European Journal of Medicinal Chemistry 44 (2009) 4034–4043

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Facile synthesis and in vitro properties of 1-alkyl- and 1-alkyl-*N*-propargyl-1,2,3,4-tetrahydroisoquinoline derivatives on PC12 cells

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ARTICLE INFO

Article history:
Received 27 August 2008
Received in revised form
18 April 2009
Accepted 23 April 2009
Available online 5 May 2009

Keywords:
Pictet–Spengler reaction
1,2,3,4-Tetrahydroisoquinoline
Structure–activity relationships
Cytotoxicity
Parkinson's disease
Cell protective effect
Propargyl group

ABSTRACT

The synthesis of several 1-alkyl-1,2,3,4-tetrahydroisoquinolines, which may play an important role in Parkinson's disease, has been achieved by modified Pictet–Spengler cyclization of the formyliminium ion. The direct cytotoxicity and preventative effects towards MPP^+-mediated death of PC12 cells were estimated. The cytotoxicities of 1-alkyl-TIQs were apoptotic and depended on their lipophilic properties. Conversely, introducing the *N*-propargyl substituent reduced cytotoxicity. 1-Methyl-, 1-methyl-*N*-propargyl- and 1-cyclopropyl-TIQ partially inhibited MPP^+-induced cell death, whereas relatively large alkyl substituents at the first position did not enhance the viability of PC12 cells. In summary, our findings suggest a crucial role for the *N*-propargyl functional group for the effective reduction of cytotoxicity, and show the importance of size and lipophilicity of substituents at the 1-position of 1-alkyl-TIQs.

1. Introduction

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a remarkably selective dopaminergic neurotoxin that produces a clinical syndrome similar to Parkinson's disease (PD) [1]. However, because MPTP is an exogenous compound, it is possible that similar endogenous toxins accumulate in the central nervous system and cause PD. It has been reported that 1,2,3,4-tetrahydroisoquinoline (TIQ) and its derivatives, which possess structural similarity to MPTP, may act as endogenous or environmental neurotoxins causing PD [2,3]. One TIQ derivative, 1-benzyl-TIQ, exists naturally in animal brain tissue and was found to induce Parkinsonism in monkeys and mice [3,4]. 1-Benzyl-TIQ has been implicated as a neurotoxin causing PD in humans because its concentration in the cerebro-spinal fluid of Parkinsonian patients is approximately three times that found in healthy individuals [3]. In contrast, 1-methyl-TIQ is present in normal mouse brain, and has been shown to prevent TIQ- and 1-benzyl-TIQ-induced behavioral abnormalities in rodents [3,5]. These findings suggest that the substituents attached to the 1-position of the TIQ ring system may influence the pharmacological properties and biological activities of TIQ derivatives.

Methods for synthesizing the isoquinoline ring system include the Pictet–Spengler reaction [6], the Bischler–Napieralski reaction [7] and the Pomeranz–Fritsch reaction [8,9]. In addition to these methods, the synthesis of 1-methyl-TIQ, 1-phenyl-TIQ and 1-benzyl-TIQ using a Pummerer-type cyclization reaction as the key step has been previously reported [10,11]. Of these reactions, the Pictet–Spengler reaction and Pummerer-type cyclization reaction have proven effective for synthesizing TIQs [12–14]. The simple endogenous TIQs are, in fact, proposed to be biosynthesized from imines derived from 2-phenylethylamine and formaldehyde, acetaldehyde or phenylacetaldehyde through a mechanism similar to that in the Pictet–Spengler reaction [15]. This biogenetic mechanism is not necessarily consistent with the observation that the Pictet–Spengler cyclization is very sensitive to the aromatic substituents of the 2-arylethylamine moiety of imines, and that an electron-donating hydroxyl or alkoxy group at the *para* position of the cyclization site is the minimum requisite for cyclization [16–18]. Cyclization of an

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doi:10.1016/j.ejmech.2009.04.035

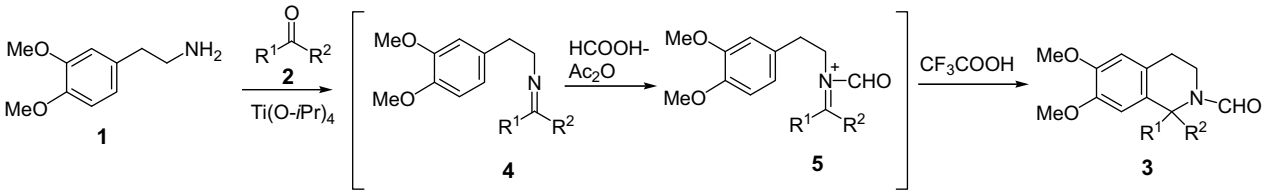

Fig. 1. Synthesis of *N*-formyl 1,1-disubstituted 1,2,3,4-tetrahydroisoquinlines *via* a modified Pictet–Spengler reaction, described previously.

imine that lacks an electron-donating group in the aromatic ring requires drastic reaction conditions. Yokoyama and coworkers reported the use of the very strong acid, trifluoromethane sulfonic acid (TFSA), to generate the dicationic species to promote the cyclization reaction for the synthesis of 1-substitued TIQs [19].

Recently, we developed a highly efficient method for synthesizing *N*-formyl 1,1-disubstituted TIQs (**3**) using a modified Pictet–Spengler reaction. In this reaction, cyclization was accelerated by the presence of the *N*-formyliminum ion (**5**) intermediate formed *in situ* from imine (**4**) of arylethylamine (**1**) using acetic-formic anhydride [20]. The high reactivity of the formyliminium ion (**5**) was apparent since **5** readily caused cyclization in the presence of the relatively weak acid, trifluoroacetic acid (TFA) (Fig. 1).

We also previously showed that the cytotoxicity of various 1-alkyl-TIQ derivatives, and the size and electron-donating properties of its functional groups, may influence the cytotoxic properties of the compound [21].

In the present study, we describe a convenient protocol for the synthesis of several 1-alkyl-TIQs (**11**) using a modified Pictet–Spengler reaction, and estimate their cytotoxicity effects on PC12 cells. Selegiline, an *N*-propargyl-containing compound with some structural resemblance to TIQ (**12**), is used as a therapeutic drug for PD, and it has been reported that the *N*-propargyl functional group plays a crucial neuroprotective role [22]. Thus, we also synthesized 1-alkyl-*N*-propargyl-TIQ and evaluated the effects of this and other TIQ derivatives on improving cell viability.

2. Chemistry

Modified Pictet–Spengler reaction conditions were applied to imine (**8**) obtained from 2-phenylethylamine (**6**) and aldehyde (**7**). The formyliminium ion (**9a**) was prepared by condensation of **6** and benzaldehyde (**7a**) by heating at 80 °C in titanium(IV) isopropoxide for 1 h, followed by treatment with acetic-formic anhydride prepared from formic acid (300 eq) and acetic anhydride (100 eq) at 70 °C for 3 h. and the *in situ* formed **9a** was treated with TFA (200 eq) by heating at 70 °C for 18 h to produce *N*-formyl-1-phenyl TIQ (**10a**) in 99% yield. The use of formic acid instead of TFA gave **10a** in only 7% yield.

The formyliminium ion (**9b**–**g**) and (**9j**, **k**) prepared *in situ* from **6** and other acyclic and cyclic aldehydes such as paraldehyde (**7b**), propionaldehyde (**7c**), *n*-butylaldehyde (**7d**), *n*-pentanal (**7e**), *n*-hexanal (**7f**), *n*-heptanal (**7g**), cyclopentanecarbaldehyde (**7j**) and cyclohexanecarbaldehyde (**7k**) gave corresponding *N*-formyl 1-alkyl-TIQs (**10b**–**g** and **10j**, **k**) in excellent to moderate yields under similar reaction conditions. Product yields are summarized in Table 1. A unique case is the reaction of **6** with cyclopropanecarbaldehyde (**7h**). This reaction yielded the cyclized products 1-(3-hydroxypropyl) TIQ (**10l**) and 1-(3-acetoxypropyl) TIQ (**10m**), formed by the acid catalyzed hydrolytic cleavage of the cyclopropane ring followed by acetylation with acetic anhydride.

TIQs (**10a**–**d**) and (**10j**, **k**) obtained as described above were identical with those obtained from the Pummerer-type cyclization reactions described previously [21].

Hydrolytic deprotection of the *N*-formyl group gave the corresponding 1-alkyl-TIQ (**11**). Moreover, TIQs (**12**) were generated from propargylamines such as Selegiline, which is a potent neuroprotective agent, by the propargylation of the nitrogen atom at the 2-position with propargylbromide and cesium carbonate in acetonitrile (Fig. 2).

The synthesized TIQs (**11**, **12**) were biochemically evaluated for their neuroprotective effects.

