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Original article

Synthesis and in vitro cytotoxicity of 1,2,3,4-tetrahydroisoquinoline derivatives

Toshiaki Saitoh [a], Kenji Abe [b], Masami Ishikawa [a], Masanao Nakatani [c], Seiichiro Shimazu [c], Noriyuki Satoh [c], Fumio Yoneda [c], Kyoji Taguchi [d], Yoshie Horiguchi [d,*]

[a] *Department of Organic Reaction Chemistry, Showa Pharmaceutical University, 3-3165 Higashitamagawagakuen, Machida, Tokyo 194-0042, Japan*
[b] *School of Pharmaceutical Sciences, Ohu University, 31-1 Tomitamachi, Koriyama, Fukushima 963-8611, Japan*
[c] *Research Institute, Fujimoto Pharmaceutical Corp., Matsubara, Osaka 580-8503, Japan*
[d] *Department of Medicinal Chemistry, Showa Pharmaceutical University, 3-3165 Higashitamagawagakuen, Machida, Tokyo 194-0042, Japan*

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Abstract

Several 1-alkyl-1,2,3,4-tetrahydroisoquinoline (TIQ) derivatives, which may play a role in Parkinson's disease, have been synthesized via Pummerer-type cyclization of the sulfonium ion formed in situ from *N*-formyl sulfoxide. Using an in vitro trypan blue exclusion assay, high concentrations of TIQ derivatives possessing bulky alkyl group substituents such as 1-cyclobutyl-, 1-cyclohexyl-, 1-phenyl- or 1-benzyl- at the C-1 position were found to significantly affect the viability of PC12 cells. Moreover, TIQ derivatives that moderately or strongly induced apoptosis (e.g., 1-phenyl-TIQ and 1-cyclohexyl-TIQ, respectively) paralleled the results obtained using the trypan blue exclusion assay. These results suggest that the size and electron-donating properties of functional groups may affect the cytotoxicity of TIQ derivatives.

Keywords: Pummerer-type cyclization; 1,2,3,4-Tetrahydroisoquinoline; Structure–activity relationships; Cytotoxicity; Parkinson's disease

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized clinically by bradykinesia, rigidity and tremor, and pathologically by the death of dopaminergic neurons in the *substantia nigra pars compacta*; additionally, Lewy bodies may be present in surviving neurons. It has been reported that 1,2,3,4-tetrahydroisoquinoline (TIQ) derivatives may act as endogenous or environmental neurotoxins causing PD [1,2]. One TIQ derivative, 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1-benzyl-TIQ), occurs naturally in animal brain tissue and was found to induce parkinsonism in monkeys and mice [2,3]. 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 [2]. 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 rats [2,4]. Furthermore, the concentration of 1-methyl-TIQ is markedly lower in brain tissue from Parkinson's patients compared to healthy individuals [5]. We have previously shown that 1-methyl-TIQ inhibits TIQ-induced bradykinesia, and have also shown that (*R*)-1-methyl-TIQ is a more potent inhibitor than (*S*)-1-methyl-TIQ in mice [6]. Recently, (*R*)-1-methyl-TIQ was found to exert a dose-dependent neuroprotective effect, while (*S*)-1-methyl-TIQ had little effect [7]. However, additional studies using in vivo binding of radioligands to presynaptic dopamine transporters in the mouse striatum showed that (*S*)-1-methyl-TIQ had an enhanced protective effect on TIQ-induced loss of ligand-transporter binding compared to (*R*)-1-methyl-TIQ [8]. These findings suggest that both (*R*)- and (*S*)-enantiomers may be endogenous agents that prevent PD by different mechanisms. Thus, 1-methyl-TIQ may play an important role in the prevention of the onset of neurotoxin-induced parkinsonism, and suggests that the substituent group at the C-1-position of the TIQ ring system may have biologically-important implications.

* Corresponding author. Tel.: +81 42 721 1570; fax: +81 42 721 1570.
E-mail address: horiguti@ac.shoyaku.ac.jp (Y. Horiguchi).

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The efficient 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 [9]. In this cyclization reaction, the sulfonium ion formed in situ from a sulfoxide is a powerful electrophile that reacts with nucleophilic carbon species. In a series of papers we reported the syntheses of TIQs [10–13], 1,2,3,4-tetrahydroquinolines [14], 2,3,4,5-tetrahydro-1*H*-3-benzazepines [15], 1,2,4,5-tetrahydro-3*H*-3-benzazepines [16], 2,3,4,5-tetrahydro-1*H*-2-benzazepines [17], 2-quinolones [18], erythrinan [19], homoerythrinan [20], isopavine and pavine alkaloids [21], and heteroaromatic analogues of TIQ [22]. All compounds were synthesized using a Pummerer-type cyclization reaction, thus demonstrating the utility of this reaction for the synthesis of various heterocyclic compounds.

In the present study, we describe the synthesis of several 1-alkyl-TIQs and investigate their neurotoxicity in PC12 pheochromocytoma cells.

2. Chemistry

Alkyl-TIQs (1-ethyl-, 1-propyl-, 1-cyclopropyl-, 1-cyclobutyl-, 1-cyclohexyl-TIQ **10e–i** and 6-fluoro-1-methyl-TIQ **10j**), were synthesized from *N*-formyl sulfoxides **5e–j** using a Pummerer-type cyclization reaction as a key step (Fig. 1). Substrates for the Pummerer-type cyclization reaction, compounds **5e–j**, were prepared from alkyl phenyl ketones **1e–j** in three steps. Condensation of **1** and 2-phenylsulfanylethylamine **2** in titanium(IV) isopropoxide, followed by $NaBH_4$ reduction of the resulting imines, afforded amines **3e–j**. Formylation of amine **3** by treatment with acetic-formic anhydride, prepared from acetic anhydride and formic acid, followed by oxidation of the resulting *N*-formyl sulfide with $NaIO_4$, produced the corresponding sulfoxide **5e–j** in high overall yields (see experimental).

As previously described [10,11], sulfoxides lacking a methoxy group as an 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). However, cyclization of these sulfoxides can be achieved by using boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) as an additional acid.

Sulfoxide **5e** in benzene was treated with TFAA (5 mol eq.) for 0.5 h, then $BF_3{\cdot}Et_2O$ (3 mol eq.) was added; the expected 1-ethyl-2-formyl-4-phenylsulfanyl-TIQ **6e** was obtained in 99% yield after 1.0 h. Sequential treatment of sulfoxides **5f–h** with TFAA and $BF_3{\cdot}Et_2O$ gave the corresponding 1-alkyl-2-formyl-4-phenylsulfanyl-TIQs **6f–h** in excellent yield. However, under these reaction conditions **5i,** steric hindrance caused by the bulkiness of cyclohexane ring resulted in only 14% yield of the expected product, 1-cyclohexyl-2-formyl-4-phenylsulfanyl-TIQ **6i,** and 44% yield of aldehyde **7i.** The cyclization of **5i** was improved by adding trifluoromethane sulfonic acid (TFSA), an exceptionally strong acid, rather than $BF_3{\cdot}Et_2O$; under these conditions, **6i** was obtained in 63% yield. Pummerer-type cyclization of **5j** also inefficient due to decreased nucleophilicity of the aromatic ring caused by the introduction of a highly electron-withdrawing fluorine in the aromatic ring. Therefore, cyclization of **5j** was achieved using

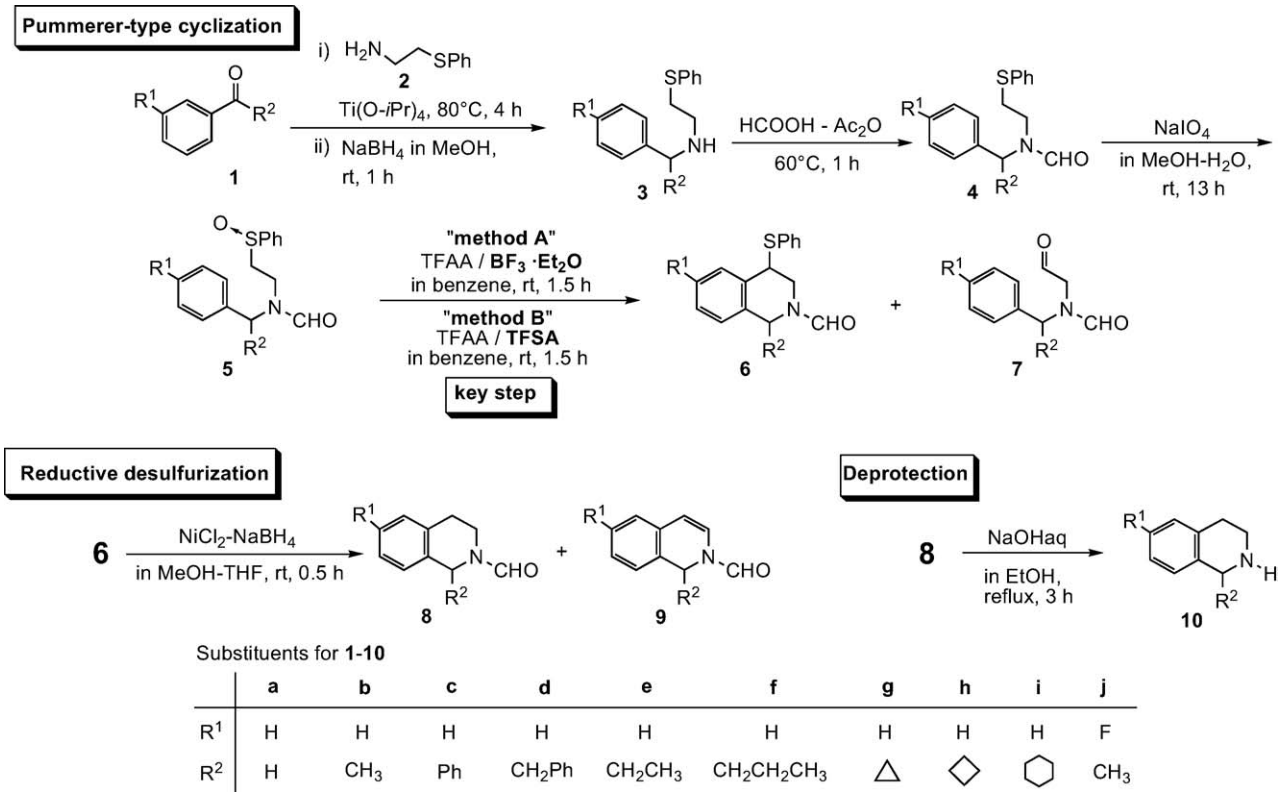


Fig. 1. Synthesis of 1-alkyl-1,2,3,4-tetrahydroisoquinolines.

