

Tumor-specific Cytotoxic Activity of 1,2,3,4-Tetrahydroisoquinoline Derivatives against Human Oral Squamous Cell Carcinoma Cell Lines

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Abstract. *The tumor-specific cytotoxicity and the type of cell death induced by thirty-eight newly synthesized tetrahydroisoquinoline derivatives in human oral squamous cell carcinoma cell lines were investigated. 6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl(3,4-dimethoxyphenyl) methanone that has bulky substituents (such as 3,4-dimethoxybenzoyl group) (TQ9) and ethyl 2-benzyloxycarbonyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylate that has ethoxycarbonyl group and benzyloxycarbonyl group (TD13) showed the highest tumor-specific cytotoxicity (TS=12.5 and 5.3, respectively). This supports the importance of molecular size for the cytotoxicity induction. TQ9 induced internucleosomal DNA fragmentation and caspase-3 activation only marginally in HL-60 cells, whereas it enhanced the formation of acidic organelles (stained with acridine orange) without induction of DNA fragmentation or caspase-3 activation in human squamous cell carcinoma cell lines (HSC-2, HSC-4), suggesting the induction of autophagy in the latter cells.*

1-Methyl-1,2,3,4-tetrahydroisoquinoline is the only endogenous Parkinsonism-preventing agent discovered to date (reviewed in (1)). The incorporation of the 1,2,3,4-tetrahydroisoquinoline (TIQ) moiety is an important synthetic strategy in drug discovery (2). The high therapeutic properties of the related drugs have encouraged medicinal chemists to synthesize a large number of novel chemotherapeutic agents.

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Pharmaceutical properties including antineoplastic (3, 4), nitric oxide (NO) inhibitory (5), histamine H₃ antagonist and serotonin reuptake inhibitory (6), α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor antagonistic (7), bradycardic (8), orexin-2 receptor-selective antagonistic (9), multidrug resistance (MDR) reversal (10-12), γ -secretase inhibitory (13), kinase insert domain-containing receptor (KDR) inhibitory (14), and antidiabetic activities (15), are all unique characteristics known for newly synthesized TIQ derivatives. On the other hand, the TIQ family of alkaloids also includes potent cytotoxic agents that display a range of biological properties such as antitumor and antimicrobial activities (16).

There is real perceived need for the discovery of new compounds endowed with antitumor activity, possibly acting through mechanisms of action which are distinct from those of well-known classes of antitumor agents. Through the various molecules designed and synthesized for this aim, it was demonstrated that *N*-acyl-TIQ derivatives could be considered as future anticancer candidates. As a part of our exhaustive search for the substances that show selective cytotoxicity against human oral squamous cell carcinoma (OSCC) cell lines, we investigated the tumor-specific cytotoxicity and the type of cell death induced in OSCC cells by thirty-eight newly synthesized TIQ derivatives, **TQ1-19** (Figure 1A) and **TD1-19** (Figure 1B) (Table I).

Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: Dulbecco's modified Eagle's medium (DMEM) (GIBCO BRL, Grand Island, NY, USA); RPMI-1640 medium, fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), acridine orange (Sigma-Aldrich Inc., St. Louis, MO, USA); dimethyl sulfoxide (DMSO) (Wako Pure Chem. Ind., Osaka, Japan).

