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ORIGINAL PAPER

# Biological activity and molecular docking studies of some new quinolines as potent anticancer agents

Tuğba Kul Köprülü[1] · Salih Ökten[2] · Vildan Enisoğlu Atalay[3] · Şaban Tekin[4] · Osman Çakmak[5]

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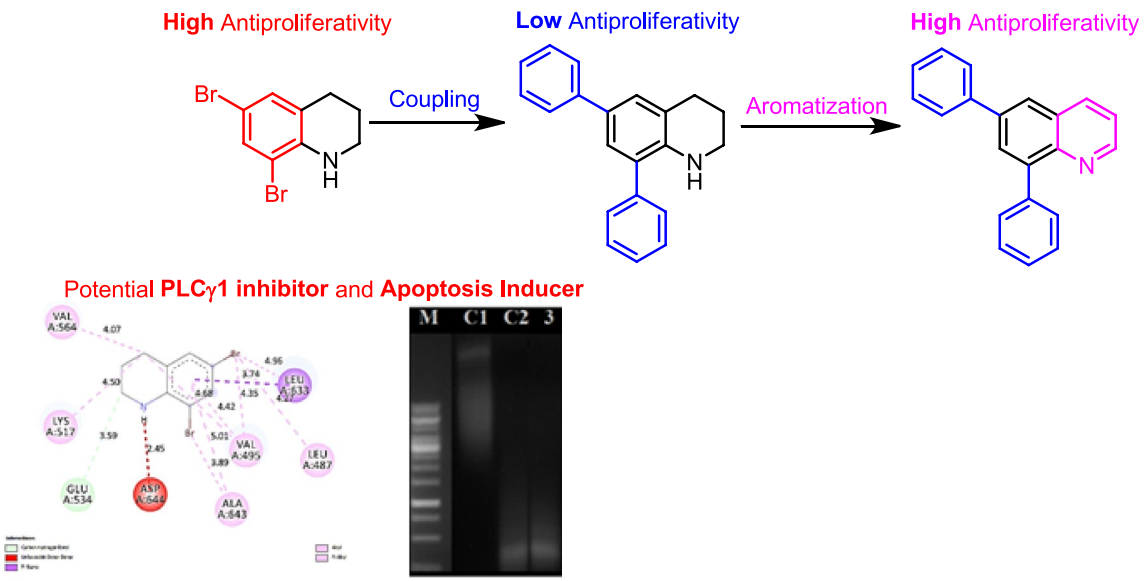
## Abstract

The objective of this study is to investigate the antiproliferative and cytotoxic properties and the action mechanism of substituted quinoline and tetrahydroquinolines **3**, **4**, **5**, **7**, and **8** against rat glioblastoma (C6), human cervical cancer (HeLa), human adenocarcinoma (HT29) cancer cell lines by BrdU Cell Proliferation ELISA, Lactate Dehydrogenase, DNA laddering and Topoisomerase I assays. The results of the study showed that 6,8-dibromotetrahydroquinoline **3** possess in vitro antiproliferative activity against C6, HeLa, and HT29 cell lines while morpholine/piperazine substituted quinoline **7** and **8** showed selective antiproliferative activity on C6 cell line with $IC_{50}$ values 47.5 and 46.3 µg/mL, respectively. Moreover, 6,8-dibromoTHQ **3** caused DNA fragmentation while it did not inhibit the Topoisomerase I (Topo I) enzyme. On the other hand, compound **8** did not cause DNA laddering while **8** inhibited the Topo I enzyme. According to these results, 6,8-dibromoTHQ **3** stimulates apoptosis on the C6 cell line while 6,8-dibromo-3-morhonilylquinoline (**8**) inhibits the Topo I enzyme to cause antiproliferative activity.