Table 1
Yields of products for compound **6–10**

	Substituents			Yields of Products (%)				
				Pummerer reaction		Desulfurization		Hydrolysis
	R^1	R^2	Method	**6**	**7**	**8**	**9**	**10**
e	H	Et	A	99	---	83	9	59
f	H	*n*-Pr	A	99	---	83	2	99
g	H		A	99	---	71	6	87
h	H		A	99	---	78	---	71
i	H		A	14	44	73	---	86
			B	63	---			
j	F	Me	A	13	45	87	---	91
			B	63	---			

TFSA, affording 6-fluoro-2-formyl-1-methyl-TIQ **6j** in 63% yield. Reductive removal of the phenylthio group of TIQ **6** readily proceeded in the presence of $NiCl_2/NaBH_4$ in $MeOH/H_2O$ to give 1-alkyl-2-formyl-TIQs **8** in good yield, although in some cases 1,2-dihydroisoquinolines **9** were produced as by-products (Fig. 1). Deprotection of the *N*-formyl group was achieved in the conventional manner, as described below. Alkaline hydrolysis of 1-alkyl-2-formyl-TIQs **8** gave 1-alkyl-TIQs **10** in excellent yield (Fig. 1). The yields of all products are summarized in Table 1.

We have previously reported the synthesis of chiral TIQs, (*R*)- and (*S*)-1-methyl TIQs **16a**, **b**, from (*R*)- and (*S*)-1-phenylethylamines **11a**, **b** via Pummerer-type cyclization in 6 steps as shown in Fig. 2 [12].

3. Biological results and discussion

3.1. Effect of cell culture conditions on the viability and differentiation of PC12 cells

Using pheochromocytoma cells (PC12 cells), the 1-alkyl-TIQs described above were tested in vitro for their cytotoxic activity and their effect on cell differentiation**.** We first examined the relationship between cell culture conditions and PC12 cell viability. In differentiation medium (DM; Dulbecco's modified Eagle's medium (DMEM) containing serum and nerve growth factor (NGF)), over 95% of PC12 cells survived (data not shown). Viability decreased when serum was removed from the medium (NGF + DMEM), and when the cells were grown in trophic withdrawal medium (DMEM without serum and NGF). The reduction in cell viability was statistically significant (Table 2; the viability of the control (DM-cultured cells) is taken as 100%). Outgrowth of neurites was also significantly decreased if serum and/or NGF were removed from the medium. In trophic withdrawal medium, the percentage of cytoplasmic neurite-bearing cells was significantly reduced. These results suggest that the ratio of viable and neurite-bearing cells depends on the presence of trophic factors in the growth medium (Table 2).

3.2. Effects of TIQ derivatives on PC12 cell viability

The effect of each TIQ derivative on trophic withdrawal-cultured PC12 cells was estimated as the percentage of trophic withdrawal-cultured cell viability in the absence of any TIQ. Cell viability was unaffected by low (10^{-5}M) and medium (10^{-4}M) concentrations of TIQ derivatives **(10a–10j)**. How-

Table 2
Effects of serum and NGF on the growth of PC12 cells

Treatment	% Of trophic withdrawal (trypan blue exclusion)	% Of trophic withdrawal (bearing neurite)
Serum + NGF (as differential medium, DM)	100 ± 0.5	100 ± 4.0
Without serum (added NGF only)	92.7 ± 0.4 **	83.2 ± 3.2 **
Without serum and NGF (as trophic withdrawal)	76.9 ± 0.9 **	57.6 ± 2.2 **

** $P < 0.01$, vs. Serum + NGF. Data are shown as mean ± S.E.

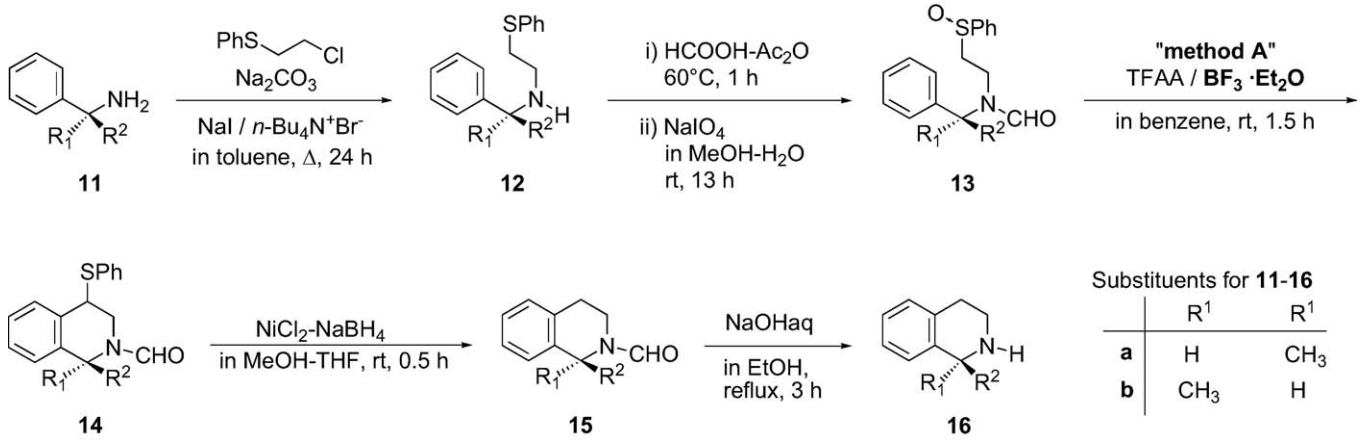


Fig. 2. Synthesis of (*R*)-(*S*)-1-methyl-1,2,3,4-tetrahydroisoquinoline (**16**).

ever, high concentrations (10^{-3}M) of TIQ derivatives with relatively large substituents (e.g., 1-cyclobutyl- **10h**, 1-cyclohexyl- **10i**, 1-phenyl- **10c** or 1-benzyl-TIQ **10d)** at the C-1 position significantly decreased cell viability. Small substituents at the C-1 position such as TIQ **10a**, 1-methyl- **16a** and **16b (10b)**, 1-ethyl- **10e,** 1-propyl- **10f** and 1-cyclopropyl-TIQ **10g** had no effect on cell viability even at high concentrations; the addition of fluorine to small substituent groups attached to the TIQ (e.g. , 6-fluoro-1-methyl-TIQ **10j**) also had no effect. One TIQ derivative, 1-methyl-6,7-dihydroxy-TIQ (salsolinol), induced cell death at lower concentrations than did TIQ derivatives with large substituents. Of the 1-alkyl-TIQs synthesized and tested, only TIQ **10a** protected cells from the effects of trophic withdrawal. These results suggest that the size of the substituent at the C-1 position of TIQs affects cell viability (Table 3).

3.3. Effects of TIQ derivatives on the differentiation of PC12 cells

The effect of TIQ derivatives on the outgrowth of neurites was more pronounced than their effect on cell viability. TIQ derivatives that reduced cell viability at high concentration, such as 1-cyclohexyl- **10i** and 1-benzyl-TIQ **10d**, significantly decreased neurite-bearing cells at lower concentrations. Other TIQs that reduced cell viability (e.g., 1-phenyl- **10c**, 1-cyclobutyl-TIQ **10h** and salsolinol) also inhibited neuronal differentiation, as did TIQ derivatives that did not influence cell viability (e.g., (*R*)- and (*S*)-1-methyl- **16a** and **16b**, 1-ethyl- **10e**, 1-propyl- **10f**, 1-cyclopropyl- **10g** and 6-fluoro-1-methyl-TIQ **10j**). In contrast, low concentrations of 1-cyclobutyl-TIQ **10h** showed increasing effects on neurite-bearing cells (Table 3).