3. Biological results and discussion

3.1. Effects of 1-alkyl-TIQ on PC12 cell viability

The effect of each 1-alkyl-TIQ derivative on the viability of PC12 cells was estimated by comparing viability to that of untreated control cells (whose viability was defined as 100%). Cell viability was unaffected except in the highest concentration of 1-methyl-TIQ, the smallest C-1 substituent derivative. However, potent cytotoxicity was observed proportional to the size of the linear extended alkyl C-1 substituent. 1-Normal (*n*)-hexyl-TIQ, the largest substituent TIQ derivative investigated in the present study, resulted in significantly reduced cell viability except at the lowest concentration tested (Fig. 3A).

Annulated substituents at the 1-position also reduced cell viability, depending on the carbon number in the substituent. Similar to 1-*n*-hexyl-TIQ, 1-cyclo (*cy*)-hexyl-TIQ resulted in significantly reduced cell viability except at the lowest concentration tested (Fig. 3B).

We calculated the concentration resulting in 50% toxicity (TC_{50}) and computer calculated the log *P* value (used as a parameter for liposolubility) for every TIQ derivative; the correlation found between these parameters is shown in Fig. 4. This result suggests that the cytotoxicity of a 1-alkyl-TIQ derivative is proportional to its liposolubility.

3.2. Effects of 1-alkyl-N-propargyl-TIQ derivatives on PC12 cell viability

1-Alkyl-TIQ derivatives substituted with the *N*-propargyl group showed weaker cytotoxicity than 1-alkyl-TIQ derivatives, and

Table 1
Synthesis of *N*-formyl 1-substituted TIQs **10**.

Run	Aldehyde **7**		Product yields (%)			
		R				
1	**7a**	Ph	**10a**	99		
2	**7b**	methyl	**10b**	57		
3	**7c**	ethyl	**10c**	49		
4	**7d**	*n*-propyl	**10d**	48		
5	**7e**	*n*-butyl	**10e**	76		
6	**7f**	*n*-pentyl	**10f**	87		
7	**7g**	*n*-hexyl	**10g**	90		
8	**7h**	cyclopropyl	**10l**	64	**10m**	33
9	**7j**	cyclopentyl	**10j**	71		
10	**7k**	cyclohexyl	**10k**	95		

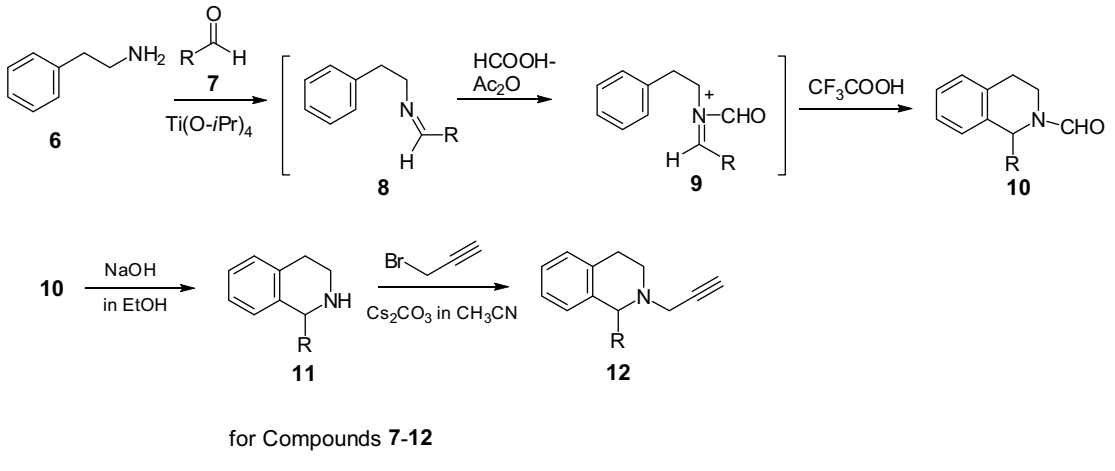

Fig. 2. Synthesis of 1-alkyl-2-propargyl-1,2,3,4-tetrahydroisoquinolines **12**, using a modified Pictet–Spengler cyclization reaction.

significantly reduced viability of PC12 cells was observed only at the highest concentration tested or, for several derivatives, also at the second highest concentration tested (Fig. 5A and B). This suggests that the *N*-propargyl group helps reduce the cytotoxicity of 1-alkyl-TIQ derivatives. This cytotoxicity-decreasing effect became evident when *N*-propargyl derivatives were compared to potent cytotoxic 1-alkyl-TIQs. For example, *N*-propargylation changed the properties of two compounds that induced cell death

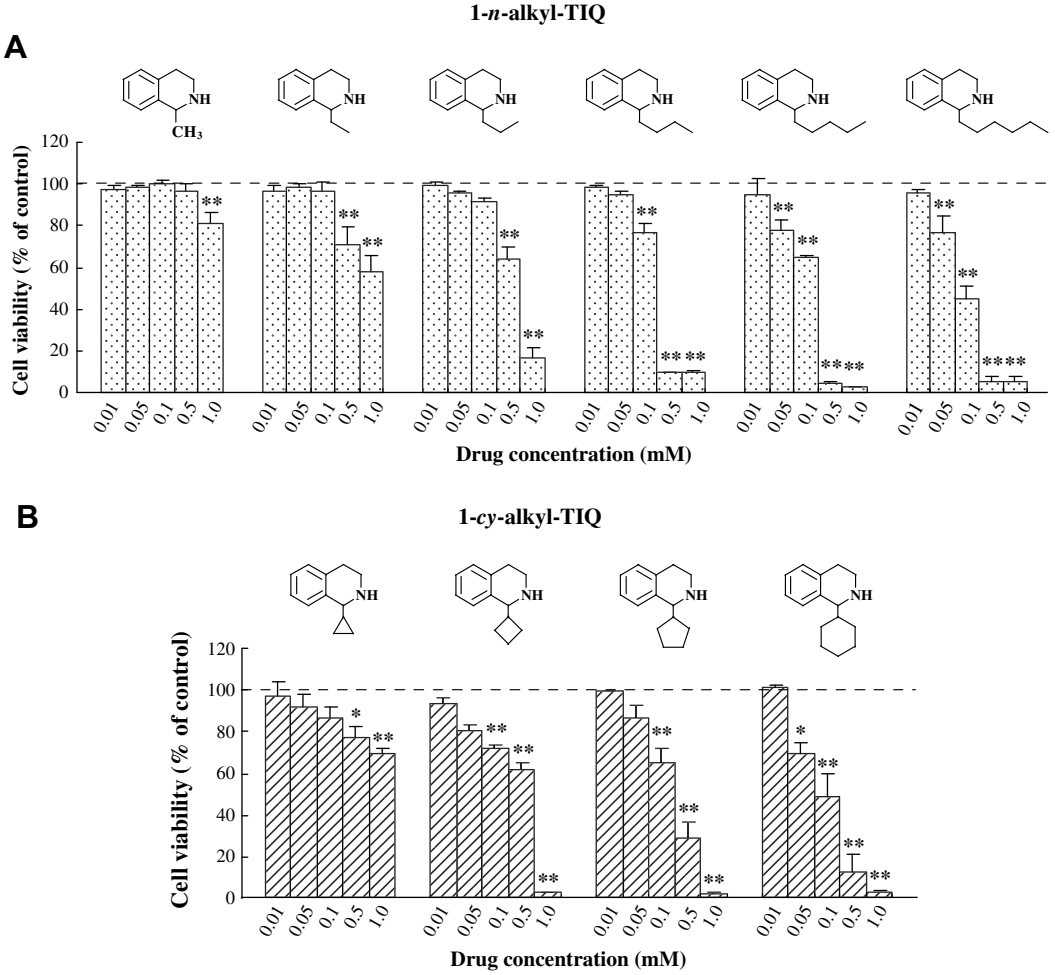

Fig. 3. Cytotoxic effects of various 1-alkyl-TIQs on cultured PC12 cells. Cells were exposed to each compound for 48 h, and cell viability is presented as a percentage of the untreated control cells (defined as 100% viable). Data are expressed as means ± standard error (S.E., $n = 3$–6). A: normal (*n*)-alkyl substituents and B: cyclo (*cy*)-alkyl substituents of TIQs were estimated. Asterisks show significant differences from control cell viability ($^{*}P < 0.05$, $^{**}P < 0.01$).

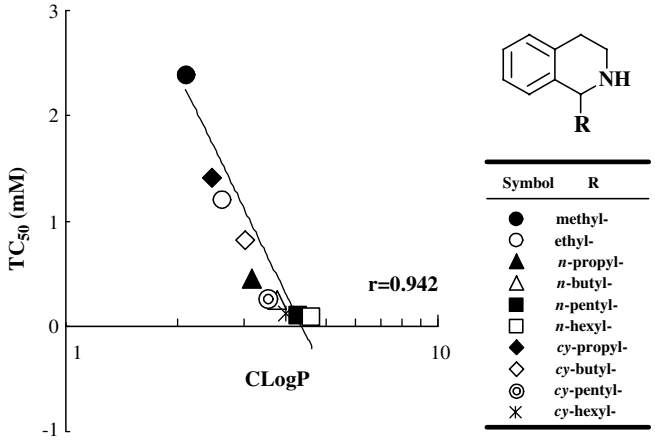

Fig. 4. Correlation diagram between log *P* values (as an indicator of liposolubility) and 50% toxicity concentration (TC_{50}) of 1-alkyl-TIQ derivatives. *n*: normal, *cy*: cyclo-.

at high concentrations, 1-*n*-butyl-TIQ and 1-*cy*-hexyl-TIQ. The cytotoxicity of these compounds was almost completely abolished by introducing the propargyl group at the *N*-position of the TIQ ring (Fig. 6). Thus, it appears that a crucial relationship exists between the liposolubility property (C-number) or the size of the functional group at the 1-position of 1-alkyl-TIQ derivatives, and the cytotoxicity-reducing effect of the *N*-propargyl substituent.