## Graphic abstract




**Keywords** Quinoline · Tetrahydroquinoline · Anticancer activity · Cytotoxicity · Molecular docking

✉ Tuğba Kul Köprülü
tugbakul.koprulu@sbu.edu.tr

✉ Salih Ökten
salihokten@kku.edu.tr

Extended author information available on the last page of the article

## Introduction

The aromatic heterocycles display physicochemical properties with relevance in the design of new drug candidates. Especially, nitrogen-containing heterocycles have drawn

the attention for a long time due to their broad range of biological and pharmacological properties [1, 2]. Among heterocyclic compounds, quinolines play important roles in the development of new drugs and are a very useful scaffold for the design of promising versatile drug candidates [3–5]. The bioactive quinolines substituted with different groups exhibit diverse pharmacological properties such as antimalarial [6], antibacterial [5, 7], antifungal [8], anticonvulsant [9], anti-inflammatory [10], antiprotozoal [11] anticholinesterase [5, 12] and antiviral [13]. The quinoline is also the effective structure for some important anticancer drug candidates [14–16]. Moreover, biologically natural, semi-synthetic, and synthetic active molecules based on many quinoline scaffolds have high antiproliferative and antitumor properties through various mechanisms of action such as cell cycle arrest, apoptosis, disruption of cell migration, inhibition of angiogenesis [2, 15, 17, 18]. Several drugs containing the quinoline ring motif such as Topotecan, Camptothecin, Irinotecan, and Belotecan are used in clinical treatment as anticancer agents [19]. The natural alkaloid Camptothecin and its semi-synthetic analog Topotecan are two examples of cytotoxic quinoline with the antitumor property through inhibition of DNA enzyme topoisomerase I [20]. Numerous quinoline derivatives were reported as anticancer agents through variable mechanisms like tubulin inhibition [21], carbonic anhydrase inhibition [22] cMet kinase inhibition [23], VEGFR inhibition [24], increase in the protein expression of Bad, Bax and decrease in Bcl-2, and PARP with apoptotic cell death [25].

Due to many of the substituted quinolines that have been used in medical applications, the synthesis of novel quinoline derivatives has attracted a lot of attention from researchers. Quinolines containing different groups have been extensively studied by our group. As a result of the studies, the potential anticancer activity properties of some novel quinoline derivatives have been reported until date by our group [1, 2, 5, 26].

The inhibition of cholinergic and metabolic enzymes (acetylcholinesterase, cytosolic carbonic anhydrase and α-glucosidase enzymes) in the human body has an important role in cancer treatment. The cytotoxic chemotherapeutic agents have side effects like cholinergic syndromes. High dose usage of CPT-11, Camptothecin derivative, lead to cholinergic syndrome by direct interaction of the drug or its metabolites with acetylcholinesterase (AChE). However, in the literature, kinetic studies with Camptothecin bearing 4-piperidinopiperidine moiety indicated that this moiety was primarily responsible for AChE inhibition with the, the major determinant in the loss of enzyme activity [27]. Moreover, α-glucosidase in tumor cells is essential for the metastatic process through the cellular interaction with collagen type 1 and IV [28]. Metastasis was also inhibited by disturbing the carbohydrate structure on the surface of neoplastic cells. Glycosidase inhibitors also suppress the metastatic potential of malignant cells by perturbing synthesis of the correct carbohydrate arrangement [29]. On the other hand, there are many connections between carbonic anhydrase (CA) and cancer. Some CA isozymes are predominantly found in cancer cells [30, 31] and these enzymes can help maintain a physiological intracellular pH while simultaneously contributing to an acidic extracellular pH, leading to tumor cell survival. Thus, this acidic pH promote tumor cell invasion and metastasis [32]. CA inhibitors might provide useful tools for controlling the pH (im)balance of tumor cells and developing novel diagnostic or therapeutic applications for the management of some tumors in hypoxic cancers [33].

In this study, it was aimed to determine the cytotoxic and antiproliferative activities of recently synthesized morpholine/piperazine/phenyl substituted quinoline derivatives [12], showing significant inhibition against AChE, cytosolic carbonic anhydrase (hCA I and hCA II) and α-Glu, against some cancer cells and to reveal their mechanisms of action, due to that the inhibition of metabolic enzymes has an important place in cancer research and quinolines display a broad range of biological activity. In addition, the activity of the substituted quinoline derivatives was supported by molecular docking.