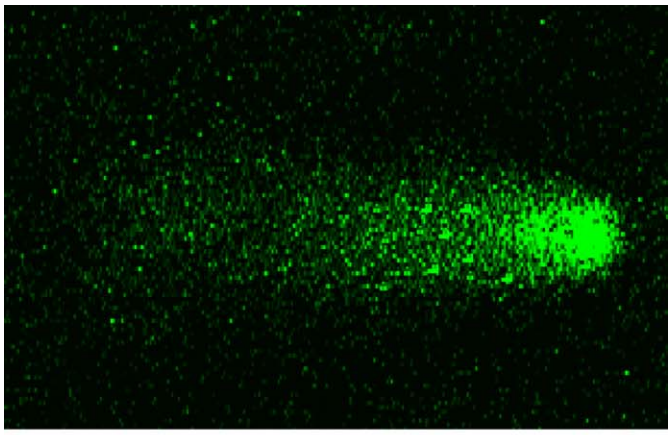
3.4. Evaluation of the cytotoxic effects of TIQ derivatives using the comet assay

At high concentrations (10^{-3} M), 1-phenyl- **10c** and cyclohexyl-TIQ **10i**, were clearly cytotoxic (cell viability was 41.4 ± 10.3% and 0.2 ± 0.2%, respectively; Table 3). The ability of these compounds to induce DNA damage in individual cells was evaluated using the comet assay. Fig. 3A depicts a typical migrated cell nucleus with fragmented DNA caused by treating with 1-cyclohexyl-TIQ **10i**. Small portions of DNA remain at

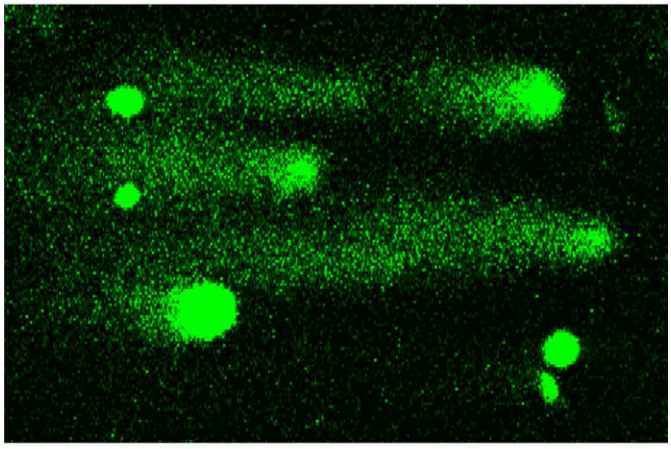
Table 3
Effects of TIQ derivatives on trophic withdrawal-induced apoptosis and differentiation in PC12 cells

Drugs		Concentration	*n*	% Of trophic withdrawal	% Of trophic withdrawal
Name	Compound	(M)		(trypan blue exclusion)	(bearing neurite)
		10^{-5}	8	95.2 ± 3.1	75.4 ± 14.2*
Salsolinol	–	10^{-4}	8	0.0 ± 0.0**	0.0 ± 0.0**
		10^{-3}	8	0.3 ± 0.2**	0.0 ± 0.0**
		10^{-5}	16	106.5 ± 2.2	N.D.
TIQ	**10a**	10^{-4}	12	106.6 ± 1.8	N.D.
		10^{-3}	12	114.6 ± 2.5**	N.D.
		10^{-5}	4	94.8 ± 5.5	89.7 ± 5.0
(*R*)-1-methyl-TIQ	**16a (10b)**	10^{-4}	4	99.9 ± 3.2	83.8 ± 12.3
		10^{-3}	4	98.2 ± 2.9	45.1 ± 5.4**
		10^{-5}	4	95.5 ± 4.5	67.8 ± 10.5
(*S*)-1-methyl-TIQ	**16b (10b)**	10^{-4}	4	101.1 ± 2.3	89.6 ± 11.5
		10^{-3}	4	97.3 ± 4.7	38.4 ± 6.3**
		10^{-5}	12	105.2 ± 3.1	81.5 ± 5.6
1-Phenyl-TIQ	**10c**	10^{-4}	12	114.2 ± 2.7**	41.0 ± 3.3**
		10^{-3}	12	41.4 ± 10.3**	3.6 ± 1.5**
		10^{-5}	12	95.0 ± 3.0	79.9 ± 4.4
1-Benzyl-TIQ	**10d**	10^{-4}	12	107.8 ± 2.1	55.8 ± 4.6**
		10^{-3}	12	0.0 ± 0.0**	0.0 ± 0.0**
		10^{-5}	4	100.2 ± 5.0	91.9 ± 5.5
1-Ethyl-TIQ	**10e**	10^{-4}	4	104.9 ± 4.1	80.3 ± 6.7
		10^{-3}	4	88.8 ± 6.9	31.7 ± 4.1**
		10^{-5}	4	104.7 ± 4.5	77.7 ± 8.3
1-Propyl-TIQ	**10f**	10^{-4}	4	104.3 ± 2.8	65.2 ± 8.3*
		10^{-3}	4	88.8 ± 4.8	27.8 ± 4.5**
		10^{-5}	4	98.7 ± 4.0	85.2 ± 13.4
1-Cyclopropyl-TIQ	**10g**	10^{-4}	4	97.1 ± 3.4	76.2 ± 18.4
		10^{-3}	4	89.1 ± 7.2	34.8 ± 11.8**
		10^{-5}	4	104.7 ± 2.7	134.2 ± 28.0*
1-Cyclobutyl-TIQ	**10h**	10^{-4}	4	104.9 ± 2.6	75.4 ± 9.8
		10^{-3}	4	34.9 ± 7.4**	12.0 ± 8.3**
		10^{-5}	4	107.9 ± 1.6	72.7 ± 13.3
1-Cyclohexyl-TIQ	**10i**	10^{-4}	4	110.2 ± 4.8	35.3 ± 5.9**
		10^{-3}	4	0.2 ± 0.2**	0.0 ± 0.0**
		10^{-5}	4	100.6 ± 2.5	82.8 ± 13.1
6-Fluoro-1-methyl-TIQ	**10j**	10^{-4}	4	95.8 ± 4.8	63.2 ± 11.0*
		10^{-3}	4	87.9 ± 8.0	35.8 ± 4.1**

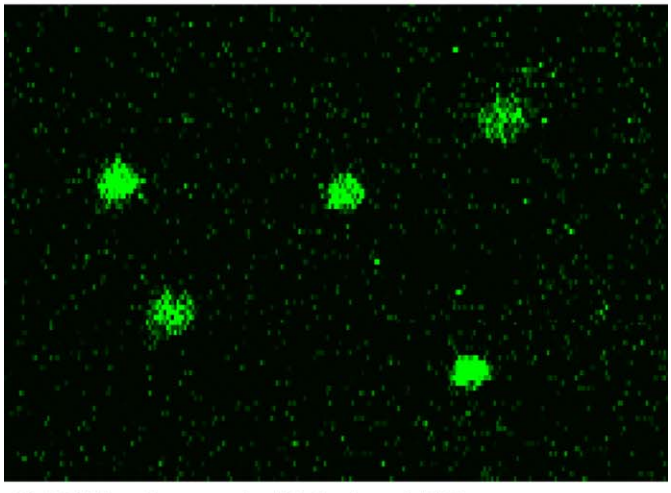
N.D.: not done, *$P < 0.05$ and **$P < 0.01$, vs. wo/Serum and NGF. Data are shown as mean ± S.E.



A: A typical apoptotic damaged PC12 cell, exhibiting the DNA 'comet tail'



B: PC12 cells treated with 1-cyclohexyl-TIQ



C: PC12 cells treated with 1-phenyl-TIQ

Fig. 3. Fluorescence photomicrograph of a comet in PC12 cells after a 3-h exposure to 1-cyclohexyl- or 1-phenyl-TIQ. Panel A shows a comet typically observed in cells treated with high concentrations (10^{-3} M) of 1-cyclohexyl-TIQ. A small portion of the DNA remains at the origin, but most of the DNA has fragmented and migrated to form the comet tail. In the present study, damaged cells such as this are termed apoptotic damaged cells. Panels B and C shows representative photomicrographs of PC12 cells treated with either 1-cyclohexyl-TIQ (B) or 1-phenyl-TIQ (C). Images were obtained using a fluorescence microscope at × 200 magnification and at a wavelength of 568 nm.

Table 4
Evaluation of 1-phenyl- and 1-cyclohexyl-TIQ-induced PC12 cell damage assessed using the comet assay

Drugs		Concentration	*n*	Length and width ratio	The frequency of tailed cells
Name	Compound	(M)		of DNA mass	(%)
Non-treat	–	–	8	1.10 ± 0.04	8.3 ± 5.4
1-Phenyl-TIQ	**10c**	10^{-3}	12	1.37 ± 0.15	23.2 ± 2.2
1-cyclohexyl-TIQ	**10i**	10^{-3}	4	3.12 ± 0.30**	69.6 ± 5.1**

**$P < 0.05$ and **$P < 0.01$, vs. non-treated cells. Data are shown as mean ± S.E.

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' is considered apoptotic. The ratio of the length of the mass of DNA in the tail to the width of the DNA mass in the tail, together with the frequency of cells exhibiting tails, was used to estimate the cytotoxicity of the TIQ derivatives described above. 1-Cyclohexyl-TIQ **10i** significantly increased both the length: width ratio and the frequency of cells with tails (Fig. 3A, B and Table 4), whereas 1-phenyl-TIQ **10c** was significantly less cytotoxic (Fig. 3C and Table 4). These observations are in agreement with results obtained using the trypan blue exclusion assay (Table 3) and suggest that both the size of the functional groups, and the presence of electron-donating group(s) attached to the isoquinoline structure, may affect the toxicity of TIQs towards PC12 cells. This tentative conclusion is further supported by the fact that although high concentrations of 1-methyl-TIQ did not induce cell death, dihydroxide-1-methyl-TIQ (salsolinol) is a potent cytotoxin (Table 3).

4. Conclusions

A number of 1-alkyl-TIQ derivatives that may be implicated in PD were synthesized using a Pummerer-type cyclization reaction. Cyclizations involving sterically-hindered 1-alkyl substituents were accelerated with TFSA. High concentrations of TIQ derivatives with large substituents at the C-1 position significantly affected PC12 cell viability; in addition, the electron-donating properties of functional groups may also contribute the cytotoxicity of TIQ derivatives. Further investigations into the synthesis and biological activity of TIQ derivatives with different C-1 substituents may be useful for developing new therapeutic agents for treating PD.

5. Experimental

5.1. Chemistry

Unless otherwise noted, the following procedures were adopted. Melting points were taken on a Yanagimoto SP-M1 hot-stage melting point apparatus and are uncorrected. IR spectra were measured as films for oils and gums, and KBr disks for solids, with a HORIBA FT-710 Fourier transform infrared spectrophotometer, and the values are given in cm^{-1}. NMR spectra were measured on a JEOL JNM-AL300 (^{1}H-NMR: 300 MHz, ^{13}C-NMR: 75 MHz) NMR spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard, and the chemical shifts are given in δ values. Low-resolution electron impact ionization mass spectra (LR-EIMS) were taken on a JEOL JMS-AM20 mass spectrometer at 70 eV using a direct inlet probe. High-resolution EIMS (HR-EIMS) and low-resolution chemical ionization mass spectra (LR-CIMS) were taken on a JEOL JMS-D300 mass spectrometer at 30 eV (EIMS) or at 270 eV [(CIMS), reactant gas: iso-butane] using a direct inlet probe. High-resolution fast atom bomb ionization mass spectra (HR-FABMS) were measured with a JEOL HX-110A mass spectrometer (reactants gas: xenon, matrix: glycerol). TLC was performed on Merck pre-coated Silica gel 60 F_{254} plates. Column chromatography was carried out with silica gel (Wakogel C-200). Flash column chromatograph was performed with silica gel (KANTOH Silica gel 60N (40–100 μm)). The organic extract from each reaction mixture was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to dryness.