3.3. Apoptotic induction of 1-alkyl-TIQ

At high concentrations, 1-*n*-hexyl- and 1-*cy*-hexyl-TIQ were clearly cytotoxic (as shown in Fig. 3A and B). The ability of these compounds and 1-methyl-4-phenylpyridinium ion (MPP^+) to induce DNA damage in individual cells was evaluated using the comet assay. Fig. 7A depicts untreated control cells, and typical migrated cell nuclei with fragmented DNA caused by treating with MPP^+. Small portions of DNA remain at the origin, but most of the DNA has fragmented and migrated to form a 'comet' tail. Diffuse, migrated DNA is indicative of apoptosis in a cell. In the present study, a cell exhibiting such a 'comet' tail was considered to be in the apoptotic state, and the whole length of the 'comet' was measured to estimate the cytotoxicity of the 1-alkyl-TIQ derivatives described above.

All of the MPP^+, 1-*n*-hexyl-TIQ and 1-*cy*-hexyl-TIQ compounds induced DNA fragmentation and extended the length of the 'comet' (Fig. 7B). To estimate cytotoxicity, we documented the length of each 'comet' at intervals of 30 μm. In MPP^+ treated cells, most 'comets' were between 60 and 90 μm long. In 1-*n*-hexyl-TIQ and 1-*cy*-hexyl-TIQ treated cells, most 'comets' were 150–180 μm and

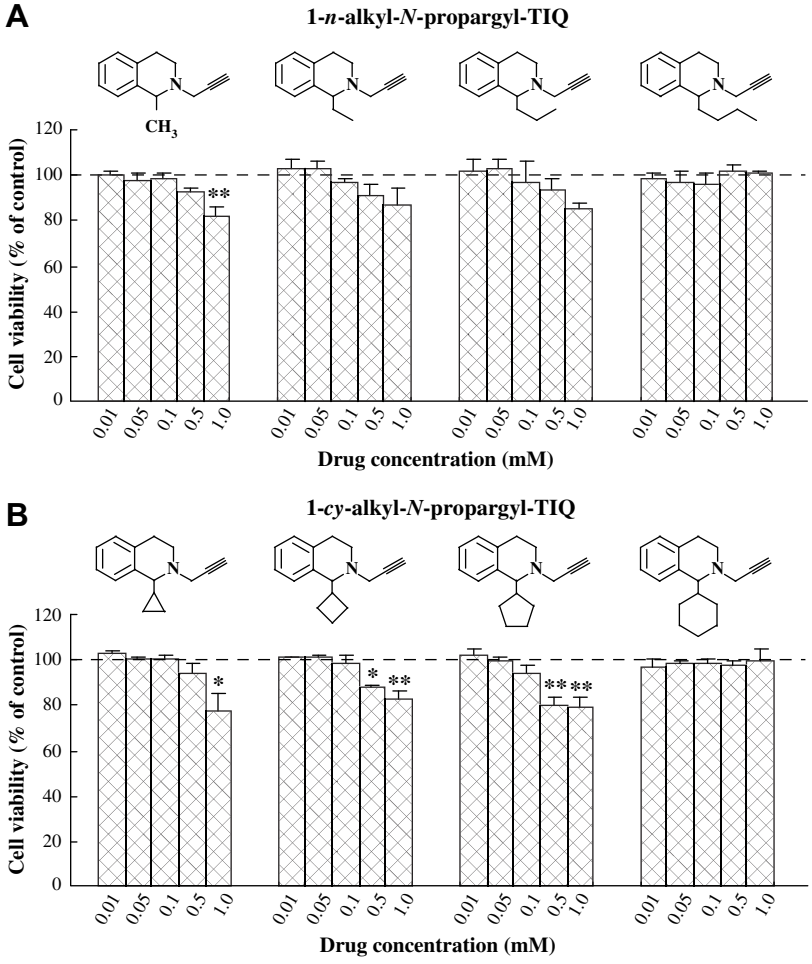

Fig. 5. Cytotoxic effects of *N*-propargyl-substituted 1-alkyl-TIQ (1-alkyl-*N*-propagyl-TIQ) derivatives on cultured PC12 cells. Each compound was incubated for 48 h with the cells, and cell viability is presented as a percentage of the untreated control (defined as 100% viable). Data are expressed as means ± standard error (S.E., $n = 3$–5). A: normal (*n*)-alkyl substituents and B: cyclo (*cy*)-alkyl substituents of *N*-propargyl-TIQs were estimated. Asterisks show significant differences from control group viability ($^*P < 0.05$, $^{**}P < 0.01$).

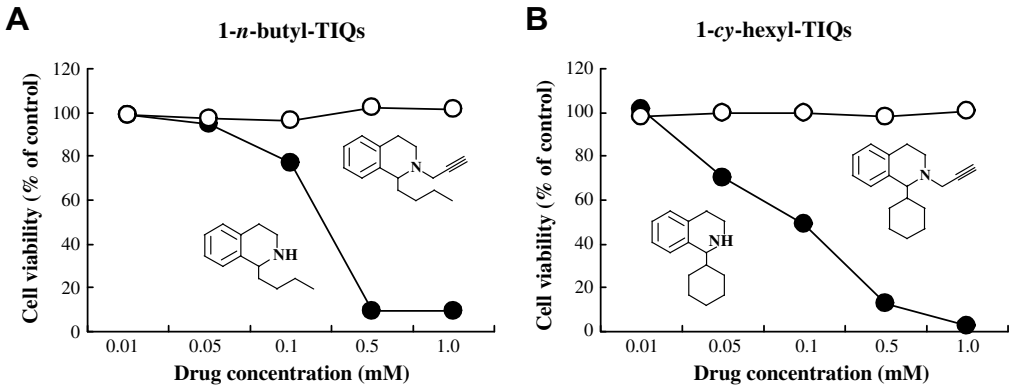

Fig. 6. Effect of *N*-propargyl substituent in 1-alkyl-TIQ derivatives on PC12 cell viability. *N*-Propargylation clearly decreases cytotoxicity of 1-alkyl-TIQ derivatives. *n*: normal, *cy*: cyclo-.

120–150 μm long, respectively. Increased 'comet' length suggested increased DNA fragmentation. Thus, these results indicate that both 1-*n*-hexyl-TIQ and 1-*cy*-hexyl-TIQ derivatives induce DNA damage in PC12 cells. This apoptotic DNA damaging effect may be related to the liposolubility (C-number) or size of the functional group at the 1-position, and may contribute to the cytotoxicity of TIQ derivatives.

3.4. Effects of 1-alkyl- and 1-alkyl-N-propargyl-TIQ derivatives on MPP^+-induced cell death

The ability of various 1-alkyl- and 1-alkyl-*N*-propargyl-TIQ derivatives to protect against MPP^+-induced cell death was evaluated. Results are expressed as the number of surviving cells treated with TIQ derivatives compared to the number of cells surviving following MPP^+ treatment alone (defined as 100%). In the 1-alkyl-TIQ (1-methyl-, ethyl-, *cy*-propyl- and *cy*-butyl-TIQ) group, only 1-methyl-TIQ significantly increased the number of surviving cells (Fig. 8A). The effects of *N*-propargyl-substituted 1-alkyl-TIQ derivatives on MPP^+-induced cell death were also investigated. Statistically significant improvements in cell viability were observed upon treatment with 1-methyl-*N*-propargyl- and 1-*cy*-propyl-*N*-propargyl-TIQ. When the substituent was increased by one carbon at the 1-position, i.e. 1-ethyl-*N*-propargyl-TIQ and 1-*cy*-butyl-*N*-propargyl-TIQ, the cells were no longer protected (Fig. 8B). The importance of the liposolubility properties of 1-alkyl-*N*-propargyl-TIQ derivatives and their ability to reduce cytotoxicity will be investigated in future studies. However, the present results indicate that the size of the C-1 substituent, rather than its liposolubility property, is the most important parameter determining the ability of 1-alkyl-*N*-propargyl-TIQs to protect cells against MPP^+-induced death.