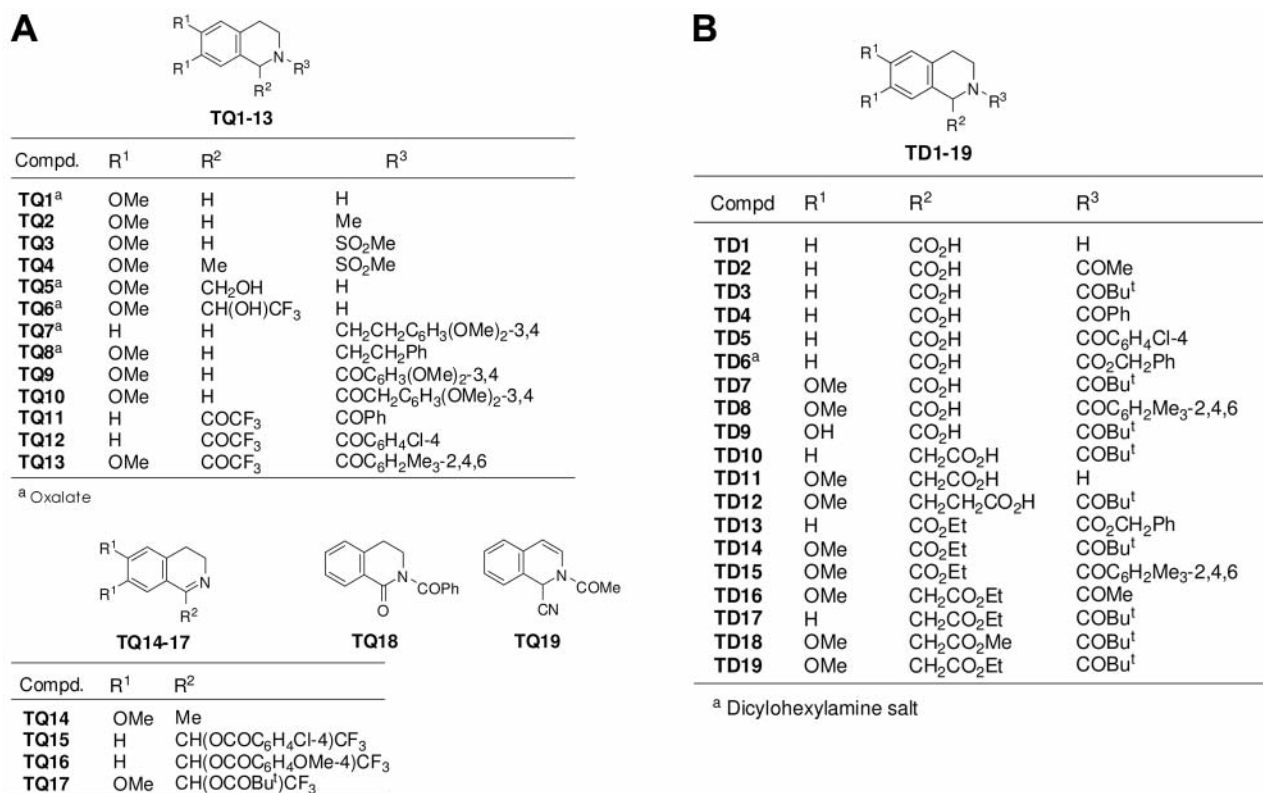


Figure 1. The structure of 1,2,3,4-tetrahydroisoquinoline derivatives. (A) **TQ1-19**, (B) **TD1-19**.

Preparation of 1,2,3,4-tetrahydroisoquinoline (TIQ) derivatives. Thirty-eight 1,2,3,4-tetrahydroisoquinoline derivatives (**TQ1-19** and **TD1-19**) (Table I) were prepared as described elsewhere (17-30).

Cell culture. Three human oral tumor cell lines (HSC-2, HSC-3, HSC-4) and three human normal cell types (HGF, HPC, HPLF) were cultured in DMEM supplemented with 10% heat-inactivated FBS. Human promyelocytic leukemic HL-60 cells were cultured in RPMI-1640 supplemented with 10% heat-inactivated FBS. Tumor cell lines were obtained from Riken Cell Bank. Normal cells were prepared from periodontal tissues according to the guideline of the Institutional Board of Meikai University Ethics Committee (Number A0808) with informed consent from the patients. Since HGF, HPC and HPLF cells have a limited lifespan due to *in vitro* senescence (31), these cells were used at the population doubling level of 7-11 for the present study.

Assay for cytotoxic activity. Cells (except for HL-60 cells) were inoculated at 3×10^3 cells/0.1 ml in 96-microwell plates (Becton Dickinson Labware, NJ, USA), unless otherwise stated. After 48 hours, the medium was removed by suction with an aspirator, and replaced with 0.1 ml of fresh medium containing different concentrations of test compounds. The first well contained a 400 μ M sample and was diluted 2-fold sequentially, with triplicate wells for each concentration. Cells were incubated for another 48 hours, and the relative viable cell number was then determined by MTT method. In brief, cells were replenished with fresh culture medium containing

0.2 mg/ml MTT and incubated for another 4 hours. The cells were lysed with 0.1 ml of DMSO, and the absorbance at 540 nm of the cell lysate was determined using a microplate reader (Biochromatic Labsystem, Helsinki, Finland) (32). A_{540} of control cells was usually in the range from 0.50 to 1.2.