5.2. Compounds

With regard to compounds **3b**–**10d,** see references [9], and **11**–**16**, see references [12].

5.3. Preparation of *3e.* Typical procedure

A mixture of **1e** (13.4 g, 0.10 mol), 2-phenylsulfanylethylamine (15.3 g, 0.10 mol), and titanium(IV) isopropoxide (43.0 g, 0.15 mol) was heated at 80°C for 4.0 h under an argon atmosphere. After cooling, the reaction mixture was diluted with MeOH (500 ml). To this solution, $NaBH_4$ (7.50 g, 0.20 mol) was added in small portions while cooling the reaction mixture on ice. The reaction mixture was stirred at room temperature for 1.0 h and concentrated in vacuo. Water (ca. 40 ml) was added to the residue, and the mixture was diluted with MeOH (ca. 500 ml). After removal of precipitated inorganic materials by filtration, the filtrate was concentrated in vacuo. The residue was dissolved in water extracted with $CHCl_3$. The residue was distilled under reduced pressure (180–185°C/5 mmHg) to give **1-ethyl-1-phenylmethyl-(2-phenylsulfanyl)ethylamine 3e** (25.2 g, 93%) as a pale yellow oil. ^{1}H-NMR: 0.80 (3H, t, J = 7 Hz, $CH_2C\underline{H}_3$), 1.50–1.85 (2H, m, $C\underline{H}_2CH_3$), 2.58–0.73 (2H, m, $SCH_2C\underline{H}_2$), 2.90–3.09 (2H, m, $SC\underline{H}_2CH_2$), 3.46 (1H, dd, J = 8, 6 Hz, C$\underline{H}$Ph), 7.17–7.28 (10H, m, PhHx2). ^{13}C-NMR: 10.8 ($CH_2\underline{C}H_3$), 31.0 ($\underline{C}H_2CH_3$), 34.3 ($SCH_2\underline{C}H_2$), 45.8 ($S\underline{C}H_2CH_2$), 64.5 (C$\underline{H}$Ph), 126.0 (PhCH), 126.9 (PhCH), 127.3 (PhCHx2), 128.3 (PhCHx2), 128.8 (PhCHx2), 129.5 (PhCHx2), 135.8 (PhC), 143.8 (PhC). LR-CIMS *m/z*: 272 (MH^+).

5.3.1. 1-Phenylmethyl-1-propyl-(2-phenylsulfanyl)ethylamine *3f*

Yield, 94%. A pale yellow oil (180–185°C/3 mmHg). ^{1}H-NMR: 0.86 (3H, t, J = 7 Hz, $CH_2CH_2C\underline{H}_3$), 1.07–1.36 (2H, m,

$CH_2CH_2CH_3$), 1.52–1.69 (2H, m, $CH_2CH_2CH_3$), 2.61–2.72 (2H, m, SCH_2CH_2), 2.93–3.01 (2H, m, SCH_2CH_2), 3.55 (1H, dd, J = 7, 6 Hz, CHPh), 7.13–7.55 (10H, m, PhHx2). ^{13}C-NMR: 14.0 ($CH_2CH_2CH_3$), 19.5 ($CH_2CH_2CH_3$), 34.3 (SCH_2CH_2), 40.5 ($CH_2CH_2CH_3$), 45.7 (SCH_2CH_2), 62.7 (CHPh), 126.1 (PhCH), 126.9 (PhCH), 127.2 (PhCHx2), 128.3 (PhCHx2), 128.8 (PhCHx2), 129.5 (PhCHx2), 135.8 (PhC), 144.1 (PhC). LR-CIMS *m/z*: 286 (MH^+).

5.3.2. 1-Cyclopropyl-1-phenylmethyl-(2-phenylsulfanyl) ethylamine **3g**

Yield, 90%. A colorless oil (190–195°C/4 mmHg). ^{1}H-NMR: 0.18–0.25 (1H, m, cyclopropyl), 0.27–0.32 (1H, m, cyclopropyl), 0.36–0.43 (1H, m, cyclopropyl), 0.53–0.62 (1H, m, cyclopropyl), 1.02–1.13 (1H, m, cyclopropyl), 2.64–2.75 (2H, m, SCH_2CH_2), 2.79 (1H, d, J = 9 Hz, CHPh), 2.95–3.06 (2H, m, SCH_2CH_2), 7.14–7.32 (10H, m, PhHx2). ^{13}C-NMR: 2.56 (CH_2), 4.81 (CH_2), 18.9 (CH_2), 34.2 (SCH_2CH_2), 45.7 (SCH_2CH_2), 68.0 (CHPh), 126.0 (PhCH), 127.0 (PhCH), 127.1 (PhCHx2), 128.2 (PhCHx2), 128.8 (PhCHx2), 129.5 (PhCHx2), 135.7 (PhC), 143.6 (PhC). HR-EIMS *m/z* (M^+): Calcd for $C_{18}H_{21}NS$: 283.1395, found: 283.1397.

5.3.3. 1-Cyclobutyl-1-phenylmethyl-(2-phenylsulfanyl) ethylamine **3h**

Yield, 84%. A colorless oil (210–212°C/8 mmHg). ^{1}H-NMR: 1.53–1.81 (7H, m, cyclobutyl), 2.55–2.70 (2H, m, SCH_2CH_2), 2.92–3.07 (2H, m, SCH_2CH_2), 3.46 (1H, d, J = 9 Hz, CHPh), 7.11–7.31 (10H, m, PhHx2). ^{13}C-NMR: 17.6 (CH_2), 25.2 (CH_2), 26.5 (CH_2), 34.2 (SCH_2CH_2), 42.2 (CH), 45.6 (SCH_2CH_2), 68.8 (CHPh), 126.0 (PhCH), 126.9 (PhCH), 127.4 (PhCHx2), 128.1 (PhCHx2), 128.7 (PhCHx2), 129.4 (PhCHx2), 135.7 (PhC), 142.2 (PhC). LR-CIMS *m/z*: 298 (MH^+).

5.3.4. 1-Cyclohexyl-1-phenylmethyl-(2-phenylsulfanyl) ethylamine **3i**

Yield, 20%. Colorless needles, m.p. 58–60°C, recrystallized from Et_2O. ^{1}H-NMR: 0.76–1.76 (10H, m, cyclohexyl), 1.93 (1H, m, cyclohexyl), 2.56 (1H, dd, J = 12, 6 Hz, SCH_2CH_2), 2.64 (1H, dd, J = 12, 6 Hz, SCH_2CH_2), 2.95 (1H, dd, J = 13, 6 Hz, SCH_2CH_2), 3.01 (1H, dd, J = 13, 6 Hz, SCH_2CH_2), 3.28 (1H, d, J = 9 Hz, CHPh), 7.11–7.29 (10H, m, PhHx2). ^{13}C-NMR: 26.2 (CH_2), 26.3 (CH_2), 26.5 (CH_2), 29.8 (CH_2), 60.2 (CH_2), 34.3 (SCH_2CH_2), 44.2 (CH), 45.9 (SCH_2CH_2), 68.4 (CHPh), 126.0 (PhCH), 126.7 (PhCH), 127.9 (PhCHx2), 127.94 (PhCHx2), 128.8 (PhCHx2), 129.5 (PhCHx2), 135.9 (PhC), 142.7 (PhC). HR-EIMS *m/z* (M^+): Calcd for $C_{21}H_{27}NS$: 325.1864, found: 325.1897.

5.3.5. 1-(4-Fluorophenyl)ethyl-(2-phenylsulfanyl)ethylamine **3j**

Yield, 83%. A pale yellow oil (185°C/4 mmHg). ^{1}H-NMR: 1.30 (3H, d, J = 7 Hz, CH_3), 1.96 (1H, br-s, NH), 2.62–2.74 (2H, m, SCH_2CH_2), 3.03 (2H, t, J = 6 Hz, SCH_2CH_2), 3.76 (1H, q, J = 6 Hz, CHAr), 6.95–7.30 (9H, m, PhH and ArH). ^{13}C-NMR: 24.4 (CH_3), 34.3 (SCH_2CH_2), 45.7 (SCH_2CH_2), 57.1 (CHPh), 115.1 (ArCHx2), 126.1 (PhCH), 128.0 (ArCHx2), 128.8 (PhCHx2), 129.5 (PhCHx2), 135.6 (PhC), 141.0 (ArC). 161.7 (ArC). HR-EIMS *m/z* (M^+): Calcd for $C_{16}H_{18}FNS$: 275.1144, found: 275.1178.