3.5. Depressive effect of 1-alkyl- and 1-alkyl-N-propargyl-TIQ derivatives towards caspase activity

TIQ derivatives that improved cell viability (i.e., 1-methyl-TIQ, 1-methy-*N*-propargyl- and 1-*cy*-propyl-*N*-propargyl-TIQ) were investigated further. The results are expressed as a ratio of caspase-3,7 activity, with untreated cells defined as 100%. Caspase-3,7 activity in MPP^+-treated cells was significantly increased (about 1.7 times higher) compared to untreated control cells. The presence of 1-methyl-, 1-methyl-*N*-propargyl- and 1-*cy*-propyl-*N*-propargyl-TIQ with MPP^+ almost entirely prevented the increased activity of caspase-3,7 (Fig. 9). This result indicates that MPP^+-induced apoptosis and apoptotic inhibition by these TIQ derivatives on PC12 cells are due at least in part to their effect on caspase-3,7 activity.

3.6. Effect of 1-methyl-N-propargyl- and 1-cy-propyl-N-propargyl-TIQ on monoamine oxidase (MAO) activity in vitro

Finally, we examined the effect of two TIQ derivatives that improved cell viability (1-methy-*N*-propargyl- and 1-*cy*-propyl-*N*-propargyl-TIQ)

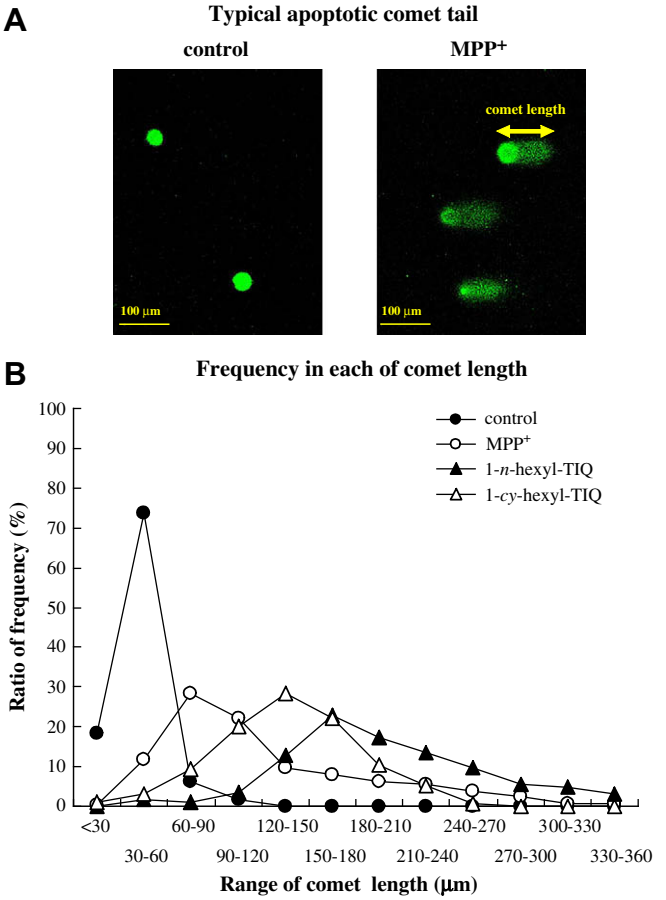

Fig. 7. 'Comet' tail formation due to fragmentation of DNA in PC12 cells caused by 3 h exposure to 1 mM MPP^+, 1-normal (*n*)-hexyl- and 1-cyclo (*cy*)-hexyl-TIQ. A: The absence of 'comets' in control cells and typically observed 'comets' in cells treated with MPP^+. Images were obtained using a fluorescence microscope at ×200 magnification and a wavelength of 568 nm. In 'comets', a small portion of the DNA remains at the origin, but most of the DNA fragments migrate to form the 'comet' tail. In the present study, damaged cells such as these are termed apoptotic damaged cells. B: The measured length of a 'comet' at intervals of 30 μm in cells that produced 'comet' tails upon treatment with MPP^+, 1-*n*-hexyl- and 1-*cy*-hexyl-TIQ.

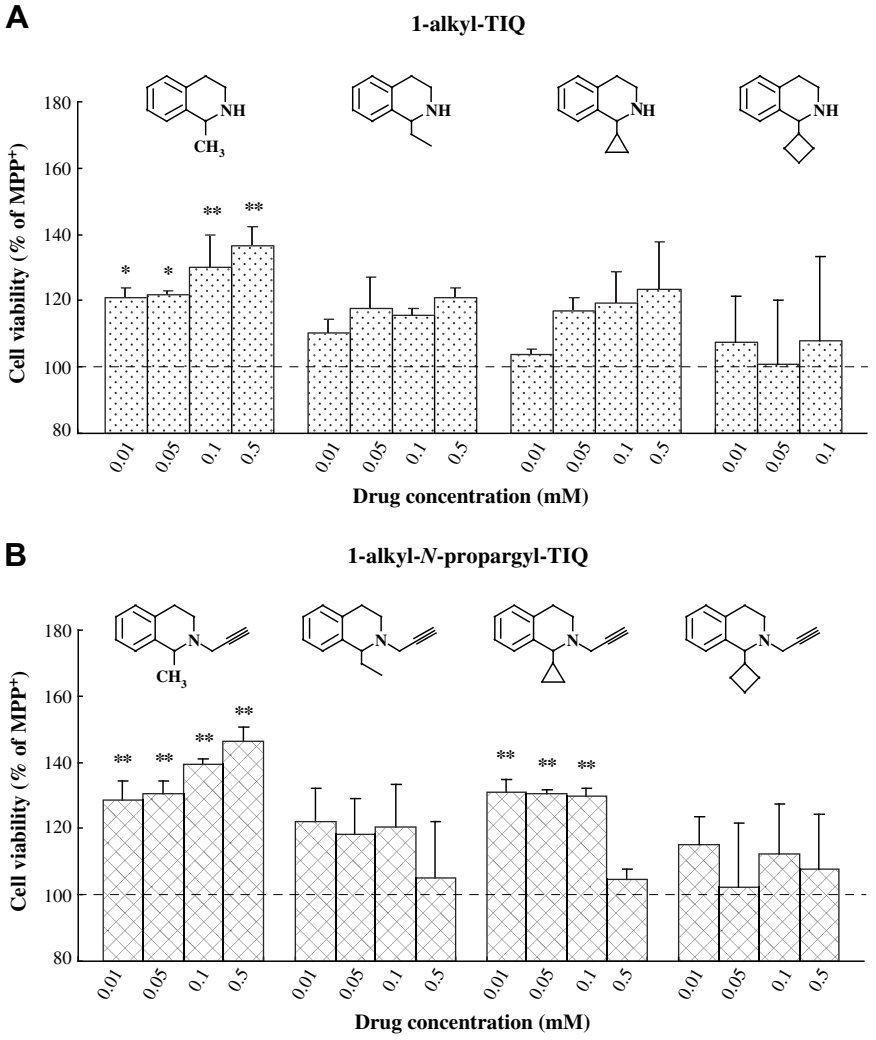

Fig. 8. Inhibitory effect of 1-alkyl- and 1-alkyl-*N*-propargyl-TIQ derivatives on MPP^+-induced cell death. Each compound was incubated for 48 h with cells together with MPP^+ (0.25 mM), and cell viability is presented as a percentage of the cells incubated with MPP^+ alone (defined as 100%). Data are expressed as means ± standard error (S.E., $n = 3$–4). 1-Alkyl-TIQs (A) and 1-alkyl-*N*-propargyl-TIQs (B) were estimated. Asterisks show significant differences from control cell viability ($^*P < 0.05$, $^{**}P < 0.01$).

on MAO activity in vitro. Selegiline was used as a positive control to selectively inhibit MAO-B. Results are expressed as the ratio of MAO activity, in which cells treated with vehicle are defined as 100% active. Selegiline potently inhibits MAO-B activity in the case of both substrates (tyramine and benzylamine) but does not affect MAO-A activity. Both 1-methy-*N*-propargyl- and 1-*cy*-propyl-*N*-propargyl-TIQ did not influence MAO activity, irrespective of their subtype and substrate (Table 2). These results show that these compounds, which help protect PC12 cell viability from MPP^+ cytotoxicity, may not inhibit dopaminergic metabolism. Selegiline helps retain dopamine at synapses due to its specific inhibition of MAO-B activity, and has been used as a putative neuroprotective agent to treat PD [23]. However, since 1-methy-*N*-propargyl- and 1-*cy*-propyl-*N*-propargyl-TIQ did not change MAO-A and MAO-B activities, it is possible that the cytoprotective effect of these compounds may follow a different mechanism(s) from Selegiline and does not depend on inhibition of MAO-B. Designing compounds that cytoprotect cells from cytotoxic substances without MAO inhibition may lead to new drugs for treating PD.

4. Conclusions

A number of 1-alkyl- and 1-alkyl-*N*-propargyl-TIQ derivatives potentially useful for treating PD were synthesized from phenylethylamine and aldehyde using a modified Pictet–Spengler reaction. The cyclization reaction was accelerated by the presence of *N*-formyliminium ion (**9**) as an intermediate, was carried out using a one-pot procedure, and was shown to be a convenient and effective method for preparing various TIQs.