## Material and methods

### Synthesis of the quinoline compounds

This study was carried out with phenyl substituted tetrahydroquinoline and morpholinyl and piperazinyl quinoline derivatives (**5**) reported in our previous papers [4, 12, 34, 35]. In brief, the synthesis of 6-bromo-1,2,3,4-tetrahydroquinoline (**2**) and 6,8-dibromo-1,2,3,4-tetrahydroquinoline (**3**) via direct bromination in ionic conditions [27] and their phenyl substituted derivatives (**4** and **5**, respectively) via Suzuki Cross-coupling reactions were reported recently by our previous publication [4]. Also, 3-morpholinyl- (**7**) and 3-piperazinyl-6,8-dibromoquinoline (**8**) were prepared by treatment of 3,6,8-tribromoquinoline (**6**) and morpholine or piperazine under microwave radiation according to our reported procedure [12]. The isolated compounds (**2–5**, **7–8**) were fully characterized with a melting point, elemental analysis, FT-IR, $^{1}$H NMR, $^{13}$C NMR, HMBC spectroscopy in these papers [4, 12, 34, 35].

### Cell culture

The HeLa, HT29 and C6 cancer cell lines used in the study were obtained from the American Type Culture Collection (ATCC, Rockville, USA). HT29, HeLa, and C6 cell lines were maintained in Dulbecco's modified eagle's medium

(DMEM, Sigma-Aldrich), supplemented with 10% (v/v) fetal bovine serum (Sigma-Aldrich, Germany) and 2% (v/v) PenStrep solution (Sigma-Aldrich). At 80% confluence, cells were detached from the flasks using 4 mL of trypsin–EDTA (Sigma-Aldrich), centrifuged and the cell pellet resuspended with 4 mL supplemented DMEM. Stock solutions of the samples and 5-fluorouracil (5-FU) were prepared in sterile dimethyl sulfoxide (DMSO) and diluted with DMEM. The final concentration of dimethyl sulfoxide (DMSO, Sigma-Aldrich) was kept below 1% in all tests. Tests were carried out in triplicate for each experiment.

### BrdU cell proliferation ELISA (BCPE)

The antiproliferative effects of the quinolines were investigated against HT29, HeLa and C6 cancer cell lines using BrdU Cell Proliferation ELISA kit (Roche, USA), a colorimetric immunoassay based on BrdU incorporation into the cellular DNA, according to manufacturer's protocol. A Cell suspension containing $3 \times 10^3$ cells in 100 µL was pipetted into wells of 96-well cell culture plates (COSTAR, Corning, USA). The cells were treated with test compounds and 5-FU at final concentrations of 5, 10, 20, 30, 40, 50, 60, and 75 µg/mL. The final volume of the wells was adjusted to 200 µL by supplemented DMEM and was incubated at 37 °C with 5% $CO_2$ overnight. Cells were exposed to BrdU labeling reagent for 4 h followed by fixation in FixDenat solution for 30 min. at room temperature. Then, cells were cultured with 1:100 diluted anti-BrdU-POD for 90 min. at room temperature, substrate solution was added to each well and BrdU incorporation was measured at 450–650 nm using a microplate reader (BioTek, Epoch). The $IC_{50}$ of the test and control compounds was calculated using XLfit5 software (IDBS) and expressed in µg/mL at 95% confidence intervals.

### Lactate dehydrogenase assay

The release of lactate dehydrogenase (LDH) is an indicator of loss of membrane integrity and cell injury. LDH assay was performed using LDH Cytotoxicity Detection Kit (Roche, USA) standard protocols. Seed cells in a 96-well flat-bottom microtiter plate at a density of 5000 cells/well in 100 µL of culture medium. The cells were treated with test compounds and 5-FU at final concentrations of $IC_{50}$. The final volume of the wells was adjusted to 200 µL by supplemented DMEM. The cells then were incubated at 37℃ with 5% $CO_2$ overnight and were measured at 492–650 nm using a microplate reader (BioTek, Epoch). The percentage of LDH released was calculated as follows: (LDH activity in media) / (LDH activity in media + intracellular LDH activity) × 100.