5.4. Formylation of **3e**. Typical procedure

A solution of formic-acetic anhydride prepared from formic acid (84.0 ml, 2.22 mol) and acetic anhydride (50.6 ml, 0.74 mol), was added to **3e** (20.0 g, 74.0 mmol) at 0°C in one portion and then the mixture was heated at 60°C for 1.0 h. After removal the formic-acetic anhydride in vacuo, 10% NaOH solution was added to the residue and extracted with $CHCl_3$. The residue was chromatographed (AcOEt/hexane = 1:2) over silica gel to give ***N*-(1-ethyl-1-phenylmethyl)-*N*-(2-phenylsulfanylethyl)formamide 4e** (22.0 g, 99%) as a pale yellow oil. IR: 1664. ^{1}H-NMR: 0.95 (3H, t, J = 7 Hz, CH_2CH_3), 1.84–2.08 (2H, m, CH_2CH_3), 2.48–2.58 (1H, m, SCH_2CH_2), 2.92–3.22 (2H, m, SCH_2CH_2 and SCH_2CH_2), 3.38–3.53 (1H, m, SCH_2CH_2), 4.36, 5.48 (total 1H, each dd, J = 9, 6 Hz, CHPh), 7.15–7.37 (10H, m, PhHx2), 8.18, 8.36 (total 1H, each s, CHO). HR-EIMS *m/z* (M^+): Calcd for $C_{18}H_{21}NOS$: 299.1344, found: 299.1314.

5.4.1. N-(1-phenylmethyl-1-propyl)-N-(2-phenylsulfanylethyl) formamide **4f**

Yield, 99%. A pale yellow oil. IR: 1666. ^{1}H-NMR: 0.93–0.99 (3H, m, $CH_2CH_2CH_3$), 1.20–1.45 (2H, m, $CH_2CH_2CH_3$), 1.85–1.95 (2H, m, $CH_2CH_2CH_3$), 2.49–2.60, 2.91–3.00 (total 2H, each m, SCH_2CH_2), 3.06–3.22, 3.37–3.46 (total 2H, each m, SCH_2CH_2), 4.48, 5.58 (total 1H, each t, J = 7 Hz, CHPh), 7.13–7.35 (10H, m, PhHx2), 8.16, 8.36 (total 1H, each s, CHO). HR-EIMS *m/z* (M^+): Calcd for $C_{19}H_{23}NOS$: 313.1501, found: 313.1514.

5.4.2. N-(1-cyclopropyl-1-phenylmethyl)-N-(2-phenylsulfanylethyl)formamide **4g**

Yield, 99%. A pale yellow oil. IR: 1657. ^{1}H-NMR: 0.35–0.90 (4H, m, cyclopropyl), 1.27−1.36 (1H, m, cyclopropyl), 2.79–2.85, 2.94–3.08 (total 2H, each m, SCH_2CH_2), 3.25–3.43 (2H, m, SCH_2CH_2), 3.64, 4.70 (total 1H, each d, J = 10 Hz, CHPh), 7.12–7.39 (10H, m, PhHx2), 8.23, 8.42 (total 1H, each s, CHO). HR-EIMS *m/z* (M^+): Calcd for $C_{19}H_{21}NOS$: 311.1341, found: 311.1341.

5.4.3. N-(1-cyclobutyl-1-phenylmethyl)-N-(2-phenylsulfanylethyl)formamide **4h**

Yield, 99%. A pale yellow oil. IR: 1668. ^{1}H-NMR: 1.66–2.13 (6H, m, cyclobutyl), 2.53–2.70 (1H, m, cyclobutyl), 2.84–3.17 (3H, m, SCH_2CH_2 and SCH_2CH_2), 3.29–3.39 (1H, m, SCH_2CH_2), 4.37, 5.66 (total 1H, each d, J = 11 Hz, CHPh), 7.12–7.37 (10H, m, PhHx2), 8.18, 8.37 (total 1H, each s, CHO). HR-EIMS *m/z* (M^+): Calcd for $C_{20}H_{23}NOS$: 325.1500, found: 325.1508.

*5.4.4. N-(1-cyclohexyl-1-phenylmethyl)-N-(2-phenylsulfanylethyl)formamide **4i***

Yield, 99%. A pale yellow oil. IR: 1668. ^{1}H-NMR: 0.75–1.29 (5H, m, cyclohexyl), 1.53–1.82 (5H, m, cyclohexyl), 2.06–2.17 (2H, m, SC$\underline{H}_2$CH$_2$), 2.33–2.63, 2.92–3.09 (total 2H, each m, SCH$_2$C$\underline{H}_2$), 3.17–3.42, 3.64–3.66 (total 1H, each m, cyclohexyl), 4.02, 5.12 (total 1H, each d, J = 12 Hz, C$\underline{H}$Ph), 7.10–7.37 (10H, m, PhHx2), 8.13, 8.30 (total 1H, each s, C$\underline{H}$O). LR-CIMS *m/z*: 354 (MH$^+$).

*5.4.5. N-[1-(4-fluorophenyl)ethyl]-N-(2-Phenylsulfanylethyl) formamide **4j***

Yield, 99%. A yellow oil. IR: 1668. ^{1}H-NMR: 1.50, 1.59 (total 3H, each d, J = 7 Hz, CH$_3$), 2.60–2.66, 2.75–2.85, 2.94–3.03 (total 2H, each m, SC$\underline{H}_2$CH$_2$), 3.16–3.37 (2H, m, SCH$_2$C$\underline{H}_2$), 4.74, 5.71 (total 1H, each q, J = 7 Hz, C$\underline{H}$Ar), 7.00–7.06 (2H, m, ArH), 7.14–7.28 (7H, m, PhH and ArH), 8.13, 8.36 (total 1H, each s, C$\underline{H}$O). HR-EIMS *m/z* (M$^+$): Calcd for C$_{17}$H$_{18}$FNOS: 303.1093, found: 303.1112.

*5.5. Oxidation of **4e** with NaIO$_4$. Typical procedure*

A solution of **4e** (8.97 g, 30.0 mmol) and NaIO$_4$ (9.63 g, 45.0 mmol) in MeOH (200 ml) and H$_2$O (100 ml) was stirred at room temperature for 13 h. After removal of inorganic precipitates by filtration, the filtrate was concentrated in vacuo. The residue was extracted with CHCl$_3$. The product was chromatographed with AcOEt and AcOEt/MeOH = 9:1 to give ***N*-(1-ethyl-1-phenylmethyl)-*N*-(2-phenylsulfinylethyl)formamide 5e** (9.10 g, 96%) as a pale yellow gum. IR: 1666. ^{1}H-NMR: 0.91–1.00 (3H, m, CH$_2$C$\underline{H}_3$), 1.99–2.07, 2.42–2.51 (total 2H, each m, C$\underline{H}_2$CH$_3$), 2.79 (1H, t, J = 7 Hz, SC$\underline{H}_2$CH$_2$), 3.03–3.70 (3H, m, SC$\underline{H}_2$CH$_2$ and SCH$_2$C$\underline{H}_2$), 4.41 (1H, d, J = 8 Hz, C$\underline{H}$Ph), 7.22–7.55 (10H, m, PhHx2), 8.34, 8.37 (total 1H, each s, C$\underline{H}$O). LR-CIMS *m/z*: 316 (MH$^+$).

*5.5.1. N-(1-phenylmethyl-1-propyl)-N-(2-phenylsulfinylethyl) formamide **5f***

Yield, 99%. A pale yellow oil. IR: 1664. ^{1}H-NMR: 0.96, 0.98 (total 3H, each t, J = 7 Hz, CH$_2$CH$_2$C$\underline{H}_3$), 1.25–1.40 (2H, m, CH$_2$C$\underline{H}_2$CH$_3$), 1.93–2.03 (2H, m, C$\underline{H}_2$CH$_2$CH$_3$), 2.41–2.51, 2.76–2.80, 3.00–3.07 (total 2H, each m, SC$\underline{H}_2$CH$_2$) ,3.18–3.45, 3.60–3.70 (total 2H, each m, SC$\underline{H}_2$CH$_2$), 4.99, 4.52 (total 1H, each t, J = 7 Hz, C$\underline{H}$Ph), 7.21–7.61 (10H, m, PhHx2), 8.33, 8.37 (total 1H, each s, C$\underline{H}$O). HR-EIMS *m/z* (M$^+$): Calcd for C$_{19}$H$_{23}$NO$_2$S: 329.1450, found: 329.1450.

*5.5.2. N-(1-cyclopropyl-1-phenylmethyl)-N-(2-phenylsulfinylethyl)formamide **5g***

Yield, 99%. A pale yellow gum. IR: 1668. ^{1}H-NMR: 0.33–0.96 (4H, m, cyclopropyl), 1.32–1.56 (1H, m, cyclopropyl), 2.82–2.93 (1H, m, SC$\underline{H}_2$CH$_2$), 3.13–3.39, 3.46–3.62 (total 3H, each m, SC$\underline{H}_2$CH$_2$ and SCH$_2$C$\underline{H}_2$), 3.66, 3.73, 4.81, 4.86 (total 1H, each d, J = 10 Hz, C$\underline{H}$Ph), 7.30–7.58 (10H, m, PhHx2), 8.27, 8.33, 8.35, 8.49 (total 1H, each s, C$\underline{H}$O). LR-CIMS *m/z*: 328 (MH$^+$).

*5.5.3. N-(1-cyclobutyl-1-phenylmethyl)-N-(2-phenylsulfinylethyl)formamide **5h***

Yield, 99%. A pale yellow gum. IR: 1668. ^{1}H-NMR: 1.70–2.10 (7H, m, cyclobutyl), 2.50–2.62, 2.68–2.75 (total 1H, each m, SC$\underline{H}_2$CH$_2$), 2.92–3.12 (2H, m, SC$\underline{H}_2$CH$_2$ and SCH$_2$C$\underline{H}_2$), 3.32–3.59 (1H, m, SCH$_2$C$\underline{H}_2$), 4.39, 4.44 (total 1H, each d, J = 11 Hz, C$\underline{H}$Ph), 7.18–7.55 (10H, m, PhHx2), 8.18, 8.33, 8.36, 8.39 (total 1H, each s, C$\underline{H}$O). LR-CIMS *m/z*: 342 (MH$^+$).

*5.5.4. N-(1-cyclohexyl-1-phenylmethyl)-N-(2-phenylsulfinylethyl)formamide **5i***

Yield, 99%. A pale yellow gum. IR: 1668. ^{1}H-NMR: 0.66–4.10 (16H, m, cyclohexyl, C$\underline{H}$Ph, SC$\underline{H}_2$CH$_2$ and SCH$_2$C$\underline{H}_2$), 7.20–7.60 (10H, m, PhHx2), 8.06, 8.11, 8.28, 8.30 (total 1H, each s, CHO). LR-CIMS *m/z*: 370 (MH$^+$).