HL-60 cells were inoculated at 5×10^4 /0.1 ml in 96-microwell plates and test compounds were added. After incubation for 48 hours, the viable cell number was determined by trypan blue exclusion, under a light microscope.

The 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve. Tumor-specificity (TS) was determined by the following equation: $TS = \{ [CC_{50}(\text{HGF}) + CC_{50}(\text{HPC}) + CC_{50}(\text{HPLF})] / [CC_{50}(\text{HSC-2}) + CC_{50}(\text{HSC-3}) + CC_{50}(\text{HSC-4}) + CC_{50}(\text{HL-60})] \} \times (4/3)$.

Assay for DNA fragmentation. HSC-2 or HSC-4 cells (9×10^4) were inoculated onto 6-well plates (9.6 cm²) and left for 48 hours to allow their complete adherence to the plate. Adherent HSC-2, HSC-4 cells or HL-60 cells freshly prepared (5×10^5) were cultured for 6 or 24 hours in fresh culture medium (3 ml) without (control) or with the indicated concentrations of samples. Cells were then harvested, washed once with phosphate-buffered saline without calcium and magnesium (PBS(-)) and lysed with 50 μ l lysis buffer [50 mM Tris-HCl (pH 7.8), 10 mM EDTA-2Na, 0.5% (w/v) sodium N-laurylsarcosinate solution]. The lysate was incubated with 0.4 mg/ml RNase A and 0.8 mg/ml proteinase K for 1-2 hours at

Table I. Preparation of thirty-eight 1,2,3,4-tetrahydroisoquinoline derivatives.

Compd.	Name	Ref.
TQ1	6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline oxalate	17
TQ2	6,7-Dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline	18
TQ3	6,7-Dimethoxy-2-(methylsulfonyl)-1,2,3,4-tetrahydroisoquinoline	19
TQ4	6,7-Dimethoxy-1-methyl-2-(methylsulfonyl)-1,2,3,4-tetrahydroisoquinoline	19
TQ5	(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)methanol oxalate	20
TQ6	1-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)-2,2,2-trifluoroethanol oxalate	21
TQ7	2-(3,4-Dimethoxyphenethyl)-1,2,3,4-tetrahydroisoquinoline oxalate	22
TQ8	6,7-Dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline oxalate	23
TQ9	(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1 <i>H</i>)-yl)(3,4-dimethoxyphenyl)methanone	×
TQ10	1-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1 <i>H</i>)-yl)-2-(3,4-dimethoxyphenyl)ethanone	×
TQ11	1-(2-Benzoyl-1,2,3,4-tetrahydroisoquinolin-1-yl)-2,2,2-trifluoroethanone	24
TQ12	1-(2-(4-Chlorobenzoyl)-1,2,3,4-tetrahydroisoquinolin-1-yl)-2,2,2-trifluoroethanone	24
TQ13	1-(6,7-Dimethoxy-2-(3,4,5-trimethylbenzoyl)-1,2,3,4-tetrahydroisoquinolin-1-yl)-2,2,2-trifluoroethanone	24
TQ14	6,7-Dimethoxy-1-methyl-3,4-dihydroisoquinoline	25
TQ15	1-(3,4-Dihydroisoquinolin-1-yl)-2,2,2-trifluoroethyl 4-chlorobenzoate	24
TQ16	1-(3,4-Dihydroisoquinolin-1-yl)-2,2,2-trifluoroethyl 4-methoxybenzoate	24
TQ17	1-(3,4-Dihydroisoquinolin-1-yl)-2,2,2-trifluoroethyl pivalate	24
TQ18	2-Benzoyl-6,7-dimethoxy-3,4-dihydroisoquinolin-1(2 <i>H</i>)-one	26
TQ19	2-Acetyl-6,7-dimethoxy-1,2-dihydroisoquinoline-1-carbonitrile	27
TD1	1,2,3,4-Tetrahydroisoquinoline-1-carboxylic acid	26
TD2	2-Acetyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid	24
TD3	6,7-Dimethoxy- <i>N</i> -pivaloyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid	24
TD4	2-Benzoyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid	26
TD5	2-(4-Chlorobenzoyl)-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid	24
TD6	2-Benzoyloxycarbonyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid dicyclohexylamine	24
TD7	6,7-Dimethoxy-2-pivaloyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid	26
TD8	6,7-Dimethoxy-2-(2,4,6-trimethylbenzoyl)-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid	24
TD9	6,7-Dihydroxy-2-pivaloyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid	28
TD10	2-(2-Pivaloyl-1,2,3,4-tetrahydroisoquinolin-1-yl)acetic acid	×
TD11	2-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)acetic acid	29
TD12	3-(6,7-Dimethoxy-2-pivaloyl-1,2,3,4-tetrahydroisoquinolin-1-yl)propionic acid	×
TD13	Ethyl 2-benzoyloxycarbonyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylate	26
TD14	Ethyl 2-pivaloyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylate	26
TD15	Ethyl 2-(2,4,6-trimethylbenzoyl)-1,2,3,4-tetrahydroisoquinoline-1-carboxylate	24
TD16	Ethyl 2-(2-acetyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)acetate	×
TD17	Ethyl 2-(2-pivaloyl-1,2,3,4-tetrahydroisoquinolin-1-yl)acetate	30
TD18	Methyl 2-(6,7-dimethoxy-2-pivaloyl-1,2,3,4-tetrahydroisoquinolin-1-yl)acetate	×
TD19	Ethyl 2-(6,7-dimethoxy-2-pivaloyl-1,2,3,4-tetrahydroisoquinolin-1-yl)acetate	×