## DNA laddering assay

The DNA laddering effect of the quinoline compounds was determined using Gong's method [36] HT29 cells ($7.5 \times 10^5$ cells/well) were seeded in 25 $cm^2$ culture flasks at 37 °C in DMEM and allowed to attach overnight. After then, the cells were treated with $IC_{50}$ concentrations of **3**, **7**, and **8** compounds; the control group is represented by untreated cells, incubated only with a cell culture medium. On the second day, the cells are fixed using 70% ethanol, DNA is extracted with 0.2 M phosphate-citrate buffer at pH 7.8, and the extract is sequentially treated with RNase A and proteinase K and then subjected to electrophoresis.

### Topoisomerase I inhibition

To investigate whether quinolines **3**, **7**, and **8** are topoisomerase inhibitors, we tested these quinolines with a topoisomerase I assay kit (TG1015-2; TopoGen, Buena Vista, CO). The topoisomerase I assay is specific for measuring Topo I activity because it is based on the relaxation of supercoiled pHOT1 plasmid DNA after incubation of the Topo I enzyme. The supercoiled pHOT1 plasmid DNA was used in 20 µL reaction buffer containing 1 unit of human Topo I enzyme [37]. The $IC_{50}$ concentrations of quinolines **3**, **7**, and **8** and positive control compound (Table 2), Camptothecin (CPT) are added in the prepared reaction mixture, respectively. After 30 min of incubation at 37 °C, the reaction was stopped by adding equal volumes of stop solution. Then the reaction mixtures were analyzed on a 1% agarose gel by running 40 V for 60 min in TBE buffer. The gel was stained with ethidium bromide (1 mg/mL) and observed under UV illumination (UVP Biospectrum, Germany).

### Wound healing assay

C6 cells were seeded at a density of $3.5 \times 10^4$ cells /well in a culture-insert (ibidi culture-insert 2, ibidi GmbH, Germany) well for wound healing assay. After allowing the cells to attach overnight, the insert was removed and added 2 mL of fresh DMEM. Cells were treated with $IC_{50}$ concentration of **3** at 37 °C with 5% $CO_2$ and then cells were taken photographed using an inverted microscope (Leica DMIL, Germany).

### Statistical analysis

Results are expressed as mean ± standard deviation (SD) for at least three independent determinations. For the statistical analyses, the Statistical Package for Social Sciences was used, and standard deviation, the P-value was calculated using means, one-way analysis of variance. $P < 0.05$ was considered as significant difference.

### Geometry optimizations

In this study, a conformational search to determine the stable structure of the new synthesized five compounds was performed with a semi-empirical PM3 method [38] using the program Spartan'04 [39]. The obtained most stable conformer structures for all new synthesized molecules were optimized with a semi-empirical PM6 method [40] using the Gaussian09 suite of programs [41].

### Molecular docking procedure

In all the molecular docking studies PDB ID: 5EG3 code was selected as the crystal structure of PLCγ1, AutoDock4.2 [42, 43] and Auto Dock Tools (ADT) software were used to calculate the binding energies in the docking studies. The grid box was formed such that the active site amino acids of the proteins were in the cube measured at $40 \times 40 \times 40$ Å$^3$, each docking run was repeated 3 times.

## Results

### Chemistry

Due to that *N*-function heterocycles displayed a wide range of pharmaceutical features, phenyl substituted tetrahydroquinolines and piperazine/morpholine substituted quinolines were prepared according to our recent articles [4, 12, 35]. 6-phenyl-1,2,3,4-tetrahydroquinoline (**4**) and 6,8-diphenyl-1,2,3,4-tetrahydroquinoline (**5**) were prepared via Suzuki–Miyaura cross-coupling reactions between corresponding bromo tetrahydroquinolines (**2**, **3**) and phenylboronic acid in presence of Pd-catalyst (Fig. 1) [4]. On the other hand, the treatment of 3,6,8-tribromoquinoline (**6**) and morpholine or piperazine under microwave radiation were afforded 3-morpholinyl- (**7**) and 3-piperazinyl quinolines (**8**) in moderate yields [35].

### Anticancer activity studies

The metabolic enzyme inhibition activities of **7** and **8** against several metabolic enzymes (AChE, BChE, α-Glu, and hCA II) were determined by Ellman's method and esterase assay [12]. The results show that these compounds significantly inhibited metabolic enzymes in the nM concentration scale [12]. These results encourage us to reveal antiproliferative and cytotoxicities of substituted activities.