*5.5.5. N-[1-(4-Fluorophenyl)ethyl]-N-(2-Phenylsulfinylethyl) formamide **5j***

Yield, 86%. A pale yellow gum. IR: 1664, 1602. ^{1}H-NMR: 1.50, 1.59, 1.65, 1.70 (total 3H, each d, J = 7 Hz, CH$_3$), 2.70–3.54 (4H, m, SC$\underline{H}_2$CH$_2$ and SCH$_2$C$\underline{H}_2$), 4.76–4.82 (1H, m, C$\underline{H}$Ar), 7.01–7.10, 7.21–7.35, 7.48–7.59 (total 9H, each m, ArH and PhH), 8.14, 8.25, 8.34, 8.40 (total 1H, each s, C$\underline{H}$O). LR-CIMS *m/z*: 320 (MH$^+$).

*5.6. Pummerer reaction of sulfoxide **5e**. Typical procedure*

TFAA (2.21 ml, 15.9 mmol) was added into a solution of **5e** (1.00 g, 3.17 mmol) in benzene (50.0 ml) at room temperature. After the mixture was stirred for 0.5 h, BF$_3$·Et$_2$O (1.20 ml, 9.51 mmol) was added, and the reaction mixture was further stirred at the same temperature for 1.0 h. The reaction mixture was diluted with H$_2$O and extracted with CHCl$_3$. Purification by column chromatography (AcOEt/hexane = 1:2) of the product gave ***N*-formyl-1-ethyl-4-phenylsulfanyl-1,2,3,4-tetrahydro-isoquinoline 6e** (932 mg, 99%) as a pale yellow gum. IR: 1673. ^{1}H-NMR: 0.98, 0.99 (total 3H, each t, J = 7 Hz, CH$_2$C$\underline{H}_3$), 1.76, 1.91 (total 2H, each dq, J = 7, 4 Hz, C$\underline{H}_2$CH$_3$), 3.15, 3.72 (total 1H, each dd, J = 14, 3 Hz, C3-H), 3.55, 4.62 (total 1H, each d, J = 14 Hz, C3-H), 4.35, 4.51 (total 1H, each d, J = 3 Hz, C4-H), 4.45, 5.41 (total 1H, each dd, J = 10, 4 Hz, C1-H), 7.13–7.49, 7.68–7.69 (total 9H, each m, ArH and PhH), 8.14, 8.37 (total 1H, each s, C$\underline{H}$O). LR-CIMS *m/z*: 298 (MH$^+$).

*5.6.1. N-formyl-4-phenylsulfanyl-1-propyl-1,2,3,4-tetrahydroisoquinoline **6f***

Yield, 99%. Colorless prisms, m.p. 62–64°C. IR: 1674. ^{1}H-NMR: 0.95, 0.97 (total 3H, each t, J = 7 Hz, CH$_2$CH$_2$C$\underline{H}_3$), 1.26–1.54 (2H, m, CH$_2$C$\underline{H}_2$CH$_3$), 1.69–1.83 (2H, m, C$\underline{H}_2$CH$_2$CH$_3$), 3.17, 3.76 (1H, each dd, J = 14, 3 Hz, C3-H), 3.52, 4.61 (1H, each d, J = 14 Hz, C3-H), 4.35, 4.52 (1H, each d, J = 3 Hz, C4-H), 5.48 (1H, dd, J = 9, 5 Hz, C1-H), 7.10–7.60, 7.65–7.68 (total 9H, each m, ArH and PhH), 8.13, 8.37 (total 1H, each s, C$\underline{H}$O). LR-CIMS *m/z*: 312 (MH$^+$).

*5.6.2. N-formyl-1-cyclopropyl-4-phenylsulfanyl-1,2,3,4-tetrahydroisoquinoline **6g***

Yield, 99%. A pale yellow gum. IR: 1674. ^{1}H-NMR: 0.36–0.48 (1H, m, cyclopropyl), 0.57–0.83 (3H, m, cyclopropyl), 1.11–1.25 (1H, m, cyclopropyl), 3.38, 3.93 (total 1H, each dd, J = 14, 3 Hz, C3-H), 3.59, 4.64 (total 1H, each d, J = 14 Hz, C3-H), 4.38, 4.54 (total 1H, each br-s, C4-H), 4.70, 4.76, 4.85 (total 1H, each d, J = 9 Hz, C1-H), 7.24–7.48 (9H, m, ArH and PhH), 8.07, 8.13, 8.33 (total 1H, each s, C<u>H</u>O). HR-EIMS *m/z* (M^+): Calcd for $C_{19}H_{19}NOS$: 309.1184, found: 309.1184.

*5.6.3. N-formyl-1-cyclobutyl-4-phenylsulfanyl-1,2,3,4-tetrahydroisoquinoline **6h***

Yield, 99%. A pale yellow gum. IR: 1674. ^{1}H-NMR: 1.73–1.84 (3H, m, cyclobutyl), 1.98–2.23 (3H, m, cyclobutyl), 2.58–2.64 (1H, m, cyclobutyl), 3.18, 3.71 (total 1H, each dd, J = 14, 3 Hz, C3-H), 3.51, 4.58 (total 1H, each d, J = 14 Hz, C3-H), 4.36, 4.51 (total 1H, each d, J = 3Hz, C4-H), 4.38, 5.41 (total 1H, each d, J = 10 Hz, C1-H), 7.10–7.48 (8H, m, ArH and PhH), 7.64–7.67 (1H, m, ArH), 8.10, 8.41 (total 1H, each s, C<u>H</u>O). LR-CIMS *m/z*: 324 (MH^+).

*5.7. Pummerer reaction of sulfoxide **5i***

Method A: TFAA (1.88 ml, 13.6 mmol) was added into a solution of **5i** (1.00 g, 2.71 mmol) in benzene (50.0 ml) at room temperature. After the mixture was stirred for 0.5 h, $BF_3{\cdot}Et_2O$ (1.00 ml, 8.13 mmol) was added then the reaction mixture was further stirred at the same temperature for 1.0 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The residue was purified by chromatography (AcOEt/hexane = 1:2) to give **6i** (133 mg, 14%), **7i** (310 mg, 44%).

Method B: TFAA (1.88 ml, 13.6 mmol) was added to a solution of **5i** (1.00 g, 2.71 mmol) in benzene (50.0 ml) at room temperature. After the mixture was stirred for 0.5 h, TFSA (0.70 ml, 8.13 mmol) was added into the mixture, then the reaction mixture was further stirred at the same conditions for 1.0 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The residue was purified by chromatography (AcOEt/hexane = 1:2) to give **6i** (599 mg, 63%).

*5.7.1. N-formyl-1-cyclohexyl-4-phenylsulfanyl-1,2,3,4-tetrahydroisoquinoline **6i***

Colorless needles, m.p. 123–125°C, recrystallized from hexane–AcOEt. IR: 1674, 1660. ^{1}H-NMR: 0.90–1.23 (5H, m, cyclohexyl), 1.51–1.91 (6H, m, cyclohexyl), 3.29, 3.83 (total 1H, each dd, J = 14, 3 Hz, C3-H), 3.58, 4.65 (total 1H, each d, J = 14 Hz, C3-H), 4.33, 5.28 (total 1H, each d, J = 6 Hz, C1-H), 4.39, 4.55 (total 1H, each d, J = 3 Hz, C4-H), 7.28–7.47, 7.61–7.64 (total 9H, each m, ArH and PhH), 8.12, 8.32 (total 1H, each s, C<u>H</u>O). HR-EIMS *m/z* (M^+): Calcd for $C_{22}H_{25}NOS$: 351.1667, found: 351.1703.

*5.7.2. N-(1-cyclohexyl)ethyl-N-(2-oxothyl)formamide **7i***

A yellow gum. IR: 1734, 1674, 1653. ^{1}H-NMR: 0.81–1.33 (5H, m, cyclohexyl), 1.60–1.87 (5H, m, cyclohexyl), 2.00–2.12 (1H, m, cyclohexyl), 3.55 (1H, dd, J = 18, 2 Hz, C<u>H</u>$_2$CHO), 4.10 (1H, dd, J = 18, 2 Hz, C<u>H</u>$_2$CHO), 4.22 (1H, J = 11 Hz, C<u>H</u>Ph), 7.21–7.40 (5H, m , PhH), 8.79 (1H, s, NC<u>H</u>O), 8.89 (1H, t, J = 2 Hz, CH$_2$C<u>H</u>O). LR-CIMS *m/z*: 260 (MH^+).

*5.8. Pummerer reaction of sulfoxide **5j***

<u>Method A</u>: **5j** (500 mg, 1.57 mmol) was treated with TFAA (1.10 ml, 7.85 mmol) and $BF_3{\cdot}Et_2O$ (0.67 ml, 4.71 mmol) in benzene (25.0 ml) at room temperature to give **6j** (62.0 mg, 13%), **7j** (148 mg, 45%).

<u>Method B</u>: **5j** (500 mg, 1.57 mmol) was treated with TFAA (1.10 ml, 7.85 mmol) and TFSA (0.14 ml, 4.71 mmol) in benzene (25.0 ml) at room temperature to give **6j** (202 mg, 63%).

*5.8.1. N-formyl-6-fluoro-1-methyl-4-phenylsulfanyl-1,2,3,4-tetrahydroisoquinoline **6j***

A pale yellow gum. IR: 1670. ^{1}H-NMR: 1.44, 1.50 (total 3H, each d, J = 7 Hz, CH_3), 3.22, 3.71 (total 1H, each dd, J = 14, 3 Hz, C3-H), 3.54, 4.58 (total 1H, each d, J = 14 Hz, C3-H), 4.28, 4.43 (total 1H, each s, C4-H), 4.81, 5.49 (total 1H, each q, J = 7 Hz, C1-H), 6.87–7.65 (8H, m, ArH and PhH), 8.00, 8.42 (total 1H, each s, C<u>H</u>O). HR-EIMS *m/z* (M^+): Calcd for $C_{17}H_{16}FNOS$: 301.0937, found: 301.0932.