The cytotoxic properties of 1-alkyl-TIQ compounds on PC12 cells depended on their liposolubility properties, and introducing an

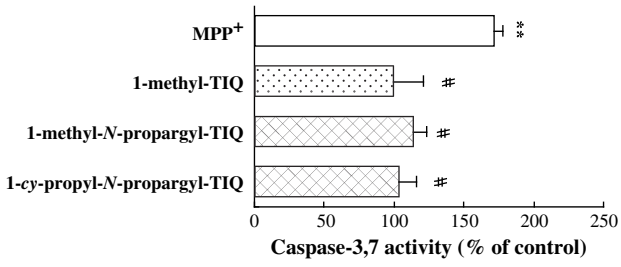

Fig. 9. Inhibitory effect of 1-methyl-, 1-methyl-*N*-propargyl- and 1-*cy*-propyl-*N*-propargyl-TIQ on MPP^+-enhanced caspase-3,7 activity. Enzyme activity in untreated cells is defined as 100%. MPP^+ significantly enhanced caspase-3,7 activity and co-addition of the above TIQ derivatives clearly depressed this activity. Data are expressed as means ± standard error (S.E., $n = 3$–4). Asterisks show significant differences compared to the untreated cells ($^{**}P < 0.01$) and the MPP^+ treated cells ($\#P < 0.05$).

Table 2
Effect of TIQ derivatives on MAO activity.

Substrate	Enzyme	MAO activity (% of vehicle control)			
		Vehicle	Selegiline	1-methyl-*N*-propargyl-TIQ	1-*cy*-propyl-*N*-propargyl-TIQ
Tyramine	MAO-B	100.0 ± 0.7	4.8 ± 0.1**	100.9 ± 0.5	98.4 ± 0.4
Benzylamine	MAO-B	100.0 ± 1.0	20.7 ± 0.3**	99.4 ± 0.8	97.5 ± 0.8
Tyramine	MAO-A	100.0 ± 1.1	94.2 ± 1.0	97.5 ± 1.2	96.2 ± 1.2

Data are expressed as means ± standard error (S.E., $n = 8$). Asterisk shows significant difference from vehicle control (**$P < 0.01$).

N-propargyl substituent reduced the cytotoxicity of 1-alkyl-TIQ. In addition, the contribution of apoptotic DNA damage was confirmed for 1-alkyl-TIQ-induced cytotoxicity. Although only 1-methyl-TIQ prevented MPP^+-induced cell death, introducing the *N*-propargyl group enhanced the cell-protecting effects of 1-*cy*-propyl-TIQ. However, such enhancement was not observed when relatively large substituents were introduced, showing that the size of the functional group at the 1-position of 1-alkyl-TIQ derivatives is important. In conclusion, these results suggest that the *N*-propargyl functional group is crucial for reducing cytotoxicity, and demonstrate the importance of the size or liposolubility of substituents at the 1-position of 1-alkyl-TIQ derivatives. Further investigations into the synthesis and biological activity of TIQ derivatives with *N*-propargyl or different C-1 substituents may help develop new agents for treating PD.

5. Experimental procedure

5.1. Chemistry

Unless otherwise noted, 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 with a HORIBA FT-710 Fourier transform infrared spectrophotometer; samples were cast as films for oils and gums, and disks were prepared for solids using KBr. The *x*-axis on the spectra is cm^{-1}. Nuclear magnetic resonance spectra were measured with a JEOL JNM-AL300 NMR spectrometer (^1H: 300 MHz, ^{13}C: 75 MHz). Samples were dissolved in chloroform-*d* with tetramethylsilane as an internal standard. Results are expressed in terms of *d* value. High resolution fast atom bomb ionization mass spectra were taken on a JEOL HX-110A mass spectrometer using *m*-nitrobenzyl alcohol as a matrix and a solvent, and results are shown as *m*/*z*.

5.1.1. General procedure for the synthesis of 1-alkyl-N-formyl-TIQ **10** using a modified Pictet–Spengler reaction

A solution of phenylethylamine (**6**) (5.52 mmol), aldehyde (**7**) (4.60 mmol) and Ti(O-*i*Pr)$_4$ (6.90 mmol) was heated at 80 °C for 1 h under an argon atmosphere. To this reaction mixture a solution of acetic-formic anhydride [prepared from formic acid (1.38 mol) and acetic anhydride (0.46 mol)] was added at 0 °C and then heated at 70 °C for 3 h. The reaction mixture was cooled to 0 °C again, trifluoroacetic acid (0.92 mol) was further added to the solution and then the resulting mixture was stirred for 16 h. The reaction mixture was diluted with MeOH (50 mL) and passed through a short silica gel column to remove TiO_2. The eluent was concentrated *in vacuo* and diluted with $CHCl_3$, washed with 10% NaOH solution and brine and then dried over Na_2SO_4. After removal of the solvent *in vacuo*, the residue was purified by chromatography on silica gel to give **10**. Yields are summarized in Table 1.

For compounds **10a**–**d** and **10j**, **k**, please refer to our previous papers [11,12,21].

5.1.1.1. Analytical data for the new compounds

*5.1.1.1.1. 1-n-Butyl-N-formyl-1,2,3,4-tetrahydroisoquinoline (**10e**).* Yield: 76%, as a colorless gum eluted with AcOEt:hexane = 1:4. IR: 1670. ^1H-NMR: 0.90, 0.94 (total 3H, each t, $J = 7$ Hz, CH_3), 1.22–1.49 (4H, m, *n*-butyl), 1.74–1.88 (2H, m, *n*-butyl), 2.75–3.15, 3.52–3.62, 3.66–3.73, 4.44–4.50 (total 4H, each m, C3-H and C4-H), 4.45–4.50, 5.38–5.43 (total 1H, each m, C1-H), 7.08–7.26, 7.36–7.49 (total 4H, each m, Ar), 8.20, 8.22 (total 1H, each s, CHO). LR-FABMS *m*/*z*: 218 (MH^+), 218 (base peak). HR-FABMS: Calcd for $C_{14}H_{20}NO$: 218.1544, found: 218.1544.

*5.1.1.1.2. N-Formyl-1-n-pentyl-1,2,3,4-tetrahydroisoquinoline (**10f**).* Yield: 87%, as a colorless gum eluted with AcOEt:hexane = 1:4. IR: 1654. ^1H-NMR: 0.88, 0.90 (total 3H, each t, $J = 7$ Hz, CH_3), 1.22–1.49, 1.68–1.86 (total 8H, each m, *n*-pentyl), 2.75–3.15, 3.12–3.61, 3.66–3.73, 4.45–4.51 (total 4H, each m, C3-H and C4-H), 4.45–4.51, 4.38–5.43 (total 1H, each m, C1-H), 7.08–7.80 (4H, m, Ar), 8.21, 8.23 (total 1H, each s, CHO). LR-FABMS *m*/*z*: 232 (MH^+), 232 (base peak). HR-FABMS: Calcd for $C_{15}H_{22}NO$: 232.1702, found: 232.1699.

*5.1.1.1.3. N-Formyl-1-n-hexyl-1,2,3,4-tetrahydroisoquinoline (**10g**).* Yield: 90%, as a yellow oil eluted with AcOEt:hexane = 1:6. IR: 1672. ^1H-NMR: 0.87, 0.89 (total 3H, each t, $J = 7$ Hz, CH_3), 1.22–1.40, 1.79–1.86 (total 10H, each m, *n*-hexyl), 2.74–3.15, 3.51–3.60, 3.61–3.72, 4.44–4.50 (total 4H, each m, C3-H and C4-H), 4.44–4.49, 5.38–5.42 (total 1H, each m, C1-H), 7.07–7.22 (4H, m, Ar), 8.20, 8.22 (total 1H, each s, CHO). LR-FABMS *m*/*z*: 246 (MH^+), 246 (base peak). HR-FABMS: Calcd for $C_{16}H_{24}NO$: 246.1858, found: 246.1855.

*5.1.1.1.4. 2-Formyl-1-(3-hydroxypropyl)-1,2,3,4-tetrahydroisoquinoline(**10l**).* Yield: 64% as a colorless prisms from AcOEt, mp 113–114 °C. IR: 3340, 1655. ^1H-NMR: 1.57–1.98 (4H, m, –$CH_2CH_2CH_2OH$), 2.75–3.03 (2H, m, C4-H), 3.07–3.17, 3.53–3.63 (total 2H, each m, C3-H), 3.68–3.77 (2H, m, –$CH_2CH_2CH_2OH$), 4.54, 5.48 (total 1H, each dd, $J = 7$ Hz, 7 Hz, $J = 5$ Hz, 9 Hz, C1-H), 7.08–7.32 (4H, m, –Ar), 8.23 (1H, s, –CHO). ^{13}C-NMR: 27.8, 28.9 ($CH_2CH_2CH_2OH$), 29.5, 29.7 (C4), 33.2, 33.3 ($CH_2CH_2CH_2OH$), 34.2, 41.0 (C3), 50.9, 57.4 (C1), 61.9, 62.4 ($CH_2CH_2CH_2OH$), 126.3, 126.6 (Ar), 126.8 (Ar), 127.1, 127.4 (Ar), 129.0, 129.2 (Ar), 132.6, 133.6 (C4a), 136.5, 136.7 (C8a), 161.6, 162.0 (CHO). LRMS *m*/*z*: 219 (M^+), 160 (base peak). HRMS: Calcd for $C_{13}H_{17}NO_2$: 219.1260, found: 219.1271.

