S.E., n = 3-5). A: 3-MeTIQs, B: 3-Me-*N*-propargyl-TIQs and selegiline were estimated. Symbols show significant difference from control (*, $p<0.05$, **, $p<0.01$) and (*RS*)-3-Me-*N*-propargyl-TIQ (#, $p<0.05$, ##, $p<0.01$) viability.

The single treatment of MPP^+ (2.0 mM) killed about 80% of the cells. We then evaluated the protective effect of 3-Me-TIQ (**2**) and 3-Me-*N*-propargyl-TIQ (**3**) against MPP^+-induced cell death. The data was calculated as the proportion of cells surviving in the presence of both 3-MeTIQ derivatives and MPP^+ compared to the proportion of cells surviving in MPP^+, which was defined as 100%. In the presence of cells and MPP^+, all the stereoisomeric 3-MeTIQs (**2**) increased cell death in dose dependent manner, even at the lowest concentration (0.01 mM). Specific contributions of optical chirality towards cytotoxicity were not observed (Fig. **5A**).

In contrast to the 3-MeTIQs (**2**), all the stereoisomers of 3-Me-*N*-propargyl-TIQ (**3**) promoted cell viability against MPP^+-induced cell death in a bell-shaped manner and with an almost equal intensity. Selegiline (**1**) also showed protec-

tive effect from MPP^+-induced cell death at the same level as 3-Me-*N*-propargyl-TIQs (**3**). The most effective 3-MeTIQ (**2**) concentration for preventing cell death was usually 1.0 mM. However, the closed cell viability rate of these compounds (154.5 - 159.1% compared to MPP^+ alone) indicates that the stereoisomeric properties of the 3-Me-*N*-propargyl-TIQs (**3**) may not contribute to the preventive effects against cell death induced by MPP^+ (Fig. **5B**).

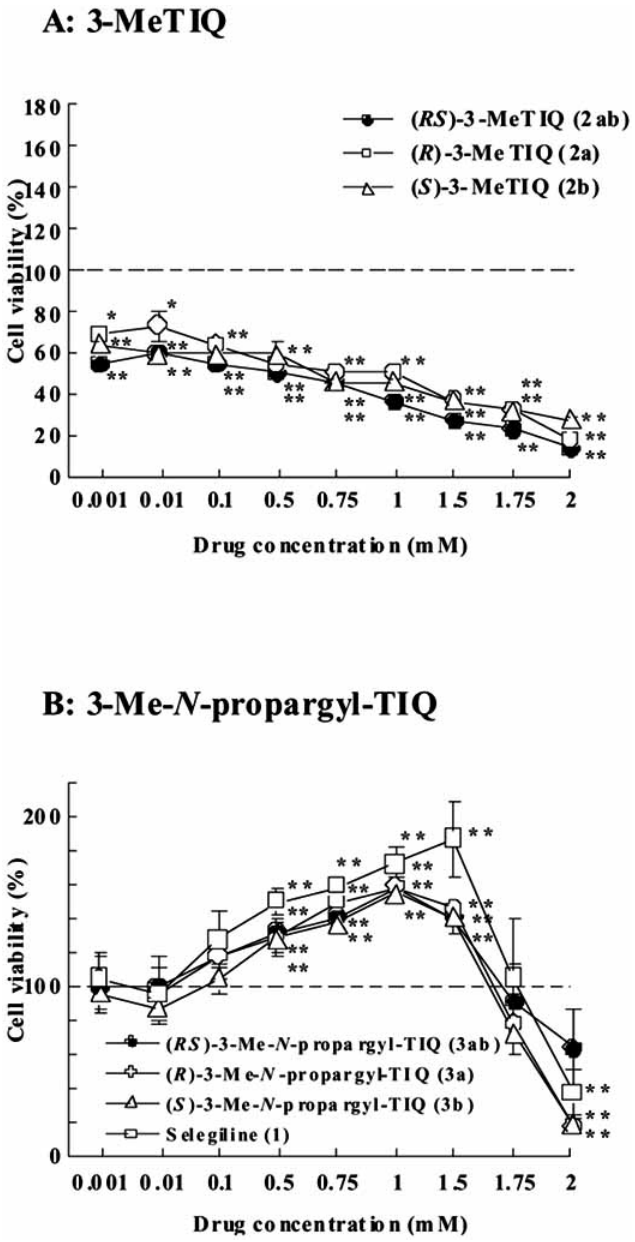


Fig. (5). Inhibitory effect of 3-MeTIQ, 3-Me-*N*-propargyl-TIQs and selegiline on MPP^+-induced cell death. The cells were exposed to each compound for 48 hr in the presence of MPP^+ (2.0 mM), and cell viability expressed as a percentage of the cell viability of MPP^+-alone treated cells (which was defined as 100%). Data express mean ± standard error (S.E., n = 4-6). A: 3-MeTIQs, B: 3-Me-*N*-propargyl-TIQs and selegiline were estimated. Asterisks showed significant differences from viability of MPP^+ alone (*, $p<0.05$, **, $p<0.01$).