×, The method of synthesis to be published elsewhere.

50°C, and then mixed with 50 µl NaI solution (7.6 M NaI, 20 mM EDTA-2Na, 40 mM Tris-HCl, pH 8.0) and 200 µl of ethanol. After centrifugation for 20 minutes at 20,000×g, the precipitate was washed with 1 ml of 70% ethanol and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA-2Na, pH 8.0). A sample (10-20 µl) was applied to 2% agarose gel electrophoresis in TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA-2Na, pH 8.0). DNA molecular marker (Bayou Biolabs, Harahan, LA, USA) and DNA from apoptotic HL-60 cells induced by UV irradiation (33) were run in parallel. After staining with ethidium bromide, DNA was visualized by UV irradiation, and photographed by CCD camera (Bio Doc-It, UVP, Inc., Upland, CA, USA).

Assay for caspase activation. HSC-2 or HSC-4 cells (6×10⁵) were inoculated on 8.5 cm plates and incubated for 48 hours to allow

their complete adherence. The adherent HSC-2 or HSC-4 cells, and HL-60 cells freshly prepared (3×10⁶ in 6-well plates) were further incubated for 6 hours in fresh medium without (control) or with the test samples. Cells were washed with PBS(-) and lysed in lysis solution [50 mM Tris-HCl (pH 7.5), 0.3% NP-40, 1 mM DTT]. After standing for 10 minutes on ice and centrifugation for 5 minutes at 21,000×g, the supernatant was collected. Lysate (50 µl, equivalent to 200 µg protein) was mixed with 50 µl lysis solution containing substrates for caspase-3 (DEVD-*p*NA (*p*-nitroanilide)). After incubation for 18 hours at 37°C, the absorbance at 405 nm of the liberated chromophore *p*NA was measured by a microplate reader (32).

Detection of acidic vesicular organelles with acridine orange staining. Acidic vesicular organelles were imaged by staining the cells with 0.1

Table II. Cytotoxicity of 1,2,3,4-tetrahydroisoquinoline derivatives (TQ compounds) on human tumor and normal cells.