*5.1.1.1.5. Acetic acid 3-(2-formyl-1,2,3,4-tetrahydroisoquinolin-1-yl)-propyl ester(**10m**).* Yield: 33% as a yellow gum. IR: 1736, 1670. ^1H-NMR: 1.69–1.98 (4H, m –$CH_2CH_2CH_2OC(O)CH_3$), 2.04, 2.06 (total 3H, each s, –$CH_2CH_2CH_2OC(O)CH_3$), 2.77–3.16 (3H, m, C3-H and C4-H), 3.51–3.61, 3.69–3.75 (total 1H, each m, C3-H), 4.09–4.17, 4.45–4.49 (total 2H, each m, –$CH_2CH_2CH_2OC(O)CH_3$), 4.50–4.55, 5.42–5.47 (total 1H, each m, C1-H), 7.09–7.33 (4H, m, –Ar), 8.23, 8.24 (total 1H, each s, –CHO). ^{13}C-NMR: 20.9, 21.0 ($CH_2CH_2CH_2OC(O)CH_3$), 25.5, 25.8 ($CH_2CH_2CH_2OC(O)CH_3$), 27.8, 29.6 (C4), 32.9, 33.1 ($CH_2CH_2CH_2OC(O)CH_3$), 34.2, 39.9 (C3), 50.5, 57.0 (C1), 63.6, 64.0 ($CH_2CH_2CH_2OC(O)CH_3$), 126.4, 126.6 (Ar), 126.7, 126.8 (Ar), 127.2 (Ar), 129.1, 129.3 (Ar), 132.8, 133.6 (C4a), 136.3, 136.4 (C8a), 161.4, 161.8 (CHO), 171.0, 171.1 ($CH_2CH_2CH_2OC(O)CH_3$). LRMS *m*/*z*: 261 (M^+), 161 (base peak). HRMS: Calcd for $C_{15}H_{19}NO_3$: 261.1366, found: 261.1396.

5.1.2. General procedure for the hydrolysis of **10**

A solution of **10** (1.90 mmol) in EtOH (15 mL)–20% NaOH (15 mL) was refluxed for 3.0 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The product was purified by column chromatography on SiO_2 (AcOEt) to give 1,2,3,4-tetrahydroisoquinoline **11**.

5.1.2.1. Analytical data for the new compounds

*5.1.2.1.1. 1-n-Butyl-1,2,3,4-tetrahydroisoquinoline (**11e**) [24–28].* Yield: 93%, as a pale yellow oil eluted with AcOEt:hexane = 1:4, HCl salt; colorless prisms from ether–MeOH, mp 127–128 °C (lit. [27] mp 143–144 °C). ^1H-NMR: 0.90–0.95 (3H, m, CH_3), 1.30–1.50 (4H, m, $(CH_2)_2$), 1.66–1.91 (2H, m, CH_2), 2.69–2.88 (2H, m, C4-H),

2.98 (1H, ddd, $J = 12$, 7, 5 Hz, C3-H), 3.24 (1H, dt, $J = 12$, 5 Hz, C3-H), 3.96 (1H, dd, $J = 9$, 4 Hz, C1-H), 7.05–7.21 (4H, m, Ar). ^{13}C-NMR: 14.1 (CH_3), 22.9 (*n*-butyl), 28.4 (*n*-butyl), 30.0 (C4), 36.2 (*n*-butyl), 41.1 (C3), 55.8 (C1), 125.7 (C7), 125.7 (C6), 126.1 (C5), 129.2 (C8), 135.1 (C8a), 139.9 (C4a). LR-FABMS *m*/*z*: 190 (MH^+), 154 (base peak). HR-FABMS: Calcd for $C_{13}H_{20}N$: 190.1556, found: 190.1604.

*5.1.2.1.2. 1-n-Pentyl-1,2,3,4-tetrahydroisoquinoline (**11f**).* Yield: 90%, as a pale yellow oil eluted with AcOEt:hexane = 1:4, HCl salt; colorless prisms from ether–MeOH, mp 83–84 °C. ^{1}H-NMR: 0.83–0.87 (3H, m, CH_3), 1.27–1.52 (6H, m, $(CH_2)_3$), 1.65–1.89 (2H, each m, CH_2), 2.69–2.88 (2H, m, C4-H), 2.99 (1H, ddd, $J = 12$, 7, 5 Hz, C3-H), 3.24 (1H, dt, $J = 12$, 5 Hz, C3-H), 3.96 (1H, dd, $J = 9$, 4 Hz, C1-H). 7.05–7.21 (4H, m, Ar). ^{13}C-NMR: 14.1 (CH_3), 22.6 (*n*-pentyl), 25.9 (*n*-pentyl), 30.1 (C4), 32.0 (*n*-pentyl), 36.5 (*n*-pentyl), 41.1 (C3), 55.8 (C1), 125.7 (C7), 125.7 (C6), 126.1 (C5), 129.2 (C8), 135.1 (C8a), 139.9 (C4a). LR-FABMS *m*/*z*: 204 (MH^+), 154 (base peak). HR-FABMS: Calcd for $C_{14}H_{22}N$: 204.1754, found: 204.1756.

*5.1.2.1.3. 1-n-Hexyl-1,2,3,4-tetrahydroisoquinoline (**11g**).* Yield: 82%, as a pale yellow oil eluted with AcOEt:hexane = 1:2, HCl salt; colorless needles from ether–MeOH, mp 123–126 °C. ^{1}H-NMR: 0.86 (3H, t, $J = 7$ Hz, CH_3), 1.25–1.53, 1.79–1.90 (total 10H, each m, *n*-hexyl), 2.69–2.88 (2H, m, C4-H), 2.98 (1H, ddd, $J = 12$, 8, 5 Hz, C3-H), 2.24 (1H, dt, $J = 12$, 5 Hz, C3-H), 3.95 (1H, dd, $J = 9$, 4 Hz, C1-H), 7.05–7.17 (4H, m, Ar). ^{13}C-NMR: 14.1 (CH_3), 22.7 (*n*-hexyl), 26.2 (*n*-hexyl), 29.5 (*n*-hexyl), 30.0 (C4), 31.8 (*n*-hexyl), 36.5 (*n*-hexyl), 41.1 (C3), 55.8 (C1), 125.7 (C7), 125.7 (C6), 126.1 (C5), 129.2 (C8), 135.1 (C8a), 139.8 (C4a). LR-FABMS *m*/*z*: 218 (MH^+), 132 (base peak). HR-FABMS: Calcd for $C_{15}H_{24}N$: 218.1909, found: 218.1886.

*5.1.3. General procedure for the synthesis of 1-alkyl-2-propargyl-TIQ **12***

A solution of propargylbromide (5.1 mmol) in acetonitrile (5 mL) was added to a solution of 1-alkyl-TIQ **11** (3.4 mmol) and cesium carbonate (6.8 mmol) in acetonitrile (20 mL) under an argon atmosphere, and the mixture was refluxed for 30 min. After cooling, the insoluble residue was removed by filtration and the filtrate was concentrated *in vacuo*. The resulting mixture was diluted with chloroform and extracted. The organic layer was concentrated *in vacuo* and purified by column chromatography on silica gel to afford 1-alkyl-2-propargyl-TIQ **12**.

5.1.3.1. Analytical data for the new compounds

*5.1.3.1.1. 1-Methyl-2-propargyl-1,2,3,4-tetrahydroisoquinoline **12b**.* Yield: 53% as a pale yellow oil eluted with AcOEt: hexane = 1:2; HCl salt; colorless prisms from MeOH–ether, mp 120–122 °C. ^{1}H-NMR: 1.40 (3H, d, $J = 7$ Hz, CH_3), 2.22 (1H, t, $J = 2$ Hz, C3′-H), 2.76–3.01 (4H, m, C3-H and C4-H), 3.50 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.67 (1H, dd, $J = 17$, 2 Hz, C1′-H), 4.00 (1H, t, $J = 7$ Hz, C1-H), 7.00–7.36 (4H, m, Ar). ^{13}C-NMR: 20.1 (CH_3), 28.5 (C4), 43.4 (C1′), 46.6 (C3), 55.4 (C1), 72.8 (C3′), 79.3 (C2′), 125.8 (C7), 126.9 (C6), 128.3 (C5), 128.6 (C8), 134.0 (C8a), 139.5 (C4a). HR-FABMS: Calcd for $C_{13}H_{16}N$: 186.1282, found: 186.1287.