In the present study, based on the structural features of selegiline (**1**) (see Fig. **1**), we synthesized 3-MeTIQs (**2**) that had the methyl group in the same position as the methyl group in selegiline (**1**). In addition, because the presence of the *N*-propargyl functional group has been shown to play an important role in any neuroprotective effect [18], the protective effects of the 3-Me-*N*-propargyl-TIQs (**3**) on MPP^+-induced cell death were also investigated. Moreover, we evaluated the four stereoisomers of the 3-MeTIQ derivatives in order to examine any structure-activity relationship, since selegiline (**1**) has previously shown different neuroprotective activities between the stereoisomers [17].

In our investigation of direct cytotoxicity, 3-MeTIQ-induced cell death did not show any optical chirality-dependent properties. The highest degree of cell death, which was about 40% and was induced by 2.0 mM 3-MeTIQs (**2**), was clearly mild compared to that induced by MPP^+, which about 80% at the same concentration. However, all of stereoisomers of 3-MeTIQ (**2**) significantly enhanced MPP^+-induced cell death, even at lower concentrations that did not induce cell death when applied alone. MPP^+ exerts its toxicity by accumulation at catecholaminergic neurons and terminals *via* the catecholamine uptake sites [22], and cell death is induced by inhibition of the respiration rate due to binding to complex I in mitochondria [23, 24]. Thus, the cell death enhancement property of the 3-MeTIQs (**2**) may be the result of acceleration or modification of this mechanism. Because the 3-MeTIQs (**2**) did not show potent cytotoxicity when applied alone, we speculate that the inhibitory effect of the 3-MeTIQs (**2**) on the mitochondrial respiration rate is also weak. Thus, the enhancement of MPP^+-induced cell death by the 3-MeTIQs (**2**) may have been caused by increasing the affinity between MPP^+ and the cells, facilitating MPP^+ accumulation or enhancing the uptake ratio of MPP^+ into the cells, i.e. accretion of MPP^+ intake.

(*RS*)- 3-Me-*N*-propargyl-TIQ (**3ab**) and (*R*)-3-Me-*N*-propargyl-TIQ (**3a**) also showed mild toxicity to PC12 cells. However, the cytotoxic effects of the (*S*)-3-Me-*N*-propargyl-TIQs (**3b**) were significantly lower than those of the above mentioned 3-Me-*N*-propargyl-TIQs (**3**). This suggests that there is a stereoisomeric aspect to the cytotoxicity of 3-Me-*N*-propargyl-TIQs (**3**). Furthermore, the cytotoxicity of the racemic compound (**3ab**), which was equal to the (*R*)-isomer (**3a**), indicates that (*S*)-3-Me-*N*-propargyl-TIQ (**3b**) did not confer resistance to cell death in racemic 3-Me-*N*-propargyl-TIQs (**3ab**).

The presence of both the 3-Me-*N*-propargyl-TIQ derivatives significantly inhibited MPP^+-induced cell death. The dose responses and the efficacy of these compounds discussed here are resembled to selegiline in the present study. Although the mechanism for this is currently unclear, our results support the suggestion that the *N*-propargyl functional group plays an important role in any neuroprotective effect [18]. However, there were no differences in the intensity of preventative effect between the racemic and optically purified 3-Me-*N*-propargyl-TIQs (**3**). These results indicate that the chirality of the methyl group that was loaded at the 3-position of *N*-propargyl-TIQ does not affect the efficacy of prevention of MPP^+-induced cell death.

In conclusion, the present study demonstrated that the cytotoxic effect of the 3-Me-TIQ (**2**) and 3-Me-*N*-propargyl-TIQs (**3**) on PC12 cells is relatively weak. The MPP^+-induced decreased viability of cells was significantly enhanced in the presence of 3-MeTIQs (**2**) and significantly inhibited by 3-Me-*N*-propargyl-TIQs (**3**). These results suggest the necessity of the *N*-propargyl group for protection of cell viability. There are many documented instances in which a slight structural difference, such as between selegiline (**1**) and 3-MeTIQ (**2**), causes pharmacological disparities. However, because of its high pharmacological potential and broad action, investigating the combination of the *N*-propargyl-group, TIQ-like structures and various constituents will increase the possibility of development of effective PD therapy.

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