### Antiproliferative activities of compounds

Recent studies, reported by our research group displayed antiproliferative activities of substituted quinolines against certain cancer cell lines using sulforhodamine-B stain (SRB), BCPE, and MTT assays [1, 2, 5, 14].

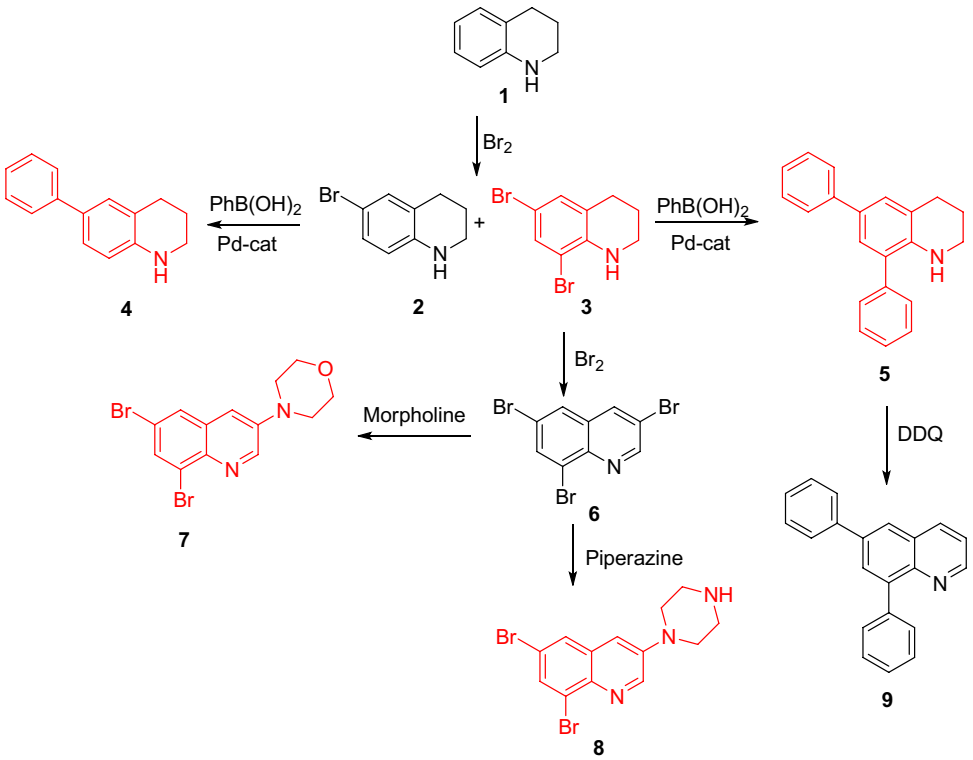



**Fig. 1** The structures of quinoline analogues, **3**, **4**, **5**, **7**, and **8**

**3** showed high inhibition activity against all cancer cell lines (HeLa, HT29, and C6) while **7** and **8** inhibited significantly the proliferation of only C6 cell lines in higher concentration (≤ 30 µg/mL) compared with the control drug, 5-FU (Fig. 2). However, the other test compounds **4** and **5** did not show any inhibition potency against any cell lines (Fig. 2).

### Cytotoxicity by LDH assay

It is an important situation for compounds having a high antiproliferative activity to have minimal toxicity against normal cells. Thus, the cytotoxic potentials of the compounds were determined using a lactate dehydrogenase (LDH) assay, indirectly demonstrating membrane damage [5]. The LDH catalyzes the interconversion of pyruvate and lactate and leaks from the cell inside to extracellular space when the cell membrane is damaged.

The results of cytoplasmic LDH activity were showed that the cytotoxicities of **3**, **7**, and **8** were lower, especially compound **5** (8%), than 5-FU at their $IC_{50}$ concentrations against HT29 cell lines (Fig. 3). **3** and **8** have lower LDH release percentages (27–33%) compared with the control drug (34%) while **7** has higher cytotoxicity (62%) (Fig. 3). On the other hand, **3** and **8** for C6 cell lines cause approximately 16–18% membrane damage at their $IC_{50}$ concentrations. However, **7** has a high LDH percentage (41%) against C6 cell lines (Fig. 3).