*5.1.3.1.2. 1-Ethyl-2-propargyl-1,2,3,4-tetrahydroisoquinoline **12c**.* Yield: 80% as a pale yellow oil eluted AcOEt: hexane = 1:2; HCl salt; colorless prisms from acetone–ether, mp 120–122 °C. ^{1}H-NMR: 0.79 (3H, t, $J = 7$ Hz, $CH_2\underline{CH_3}$), 1.73–1.88 (2H, m, $C\underline{H_2}CH_3$), 2.16 (1H, t, $J = 2$ Hz, C3′-H), 2.66–2.75, 2.77–2.82, 2.84–2.94, 2.98–3.07 (each 1H, m, C3-H and C4-H), 3.42 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.58 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.84 (1H, t, $J = 5$ Hz, C1-H), 7.04–7.15 (4H, m, Ar). ^{13}C-NMR: 8.9 ($CH_2\underline{C}H_3$), 26.9 (C4), 27.4 ($\underline{C}H_2CH_3$), 43.1 (C1′), 46.5 (C3), 60.4 (C1), 72.4 (C3′), 79.6 (C2′), 125.5 (C7), 125.6 (C6), 126.9 (C5), 128.3 (C8), 135.1 (C8a), 137.7 (C4a). HR-FABMS: Calcd for $C_{14}H_{18}N$: 200.1441, found: 200.1453.

*5.1.3.1.3. 2-Propargyl-1-n-propyl-1,2,3,4-tetrahydroisoquinoline **12d**.* Yield: 57% as a pale yellow oil eluted with AcoEt:hexane = 1:7; HCl salt; colorless prisms from acetone–ether, mp 109–110 °C. ^{1}H-NMR: 0.88 (3H, t, $J = 7$ Hz, $CH_2CH_2\underline{CH_3}$), 1.16–1.51 (2H, m, $CH_2\underline{CH_2}CH_3$), 1.65–1.84 (2H, m, $\underline{CH_2}CH_2CH_3$), 2.20 (1H, t, $J = 2$ Hz, C3′-H), 2.79 (2H, t, $J = 6$ Hz, C4-H) 2.95 (1H, dt, $J = 12$, 6 Hz, C3-H), 3.12 (1H, dt, $J = 12$, 6 Hz, C3-H), 3.45 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.58 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.87 (1H, t, $J = 5$ Hz, C1-H), 7.05–7.17 (4H, m, Ar). ^{13}C-NMR: 14.3 ($CH_2CH_2\underline{CH_3}$), 18.4 ($CH_2\underline{CH_2}CH_3$), 26.7 (C4), 37.3 ($\underline{CH_2}CH_2CH_3$), 43.2 (C1′), 45.9 (C3), 59.7 (C1), 72.4 (C3′), 80.1 (C2′), 125.7 (C7), 125.8 (C6), 127.3 (C5), 128.6 (C8), 134.8 (C8a), 138.3 (C4a). HR-FABMS: Calcd for $C_{15}H_{20}N$: 214.1596, found: 214.1574.

*5.1.3.1.4. 1-n-Butyl-2-propargyl-1,2,3,4-tetrahydroisoquinoline **12e**.* Yield: 59% as a pale yellow oil eluted with AcOEt: hexane = 1:7, *p*-TsOH salt; colorless needles from isopropanol-ether, mp 146–148 °C. ^{1}H-NMR: 0.86 (3H, t, $J = 7$ Hz, CH_3), 1.09–1.44, 1.69–1.87 (each 3H, each m, *n*-butyl), 2.01 (1H, t, $J = 2$ Hz, C3′-H), 2.80 (2H, t, $J = 6$ Hz, C4-H), 2.96 (1H, dt, $J = 12$, 6 Hz, C3-H), 3.11 (1H, dt, $J = 12$, 6 Hz, C3-H), 3.45 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.59 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.87 (1H, t, $J = 5$ Hz, C1-H), 7.05–7.18 (4H, m, Ar). ^{13}C-NMR: 14.1 (CH_3), 22.9 (*n*-butyl), 26.8 (C4), 27.2 (*n*-butyl), 34.6 (*n*-butyl), 43.2 (C′1), 46.1 (C3), 59.8 (C1), 72.5 (C′3), 80.0 (C′2), 125.7 (C7), 125.8 (C6), 127.2 (C5), 128.6 (C8), 134.8 (C8a), 138.3 (C4a). HR-FABMS: Calcd for $C_{16}H_{22}N$: 228.1751, found: 228.1773.

*5.1.3.1.5. 1-n-Pentyl-2-propargyl-1,2,3,4-tetrahydroisoquinoline **12f**.* Yield: 85% as a pale yellow oil eluted with AcOEt: hexane = 1:2. ^{1}H-NMR: 0.85 (3H, t, $J = 7$ Hz, CH_3), 1.18–1.30 (5H, m, *n*-pentyl), 1.37–1.44 (1H, m, *n*-pentyl), 1.69–1.86 (2H, each m, *n*-pentyl) 2.20 (1H, t, $J = 2$ Hz, C3′-H), 2.77–2.81 (2H, m, C4-H), 2.91–2.99 (1H, m, C3-H), 3.07–3.15 (1H, m, C3-H), 3.45 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.59 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.86 (1H, t, $J = 5$ Hz, C1-H), 7.04–7.18 (4H, m, Ar). ^{13}C-NMR: 14.1 (CH_3), 22.6 (*n*-pentyl), 24.7 (*n*-pentyl), 26.8 (C4), 32.1 (*n*-pentyl), 34.9 (*n*-pentyl), 43.2 (C′1), 46.1 (C3), 59.9 (C1), 72.4 (C′3), 80.0 (C′2), 125.7 (C7), 125.8 (C6), 127.2 (C5), 128.6 (C8), 134.8 (C8a), 138.3 (C4a). HR-FABMS: Calcd for $C_{17}H_{24}N$: 242.1909, found: 242.1917.

*5.1.3.1.6. 1-n-Hexyl-2-propargyl-1,2,3,4-tetrahydroisoquinoline **12g**.* Yield: 37% as a pale yellow oil eluted with chloroform: hexane = 1:2. ^{1}H-NMR: 0.83 (3H, t, $J = 7$ Hz, CH_3), 1.15–1.43, 1.67–1.86 (total 10H, each m, *n*-hexyl), 2.20 (1H, t, $J = 2$ Hz, C3′-H), 2.79 (2H, t, $J = 6$ Hz, C4-H), 2.95 (1H, dt, $J = 12$, 6 Hz, C3-H), 3.11 (1H, dt, $J = 12$, 6 Hz, C3-H), 3.44 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.59 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.86 (1H, d, $J = 5$ Hz, C1-H), 7.04–7.20 (4H, m, Ar). ^{13}C-NMR: 14.1 (CH_3), 22.7 (*n*-hexyl), 25.0 (*n*-hexyl), 26.8 (C4), 29.6 (*n*-hexyl), 31.8 (*n*-hexyl), 34.9 (*n*-hexyl), 43.2 (t, C′1), 46.1 (C3), 59.8 (C1), 72.5 (C′3), 80.0 (C′2), 125.7 (C7), 125.8 (C6), 127.2 (C5), 128.6 (C8), 134.8 (C8a), 138.3 (C4a). HR-FABMS: Calcd for $C_{18}H_{26}N$: 256.2065, found: 256.2039.

*5.1.3.1.7. 1-Cyclopropyl-2-propargyl-1,2,3,4-tetrahydroisoquinoline **12h**.* Yield: 40% as a pale yellow oil eluted with AcOEt: hexane = 1:8, HCl salt; colorless needles from isopropanal-ether, mp 161–164 °C. ^{1}H-NMR: 0.50–0.57, 0.63–0.76 (total 4H, each m, cyclopropyl), 0.91–1.03 (1H, m, cyclopropyl), 2.22 (1H, t, $J = 2$ Hz, C3′-H) 2.80–3.03, 3.12–3.19 (total 4H, each m, C3-H and C4-H), 3.21 (1H, d, $J = 9$ Hz, C1-H), 3.53 (1H, dd, $J = 17$, 2 Hz, C1′-H) 3.96 (1H, dd, $J = 17$, 2 Hz, C1′-H) 7.07–7.18, 7.45–7.49 (total 4H, each m, Ar). ^{13}C-NMR: 4.0 (cyclopropyl), 4.4 (cyclopropyl), 15.5 (cyclopropyl), 27.8 (C4), 43.6 (C1′), 47.3 (C3), 63.9 (C1), 73.0 (C3′), 79.8 (C2′), 125.6 (C7), 126.3 (C6), 127.4 (C5), 128.7 (C8), 134.3 (C8a), 138.2 (C4a). HR-FABMS: Calcd for $C_{15}H_{18}N$: 212.1439, found: 212.1427.