*5.8.2. N-[1-(4-fluorophenyl)ethyl]-N-(2-oxothyl)formamide **7j***

A yellow gum. IR: 1735, 1662, 1604. ^{1}H-NMR: 1.48, 1.64 (total 3H, each d, J = 7 Hz, CH_3), 3.73, 3.76 (total 1H, each d, J = 18 Hz, C<u>H</u>$_2$CHO), 3.83, 3.89 (total 1H, each d, J = 18 Hz, C<u>H</u>$_2$CHO), 4.89 (1H, q, J = 7 Hz, C<u>H</u>Ph), 7.00–7.10 (2H, m , PhH), 7.23–7.28 (2H, m, PhH), 8.12, 8.50 (total 1H, each s, NC<u>H</u>O), 9.30, 9.35 (total 1H, each t, J = 1 Hz, CH$_2$C<u>H</u>O). HR-EIMS *m/z* (M^+): Calcd for $C_{11}H_{12}FNO_2$: 209.0852, found: 209.0890.

*5.9. Reductive desulfurization of **7e**. Typical procedure*

$NaBH_4$ (1.87 g, 49.0 mmol) was added in small portions to a stirred solution of **7e** (700 mg, 2.35 mmol) and $NiCl_2{\cdot}6H_2O$ (3.92 g, 16.5 mmol) in MeOH-THF (3:1) (50.0 ml) under ice-cooling. The mixture was stirred at room temperature for further 0.5 h. To the reaction mixture, H_2O (10.0 ml) was added and filtered. The filtrate was diluted with H_2O and acidified by 5% HCl solution, and the mixture was extracted with $CHCl_3$. The residue was purified by flash column chromatography (AcOEt/hexane = 2:3) to give **8e** (369 mg, 83%) and **9e** (39 mg, 9%).

*5.9.1. N-formyl-1-ethyl-1,2,3,4-tetrahydroisoquinoline **8e***

Colorless prisms, m.p. 63–65°C, recrystallized from hexane-AcOEt. IR: 1674. ^{1}H-NMR: 0.98 (3H, t, J = 7 Hz, CH$_2$C<u>H</u>$_3$), 1.75–1.96 (2H, m, C<u>H</u>$_2$CH$_3$), 2.73–3.13, 3.51–3.73, 4.44–4.51 (total 4H, each m, C4-H and C3-H), 4.35–

4.40, 5.32–5.37 (total 1H, each m, C1-H), 7.08–7.22 (4H, m, ArH), 8.21, 8.25 (total 1H, each s, CHO). HR-EIMS *m/z* (M^+): Calcd for $C_{12}H_{15}NO$: 189.1151, found: 189.1134.

5.9.2. N-formyl-1-ethyl-1,2-dihydroisoquinoline ***9e***

A pale yellow gum. IR: 1674. ^{1}H-NMR: 0.84, 0.90, (total 3H, each t, J = 7 Hz, CH_2CH_3), 1.54–1.85 (2H, m, CH_2CH_3), 4.60 (dd, J = 8, 6 Hz) and 5.45 (t, J = 7 Hz): total 1H, C1-H, 5.91, 6.06 (total 1H, each d, J = 8 Hz, C4-H), 6.56, 7.06 (total 1H, d, J = 8 Hz, C3-H), 7.05–7.61 (4H, m, ArH), 8.16, 8.35 (total 1H, each s, CHO). LR-CIMS *m/z*: 188 (MH^+).

8f (486 mg, 83%) and **9f** (14 mg, 2%) were obtained from **6f** (900 mg).

5.9.3. N-formyl-1-propyl-1,2,3,4-tetrahydroisoquinoline ***8f***

A pale yellow gum. IR: 1674. ^{1}H-NMR: 0.96, 0.98 (total 3H, each t, J = 7 Hz, $CH_2CH_2CH_3$), 1.30–1.51 (2H, m, $CH_2CH_2CH_3$), 1.66–1.92 (2H, m, $CH_2CH_2CH_3$), 2.75–3.16, 3.52–3.73, 4.43–4.51 (total 4H, each m, C3-H and C4-H), 4.49, 5.42 (total 1H, each t, J = 7 Hz, C1-H), 7.08–7.21 (4H, m, ArH), 8.20, 8.22 (total 1H, each s, CHO). HR-EIMS *m/z* (M^+): Calcd for $C_{13}H_{17}NO$: 203.1311, found: 203.1349.

5.9.4. N-formyl-1-propyl-1,2-dihydroisoquinoline ***9f***

A pale yellow gum. IR: 1687, 1631. ^{1}H-NMR: 0.84–0.99 (3H, m, $CH_2CH_2CH_3$), 1.22–1.29 (2H, m, $CH_2CH_2CH_3$), 1.32–1.79 (2H, m, $CH_2CH_2CH_3$), 4.69 (dd, J = 7, 6 Hz) and 5.51 (t, J = 7 Hz): total 1H, C1-H, 5.22, 6.06 (total 1H, each d, J = 7 Hz, C4-H), 6.54, 7.06 (total 1H, d, J = 8 Hz, C3-H), 7.04–7.35 (4H, m, ArH), 8.14, 8.33 (total 1H, each s, CHO). LR-CIMS *m/z*: 202 (MH^+).

8g (415 mg, 71%) and **9g** (32 mg, 6%) were obtained from **6g** (700 mg).

5.9.5. N-formyl-1-cyclopropyl-1,2,3,4-tetrahydroisoquinoline ***8g***

Colorless prisms, m.p. 82–84°C, recrystallized from hexane-AcOEt. IR: 1664. ^{1}H-NMR: 0.33–0.48 (1H, m, cyclopropyl), 0.60–0.80 (3H, m, cyclopropyl), 1.22–1.31 (1H, m, cyclopropyl), 2.86–2.97 (2H, m, C4-H), 3.31–3.40, 4.38–4.45 (total 1H, each m , C3-H), 3.72–3.76 (1H, m, C3-H), 3.79, 4.83 (total 1H, each d, J = 9 Hz, C1-H), 7.08–7.32 (4H, m, ArH), 8.19, 8.38 (total 1H, each s, CHO). HR-EIMS *m/z* (M^+): Calcd for $C_{13}H_{15}NO$: 201.1151, found: 201.1119.

5.9.6. N-formyl-1-cyclopropyl-1,2-dihydroisoquinoline ***9g***

A pale yellow gum. IR: 1685, 1631. ^{1}H-NMR: 0.36–0.65 (4H, m, cyclopropyl), 1.20–1.31 (1H, m, cyclopropyl), 3.97, 5.04 (total 1H, each d, J = 8 Hz, C1-H), 5.97, 6.09 (total 1H, each d, J = 7 Hz, C4-H), 6.62, 7.08 (total 1H, each d, J = 7 Hz, C3-H), 7.10–7.30 (4H, m, ArH), 8.16, 8.38 (total 1H, each s, CHO). HR-FABMS *m/z* (MH^+): Calcd for $C_{13}H_{14}NO$: 200.1076, found: 200.1077.

8h (466 mg, 78%) was obtained from **6h** (900 mg).

5.9.7. N-formyl-1-cyclobutyl-1,2,3,4-tetrahydroisoquinoline ***8h***

A colorless gum. IR: 1672, 1653. ^{1}H-NMR: 1.76–2.20 (6H, m, cyclobutyl), 2.64–3.00, 3.13–3.22 (total 4H, each m, cyclobutyl and C4-H, C3-H), 3.49–3.68 (1H, m, C3-H), 4.33, 5.35 (total 1H, each d, J = 10 Hz, C1-H), 7.08–7.22 (4H, m, ArH), 8.21, 8.26 (total 1H, each s, CHO). HR-EIMS *m/z* (M^+): Calcd for $C_{14}H_{17}NO$: 215.1307, found: 215.1291.

8i (254 mg, 73%) was obtained from **6i** (500 mg).

5.9.8. N-formyl-1-cyclohexyl-1,2,3,4-tetrahydroisoquinoline ***8i***

A colorless gum. IR: 1670. ^{1}H-NMR: 0.93–1.26 (5H, m, cyclohexyl), 1.63–1.76 (6H, m, cyclohexyl), 2.94 (2H, t, J = 7 Hz, C4-H), 3.32–3.41, 4.14–4.22 (total 1H, each m, C3-H), 3.66–3.70, 4.13–4.20 (total 1H, each m, C3-H), 4.15, 5.14 (total 1H, each d, J = 8 Hz, C1-H), 7.08–7.36 (4H, m, ArH), 8.18, 8.23 (total 1H, each s, CHO). HR-EIMS *m/z* (M^+): Calcd for $C_{16}H_{21}NO$: 243.1623, found: 243.1641.

8j (279 mg, 87%) was obtained from **6j** (500 mg)

5.9.9. N-formyl-6-fluoro-1-methyl-1,2,3,4-tetrahydroisoquinoline ***8j***

A pale yellow oil. IR: 1616. ^{1}H-NMR: 1.46, 1.54 (total 3H, each d, J = 7 Hz, CH_3), 2.70–3.00 (2H, m, C3-H and C4-H), 3.15, 3.53 (total 1H, each ddd, J = 13, 5, 2 Hz, C3-H), 3.70, 4.45 (total 1H, each ddd, J = 13, 6, 2 Hz, C3-H), 4.77, 5.44 (total 1H, each q, J = 7 Hz, C1-H), 6.79–7.13 (3H, m, ArH), 8.14, 8.29 (total 1H, each s, CHO).