TQ	CC ₅₀ (μM)							
	Human tumor cell lines				Human normal oral cells			
	HSC-2	HSC-3	HSC-4	HL-60	HGF	HPC	HPLF	TS
1	167±24	310±58	343±11	315±79	>364	>382	>371	>1.2±0.17
2	>400	>400	>378	>395	>386	>400	>387	><1.1±0.08
3	>355	>400	>400	336±58	>400	>400	>400	>1.1±0.10
4	>395	>400	>400	293±93	>360	>390	>397	>1.1±0.16
5	176±82	248±19	175±31	193±179	>354	>400	>374	>2.0±0.66
6	>370	>400	>400	326±65	>400	>400	>400	>1.1±0.10
7	276±28	298±56	250±23	71±15	307±33	313±13	299±45	1.4±0.12
8	255±21	238±34	222±40	87±23	298±50	318±21	289±35	1.5±0.00
9	20±3.5	42±8.5	30±12	10±0.6	334±89	346±47	282±119	12.5±2.47
10	296±20	>397	358±63	98±32	279±59	314±5.5	303±67	1.1±0.22
11	116±19	83±7.0	92±11	32±10	272±7.0	282±22	249±69	3.4±0.61
12	161±21	166±8.6	148±43	38±8.1	348±48	>371	>325	>2.8±0.53
13	131±23	97±18	115±21	27±6.1	274±18	232±59	172±119	2.6±0.53
14	329±17	363±38	282±77	>373	>400	>400	>384	><1.2±0.10
15	209±19	155±5.0	153±12	43±4.0	287±39	256±26	291±28	1.9±0.15
16	105±25	80±17	80±15	32±3.2	264±97	209±93	184±103	3.0±0.57
17	152±29	141±44	182±47	30±3.8	269±88	261±37	226±92	2.0±0.06
18	266±11	240±49	248±31	85±17	287±27	306±11	273±32	1.4±0.10
19	>389	>400	>400	255±23	>364	>400	>377	><1.0±0.06

Each value represents the mean ±S.D. from three independent experiments. TS, Tumor specificity.

μg/ml acridine orange for 20 minutes. Samples were then examined under Laser Scanning Microscope LSM510 (Carl Zeiss Inc., Gottingen, Germany) using the following filters: excitation filter 488 nm, emission filter 505-530 nm and >650 nm (32).

Statistical analysis. Significance of the difference of control and treated-samples was evaluated by Student's *t*-test.

Results

Cytotoxicity and tumor-specificity. We first investigated the cytotoxicity of nineteen TQ compounds (TQ1-TQ19) (Figure 1A, Table II). Among 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines (TQ1-3, TQ8-10), the substituents at the C-2 position (R³) seem to be important. TQ9 with a 3,4-dimethoxybenzoyl group showed the highest tumor-specific cytotoxicity (TS=12.5). Introduction of -CH₂OH (TQ5), trifluoroacetyl group (TQ11, TQ12, TQ13), -CH(OCOC₆H₄-4-Cl)CF₃ (TQ15), -CH(OCOC₆H₄-4-OMe)CF₃ (TQ16) or -CH(OCOBu^t)CF₃ group (TQ17) at the C-1 position (R²) gave intermediate tumor-specificity indices (TS=>2.0, 3.4, >2.8, 2.6, 1.9, 3.0, and 2.0, respectively).

We next investigated the cytotoxicity of nineteen TD compounds (TD1-TD19) (Figure 1B, Table III). All nine compounds (TD1-9) that have a -COOH group at C-1 (R²) showed much lower cytotoxicity and tumor-specificity (TS=1.0-1.3). On the other hand, TD11 with a -CH₂COOH group (R²),

Table III. Cytotoxicity of 1,2,3,4-tetrahydroisoquinoline derivatives (TD compounds) on human tumor and normal cells.