*5.1.3.1.8. 1-Cyclobutyl-2-propargyl-1,2,3,4-tetrahydroisoquinoline **12i**.* Yield: 67% as a pale yellow oil eluted with AcOEt: hexane = 1:3; HCl salt; colorless prisms from isopropanol-ether, mp 99–102 °C. ^{1}H-NMR: 1.64–2.06 (6H, m, cyclobutyl), 2.21 (1H, t, $J = 12$ Hz, C3′-H), 2.50–2.68, 2.81–2.91 (total 3H, each m, cyclobutyl and C4-H), 2.97–3.04 (1H, m, C3-H), 3.17–3.26 (1H, m, C3-H), 3.37 (1H, dd, $J = 16$, 2 Hz, C1′-H), 3.45 (1H, dd, $J = 16$, 2 Hz, C1′-H), 3.69

(1H, d, $J = 9$ Hz, C1-H), 7.05–7.14 (4H, m, Ar). ^{13}C-NMR: 18.2 (cyclobutyl), 24.7 (C4), 27.0 (cyclobutyl), 27.5 (cyclobutyl), 41.7 (cyclobutyl), 43.5 (C1′), 44.5 (C3), 65.0 (C1), 72.0 (C3′), 81.0 (C2′), 125.4 (Ar), 126.1 (Ar), 128.1 (Ar), 128.7 (Ar), 134.0 (Ar), 136.4 (Ar). HR-FABMS: Calcd for $C_{16}H_{20}N$: 226.1602, found: 226.1532.

5.1.3.1.9. 1-Cyclopentyl-2-propargyl-1,2,3,4-tetrahydroisoquinoline ***12j***. Yield: 58% as a pale yellow oil eluted with AcOEt: hexane = 1:3. ^{1}H-NMR: 1.25–1.68, 1.84–1.86 (total 8H, each m, cyclopentyl), 1.97–2.11 (1H, m, cyclopentyl), 2.18 (1H, t, $J = 2$ Hz, C3′-H), 2.61–2.70 (2H, m, C4-H), 2.98–3.06, 3.29–3.35 (total 2H, each m, C3-H), 3.34 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.42 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.57 (1H, d, $J = 9$ Hz, C1-H), 7.11–7.16 (4H, m, Ar). ^{13}C-NMR: 24.3 (cyclopentyl), 24.4 (C4), 25.1 (cyclopentyl), 30.4 (cyclopentyl), 31.1 (cyclopentyl), 43.6 (C1′), 44.2 (C3), 47.3 (cyclopentyl), 65.0 (C1), 71.8 (C3′), 81.2 (C2′), 125.3 (C7), 126.1 (C6), 128.6 (C5), 128.8 (C8), 134.3 (C8a), 137.5 (C4a). HR-FABMS: Calcd for $C_{17}H_{22}N$: 240.1753, found: 240.1761.

5.1.3.1.10. 1-Cyclohexcyl-2-propargyl-1,2,3,4-tetrahydroisoquinoline ***12k***. Yield: 85% as a pale yellow oil eluted with benzene: hexane = 2:1; HCl salt; colorless prisms from MeOH–ether, mp 193–196 °C. ^{1}H-NMR: 0.82–1.86 (11H, m, cyclohexyl), 2.15 (1H, t, $J = 2$ Hz, C3′-H), 2.75 (2H, t, $J = 6$ Hz, C4-H), 2.91 (1H, dt, $J = 6$, 12 Hz, C3-H), 3.18 (1H, dt, $J = 6$, 12 Hz, C3-H), 3.39 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.50 (1H, dd, $J = 17$, 2 Hz, C1′-H), 3.55 (1H, d, $J = 6$ Hz, C1-H), 7.00–7.16 (4H, m, Ar). ^{13}C-NMR: 26.5 (cyclohexyl), 26.6 (cyclohexyl), 26.6 (cyclohexyl), 26.7 (C4), 30.3 (cyclohexyl), 30.3 (cyclohexyl), 44.6 (C1′), 44.8 (C3), 46.0 (cyclohexyl), 65.9 (C1), 71.9 (C3′), 80.8 (C2′), 125.0 (C7), 125.8 (C6), 128.1 (C5), 128.8 (C8), 135.7 (C8a), 136.6 (C4a). LR-FABMS *m*/*z*: 254 (MH^+), 254 (base peak). HR-FABMS: Calcd for $C_{18}H_{24}N$: 254.1909, found: 254.1922.

5.2. Biological experiments

5.2.1. 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 (Switzerland). 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.

5.2.2. Determination of cell viability

Cells used for experiments were in logarithmic growth. Viable cell ratios were determined by colorimetric assay of formazan produced by the reduction of a water-soluble tetrazolium salt, similar to the MTT assay. Cells were suspended in serum containing DMEM at a concentration of 3×10^5 cells/mL and were transferred to 96-well plates. After 24 h incubation, the supernatants were removed and various concentrations of 1-alkyl- and 1-alkyl-*N*-propargyl-TIQ derivatives in solution (prepared using non-serum DMEM) were added (100 μL/well). After 48 h incubation, Cell Counting Kit-8 solution (Dojindo, 10 μL/well) was added to each well and incubated further for 4 h at 37 °C. The absorbance at 490 nm was measured with a microplate reader (Bio-Rad, Model 550), and cell viability was evaluated in terms of A_{490} and expressed as a percentage of the untreated control.

5.2.3. Determination of apoptotic induction (comet assay)

Reagents and equipment for the comet assay were purchased from Trevigen (USA). PC12 cells (5×10^5 cells/well, suspended in PBS(−)) and MPP^+ or TIQ derivative solution (1.0 mM final concentration) were incubated in 24-well microplates at 37 °C for 3 h. After exposure, 10 μL of the cell suspension was mixed with 100 μL of 1% low-melting point agarose. The mixture (75 μL aliquots) was applied to the comet slides, and the slides were kept horizontal at 4 °C until the agarose became completely transparent. The slides were then treated with Lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris base, 1% sodium laurylsarcosinate, and 1% Triton X-100) for 1 h at 4 °C. The slides were immersed in alkaline solution (300 mM NaOH and 1 mM EDTA) at room temperature for 30 min in the dark, followed by two immersions in TBE buffer for 5 min. Electrophoresis was conducted at 15 V at room temperature for 30 min, then the slides were dipped in 70% EtOH for 5 min. After drying, DNA on the slides was stained with 50 μL of SYBR Green (0.1 μL/mL) solution.

Comet images were observed through a fluorescence microscope at ×200 magnification and 568 nm. Routinely, 200 cells were screened per sample. In selecting cells for measurement, straight line scanning of a slide was begun at an arbitrary point and cells were measured as they came into the field, provided there was no overlap with patterns from other cells. The length of the 'comet' was measured using an ocular micrometer disk.

5.2.4. Inhibitory effect of TIQ derivatives on MPP^+-induced cell death

Cell viability in the presence of 0.25 mM MPP^+ and TIQ derivatives was estimated according to the method described in Section 5.2.2. The effect of TIQ derivatives in preventing cell death was calculated as a percentage of MPP^+-induced cell death in the absence of TIQ derivatives (defined as 100%).

5.2.5. Determination of caspase activity

Cells were suspended in serum containing DMEM at a concentration of 3×10^5 cells/mL and were transferred to a Petri dish. After 24 h incubation, supernatants were removed and 0.25 mM of MPP^+ or 0.01 mM of TIQ derivative solution (final concentration, prepared by using non-serum DMEM) was added. After an additional 48 h incubation, cells were harvested and lysed using 20 mM phosphate buffer (pH 7.4) containing 0.05% Triton-X and 1 mM EDTA for 3 min. Centrifuged supernatants (4 °C, 10 min) were collected as cell extract. Enzyme assay buffer (32 μL, containing 312.5 mM HEPES (pH 7.5), 31.25% sucrose, 0.325% CHAPS), 0.5 mM Ac-DEVD-AMC (10 μL), DMSO (2 μL), 100 mM dithiothreitol (10 μL), H_2O (36 μL) and cell extract (10 μL) were mixed and incubated at 37 °C for 60 min. Fluorescence derived from 7-amino-4-methylcoumarin (AMC), released by fragmentation due to caspase-3,7 activity, was measured with a microplate reader (excitation at 360 nm and emission at 460 nm Applied Biosystems Model CytofluorII). Data are presented as a percentage compared to the activity of the untreated control (defined as 100%).

5.2.6. Determination of MAO activity

Quantitative analysis of MAO activity was conducted using a fluorescence monoamine oxidase A and B detection kit (Fluoro MAO™, Peninsula Laboratories, Inc.) according to the manufacturer's protocol. The intensity of the fluorescent dye released in the presence of H_2O_2 (upon reaction of MAO and substrate) and the kit peroxidase reagent was measured. MAO-A or MAO-B (10 μg/mL, Sigma) and corresponding substrate (0.5 mM tyramine for MAO-A and MAO-B, 2.5 mM benzylamine for MAO-B) were co-incubated with 100 nM 1-methy-*N*-propargyl- or 1-*cy*-propyl-*N*-propargyl-TIQ at 37 °C for 60 min. Fluorescence was measured using a microplate reader (excitation at 530 nm and emission at 590 nm; Applied Biosystems Model CytofluorII). Data are presented as a percentage of the vehicle control activity (defined as 100%).

5.2.7. Data analysis

Data are expressed as means ± standard error (S.E.) for each group. Each datum was first 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. A value of $P < 0.05$ was regarded as statistically significant.

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