5.10. Hydrolysis of ***8e.*** *Typical procedure*

A solution of **8e** (360 mg, 1.90 mmol) in EtOH (15.0 ml)/20% NaOH (15.0 ml) was refluxed for 3.0 h. The reaction mixture was diluted with H_2O and the mixture was extracted with $CHCl_3$. The product was purified by column chromatography (AcOEt) to give **1-ethyl-1,2,3,4-tetrahydroisoquinoline 10e** (274 mg, 59%) as a colorless oil. ^{1}H-NMR: 1.01 (3H, t, J = 7 Hz, CH_2CH_3), 1.66–1.81 (1H, m, CH_2CH_3), 1.86–1.99 (1H, m, CH_2CH_3), 2.70–2.89 (2H , m, C4-H), 2.97 (1H, ddd, J = 12, 8, 5 Hz, C3-H), 3.24 (1H, dt, J = 12, 5 Hz, C3-H), 3.90 (1H, dd, J = 9, 4 Hz, C1-H), 7.06–7.18 (4H, m, ArH). ^{13}C-NMR: 10.5 (CH_2CH_3), 29.0 (CH_2CH_3), 30.0 (C4), 41.1 (C3), 57.0 (C1), 125.7 (C7), 125.8 (C6), 126.1 (C5), 129.2 (C8), 135.2 (C8a), 139.5 (C4a). HR-FABMS *m/z* (MH^+): Calcd for $C_{11}H_{16}N$: 162.1282, found: 162.1295.

5.10.1. 1-Propyl-1,2,3,4-tetrahydroisoquinoline ***10f***

Yield, 99%. A pale yellow oil. ^{1}H-NMR: 0.98 (3H, t, J = 7 Hz, $CH_2CH_2CH_3$), 1.37–1.58 (2H, m, $CH_2CH_2CH_3$), 1.65–1.88 (2H, m, $CH_2CH_2CH_3$), 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), 3.97 (1H, dd, J = 9, 4 Hz, C1-H), 7.07–7.17 (4H, m, ArH). ^{13}C-NMR: 14.9 ($CH_2CH_2CH_3$), 19.4 ($CH_2CH_2CH_3$), 30.0 (C4), 38.7 ($CH_2CH_2CH_3$), 41.0 (C3), 55.5 (C1), 125.7 (C7), 125.7 (C6), 126.1 (C5), 129.2 (C8), 135.1 (C8a), 139.8 (C4a). HR-FABMS *m/z* (MH^+): Calcd for $C_{12}H_{18}N$: 176.1439, found: 176.1441.

*5.10.2. 1-Cyclopropyl-1,2,3,4-tetrahydroisoquinoline **10g***

Yield, 87%. A yellow oil. ^{1}H-NMR: 0.27–1.25 (5H, m, cyclopropyl), 2.62–3.37 (5H, m, C1-H, C3-H and C4-H), 7.01–7.51 (4H, m, ArH). ^{13}C-NMR: 1.7 (CH_2), 5.4 (CH_2), 17.8 (CH), 29.7 (C4), 42.5 (C3), 61.8 (C1), 125.5 (C7), 125.9 (C6), 126.1 (C5), 128.9 (C8), 134.7 (C8a), 139.1 (C4a). LR-CIMS *m/z*: 174 (MH^+).

*5.10.3. 1-Cyclobutyl-1,2,3,4-tetrahydroisoquinoline **10h***

Yield, 71%. A yellow oil. ^{1}H-NMR: 1.81–2.16 (6H, m, cyclobutyl), 2.62–2.83 (3H, m, cyclobutyl and C4-H), 2.96 (1H, ddd, J = 12, 7, 6 Hz, C3-H), 3.23 (1H, dt, J = 12, 6 Hz, C3-H), 3.83 (1H, d, J = 9 Hz, C1-H), 7.06–7.15 (4H, m, ArH). ^{13}C-NMR: 18.1 (CH_2), 25.8 (CH_2), 27.9 (CH_2), 29.9 (C4), 41.1 (C3), 41.3 (CH), 60.7 (C1), 125.3 (C7), 125.8 (C6), 125.9 (C5), 129.1 (C8), 134.7 (C8a), 138.8 (C4a). LR-CIMS *m/z*: 188 (MH^+).

*5.10.4. 1-Cyclohexyl-1,2,3,4-tetrahydroisoquinoline **10i***

Yield, 86%. A yellow oil. ^{1}H-NMR: 1.05–1.44 (6H, m, cyclohexyl), 1.70–1.97 (5H, m, cyclohexyl), 2.63–2.70 (1H, m, C4-H), 2.81–2.97 (2H, m, C3-H and C4-H), 3.24–3.31 (1H, m, C3-H), 3.90 (1H, d, J = 4 Hz, C1-H), 7.05–7.16 (4H, m, ArH). ^{13}C-NMR: 26.4 (CH_2), 26.57 (CH_2), 26.62 (CH_2), 27.0 (CH_2), 30.3 (CH_2), 30.9 (C4), 42.3 (C3), 42.9 (CH), 60.7 (C1), 125.5 (C7), 125.6 (C6), 126.0 (C5), 129.1 (C8), 136.2 (C8a) 138.5 (C4a). HR-EIMS *m/z* (M^+): Calcd for $C_{15}H_{21}N$: 215.1709, found: 215.1692.

*5.10.5. 6-Fluoro-1-methyl-1,2,3,4-tetrahydroisoquinoline **10j***

Yield, 91%. A colorless oil. ^{1}H-NMR: 1.44 (3H, d, J = 7 Hz, CH_3), 2.72 (1H, dt, J = 16, 5 Hz, C4-H), 2.86 (1H, m, C4-H), 3.00 (1H, ddd, J = 13, 9, 5 Hz, C3-H), 3.50 (1H, dt, J = 13, 5 Hz, C3-H), 4.06 (1H, q, J = 7 Hz, C1-H), 6.77 (1H, dd, J = 10, 3 Hz, C8-H), 6.84 (1H, td, J = 9, 3 Hz, C7-H), 7.09 (1H, dd, J = 9, 6Hz, C6-H). ^{13}C-NMR: 22.6 (CH_3), 30.0 (C4), 41.4 (C3), 51.1 (C1), 115.0 (C5 or C7), 127.7 (C5 or C7), 127.2 (C8), 136.0 (C8a), 136.8 (C4a), 160.8 (C6).

5.11. Biological experiments

5.11.1. Cell culture

PC12 cells were obtained from the American Tissue Type Culture Collection (reference no. CRL 1721). Dulbecco's modified Eagle's medium (DMEM), horse serum and fetal bovine serum (FBS) were purchased from GIBCO (Grand Island, NY, USA). Mouse nerve growth factor (2.5S NGF) was from Takara Shuzo Co. Ltd. (Shiga, Japan). PC12 cells were routinely maintained in DMEM containing 15% heat-inactivated serum (10% FBS, 5% horse serum) under an atmosphere containing 5% CO_2 at 37°C. The medium was changed every 2–3 days. The cells were transferred to 24-well plates and cultured for 2 days. These cells were neuronally differentiated for 5 days in DMEM containing 7% heat-inactivated serum (2% FBS and 1% horse serum) supplemented with 50 ng/ml 2.5S NGF (differentiation medium, DM). Thereafter, cells were washed repeatedly with phosphate-buffered saline (PBS, pH 7.4) to remove serum and NGF, and placed in DM, DMEM without serum (as NGF + DMEM) or DMEM without either serum or NGF (as trophic withdrawal medium). TIQs were added at final concentrations ranging from 10^{-5} to 10^{-3} M to trophic withdrawal medium, and cells were cultured for 24 h.

5.11.2. Determination of cell viability

Viable cell ratios were determined using the dye exclusion assay. This assay is based on the exclusion of trypan blue dye from viable cells. After 24 h treatment with TIQs, PC12 cells were dispersed with trypsin/EDTA, then incubated with 0.1% trypan blue for 5 min. At least 1000 cells per sample were observed under a microscope and counted as either stained or unstained. Viable cell ratios of NGF + DMEM and trophic withdrawal medium were expressed as a percentage of the DM-cultured cells' viability. The effects of TIQs on trophic withdrawal-cultured PC12 cells were estimated as the percentage of trophic withdrawal-cultured cell viability in the absence of any TIQ.

5.11.3. Measurement of neurite outgrowth

Cells bearing neurite-like processes longer than the cell body diameter (measured at the widest point), or bearing a neurite with a length at least double that of the cell diameter, were counted as differentiated. Data were expressed as the ratio of neurite-bearing cells to total cells counted. Cell aggregates were not scored, and cells with more than one neurite were only counted once. Estimates of the effects of growth medium and TIQs on neurite bearing were expressed in the same manner as "Determination of cell viability", described above.

5.11.4. Comet assay

The reagents and equipment for the comet assay were purchased from TREVIGEN (Gaithersburg, MD, USA). PC12 cells were incubated with the test substances at 37°C for 3 h. After exposure, cells were washed with PBS(-), then suspended (1×10^5 cells/100 μl PBS(–)); 50 μl of this suspension was mixed with 500 μl of 1% low-melting point agarose. The mixture (75 μl aliquots) was applied to the comet slides, and the slides were kept horizontal for 10 min at 4°C. 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, 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 SYBR Green (0.1 μl/ml) solution.

The comet image was observed through a fluorescence microscope at × 200 magnification and at 568 nm. Routinely, 250 cells (50 cells/slide) 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 and width of the DNA mass was measured using an ocular micrometer disk. In the present study, the length: width ratios of the DNA mass and the frequency of cells with prominent tails (i.e., a small portion of the DNA remains at the origin, but most of the DNA has fragmented and migrated to form a 'tail') were used for estimating the cytotoxicity of the TIQ derivatives.

5.11.5. Data analysis

Data were expressed as mean ± standard error (S.E.) for each group. The significance of difference was analyzed by Dunnett's multiple comparison test or Tukey–Kramer's multiple comparison test. A value of $P < 0.05$ was regarded as statistically significant.

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