TD	CC ₅₀ (μM)							
	Human tumor cell lines				Human normal cells			
	HSC-2	HSC-3	HSC-4	HL-60	HGF	HPC	HPLF	TS
1	>400	>400	>400	>400	>400	>400	>400	><1.0
2	>400	>400	>400	>400	>400	>400	>400	><1.0
3	>400	>400	>400	>400	>400	>400	>400	><1.0
4	>400	>400	>400	>394	>400	>400	>400	><1.0
5	>400	>400	>400	342	>400	>400	>400	><1.0
6	>400	>400	>400	199	>400	>400	>400	><1.1
7	>400	>400	>400	>400	>400	>400	>400	><1.0
8	>365	>400	>386	131	309	330	>345	<1.0
9	>364	>376	>392	146	>400	>400	>400	><1.3
10	>400	>400	>400	>360	>400	>400	>400	><1.0
11	115	275	72	108	>400	>400	>400	>2.6
12	>400	>400	>400	>396	>400	>400	>400	><1.0
13	61	63	72	10	301	272	226	5.2
14	>354	>400	>400	88	>374	>382	>396	><1.2
15	68	64	83	16	120	134	108	2.1
16	>400	>400	>400	201	>400	>400	>400	><1.1
17	258	307	300	38	325	344	324	1.5
18	>353	>400	>390	112	336	>357	>355	<1.0
19	>353	>391	>373	84	314	317	315	1.0

Each value represents the mean of triplicate assays. TS, Tumor specificity.

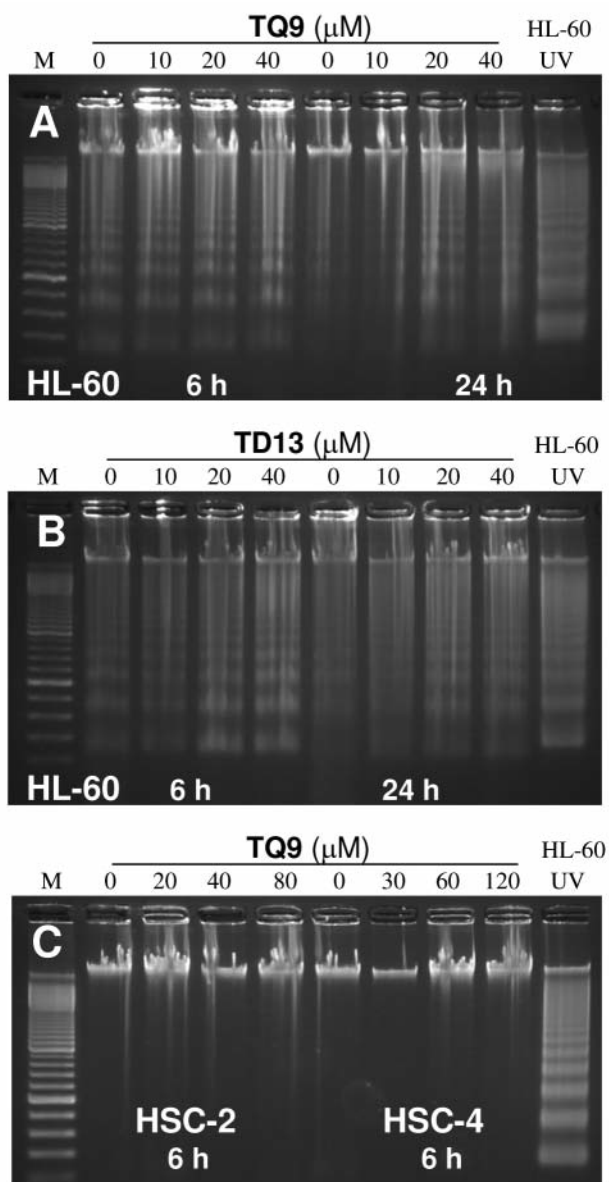


Figure 2. Induction of DNA fragmentation by two 2-aminotropolone derivatives. Near confluent HL-60 (A, B) or HSC-2, HSC-4 cells (C) were incubated for 6 or 24 hours with the indicated concentrations of **TQ9** (A, C) or **TD13** (B). DNA was then extracted and applied to agarose gel electrophoresis. M: Marker DNA. UV, DNA from the HL-60 cells exposed to 1-min UV irradiation (32).

and **TD13** and **TD15** with a $-\text{COOCH}_2\text{CH}_3$ group at C1 (R^2) showed higher TS values (TS = >2.6, 5.2 and 2.1, respectively).

Induction of apoptosis. Untreated control HL-60 cells spontaneously produced an internucleosomal DNA fragmentation pattern, suggesting relatively higher susceptibility of this cell line to apoptosis inducers. **TQ9** and **TD13** (10-40

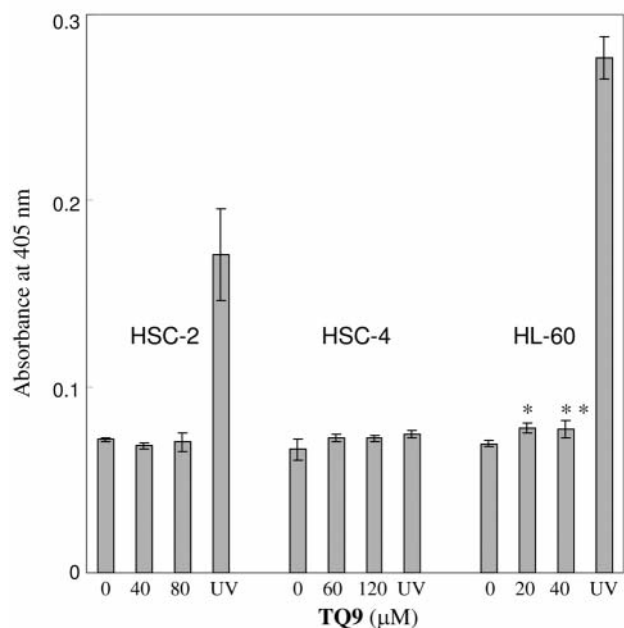


Figure 3. Effect of **TQ9** on caspase-3 activity. Near confluent HSC-2, HSC-4 and HL-60 cells were incubated for 6 hours with the indicated concentrations of **TQ9**, or exposed to 1 min UV irradiation followed by 6 hours incubation (32). Each point represents the mean \pm S.D. from triplicate assays. Significance between control and treated cells: * $p=0.046$, ** $p=0.137$.

μM) slightly enhanced the DNA fragmentation in HL-60 cells, but to a much lesser extent than that attained by UV irradiation (Figure 2A, B). **TQ9** failed to induce internucleosomal DNA fragmentation in HSC-2 and HSC-4 cells (Figure 2C).

TQ9 (20 μM) activated caspase-3 ($p < 0.05$) in HL-60 cells, but to much lesser extent than that achieved by UV irradiation (Figure 3). **TQ9** failed to activate the caspase-3 in both HSC-2 and HSC-4 cells (Figure 3). It is notable that UV irradiation activated caspase-3 to a much lesser extent in HSC-2 cells as compared to HL-60 cells, whereas it did not activate caspase-3 at all in HSC-4 cells (Figure 3).

Induction of autophagy. We next investigated whether **TQ9** induces autophagy in HSC-2 and HSC-4 cell lines. When these cells were incubated for 1 hour in Hank's balanced solution, acidic organelles (stained by acridine orange) became apparent (Figure 4D, H), confirming a previous report (34). Treatment of HSC-2 cells for 6 hours with **TQ9** (40, 80 μM) enhanced the production of acidic organelles (Figure 4B, C), as compared with control cells (Figure 4A). Untreated HSC-4 cells produced higher amounts of acidic organelles (Figure 4E). Treatment of HSC-4 cells for 6 hours with **TQ9** (120 μM) slightly enhanced the production of acidic organelles (Figure 4G). These data suggest that **TQ9** may induce autophagy in HSC-2 and HSC-4 cells.

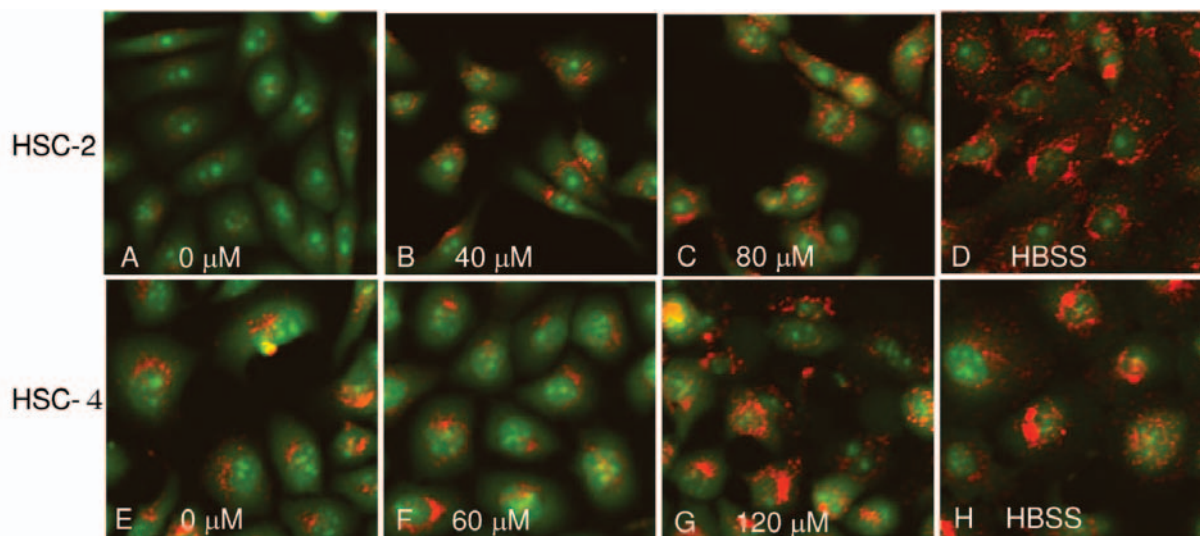


Figure 4. Induction of the production of acidic organelles by **TQ9**. HSC-2 (A-D) and HSC-4 cells (E-H) were incubated for 6 hours without (A, E) or with 40 (B), 60 (F), 80 (C), or 120 (G) μM **TQ9** in DMEM+10% FBS, or for 1 hour in Hank's balanced salt solution (D, H). The cells were stained with acridine orange for the detection of acidic organelles.

Discussion

The present study demonstrated that TIQ compounds with bulky substituents such as a 3,4-dimethoxybenzoyl group at C-2 (R^3) (**TQ9**), and an ethoxycarbonyl group at C-1 (R^2) and benzyloxycarbonyl group at C-2 (R^3) (**TD13**) showed the highest cytotoxicity and tumor-specificity (TS=12.5 and 5.3, respectively). This finding confirms a previous report that TIQ derivatives possessing bulky alkyl group substituents such as 1-cyclobutyl-, 1-cyclohexyl-, 1-phenyl, or 1-benzyl- at the C-1 position significantly showed cytotoxicity against PC12 cells (35). We also recently found a good correlation between the cytotoxicity of TQ compounds and their molecular size (such as surface area, volume and width), but not with other physicochemical descriptors (such as heat of formation, stability of hydration, dipole moment, electron affinity, ionization potential, highest occupied molecular orbital energy, lowest unoccupied molecular orbital energy, absolute hardness, molecular weight) (36).

The present study suggests that **TQ9** and **TD13** may induce little or no apoptosis in HL-60, HSC-2 and HSC-4 cells (Figure 2), but rather induce autophagy. Since acridine orange staining, however, is not a decisive method of autophagy identification, confirmation with more appropriate markers of autophagy is necessary. TIQ derivatives have been found to induce cell death *via* the decline of ATP level due to the mitochondria inhibition of complex 1, and the DNA damage (1) and inactivation of Cu,Zn-superoxide dismutase induced (37) by free radical formation. TIQ compounds have been reported to induce apoptosis in human leukemic cells

(11), dopaminergic neuroblastoma SH-SY5Y cells (38) and rat PC12 cells (39), but induced autophagy accompanied by the selective destruction of mitochondria in rat glioma cells (40). These data suggest that the type of cell death induced by TIQ derivatives depends on the cells investigated.

TIQ derivatives have well established multidrug resistance reversal activity (10-12) and synergistic effect with adriamycin and cisplatin (11). Since **TQ9** showed the highest cytotoxicity and tumor specificity, it remains to be investigated whether **TQ9** and popular antitumor agents exert synergistic effects.

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