### The evaluation of apoptotic potential, topoisomerase I inhibition and cell migration

To evaluate the apoptotic potentials of **3**, **7**, and **8** on the C6 cell line, the DNA laddering assay was used. DNA laddering assay tests whether the mechanism of antiproliferative and cytotoxic activity of **3**, **7**, and **8** involved apoptosis or not. Apoptotic DNA fragmentation of **3** was observed as a DNA laddering pattern at 180–200 bp intervals indicating the DNA damage (Fig. 5a). It was determined that **7** and **8** were not apoptotic, but **3** was induced apoptosis in the C6 cell line.

Then, the inhibition activity of **3**, **7**, and **8** on Topo I enzyme with relaxation assay was investigated on supercoiled DNA using agarose gel electrophoresis. As illustrated in Fig. 4, supercoiled pHOT1 DNA was relaxed by incubation with Topo I (Lane C1) while the control drug CPT, a quinoline derivative, displays anticancer activity as an inhibitor of DNA Topo I inhibited relaxation of supercoiled DNA (Lane C2). **8** inhibited the Topo I enzyme (Lane 8). However, **3** and **7** did not inhibit the Topo I enzyme.

Cell migration is key procedure involved in many biological processes and the wound healing assay is generally performed to determine the growth and migration ability of cancer cells in two dimensions. The ability of cancer cells to metastasize makes them a clear target of anticancer drugs. Wound healing assay was applied to observe the effect of **3** on cell migration and cells were photographed at the start of the experiment (0 h) and 18 h later. It is observed that **3** has not migration inhibitory effect (Fig. 5b).

### Molecular docking studies

Phospholipase C gamma 1 (PLCγ1) is overexpressed in many metastatic tumors [44, 45]. The inhibition of PLCγ1 in nude mice was reported to essentially suppress lung metastasis [5, 45]. The small molecule binding site of the PLCγ1 complex is in the nSH2 domain that binds the phosphorylated tyrosine 766 (pV766) residue of the tyrosine kinase domain of growth factors [5, 46]. As seen in Fig. 6, 3, 7, and 8 suitably and favorably docks into the FGFR2 binding site of PLCγ1.

## Discussion

Quinoline derivatives are attributed to promising drug candidates showing biological activity in a wide range, especially anticancer activity [2, 5, 18, 35]. In a recent report 6,8-dibromo-tetrahydroquinoline (**3**), showed significant antiproliferative activities against several cancer cell lines, HeLa, Hep3B, MCF7 and HT29 with $IC_{50}$ values ranging 4.3 and 21.8 µg/mL according to the MTT proliferation assay [5]. Also, in the first report of anticancer activity of **3**. Its inhibitory activity of proliferation of HT29, C6, and HeLa cancer cell lines was determined at 30 µg/mL and higher concentrations by BCPE assay [14].

In this study, the antiproliferativity of dibromotetrahydroquinoline **(3)** was reworked against HeLa, HT29, and C6 cell lines to compare the proliferation inhibitory effects of its corresponding derivatives bearing phenyl groups and to investigate its action mechanism. According to BCPE assay results, 6,8-dibromo-tetrahydroquinoline (**3**) inhibited the proliferation of each three types of cancer cell lines at low concentrations ($IC_{50}$ values ranging 100.0 and 144.8 µM, Table 2) compared with 5-FU. These results are confirmed in previous reports [5, 14]. 6,8-diphenylTHQ **5**, derived from 6,8-dibromoTHQ **3** and 6-phenyltetrahydroquinoline (**5**) derived from monobromo isomer of compound **3** did not show any antiproliferative activity against tested cell lines HeLa, HT29, and C6. However, the significant antiproliferative effects of the aromatic form of 6,8-diphenylTHQ **5**, 6,8-diphenylquinoline were reported against HeLa, HT29, and C6 at $IC_{50}$ values 77.6–119.5 µM [2, 26]. **9** has high cytotoxicity (48–82%), except for HeLa (3%) [2]. On the contrary, the cytoplasmic LDH assay showed that 6,8-dibromoTHQ (**3**) led to the release of LDH out of the cells at a low

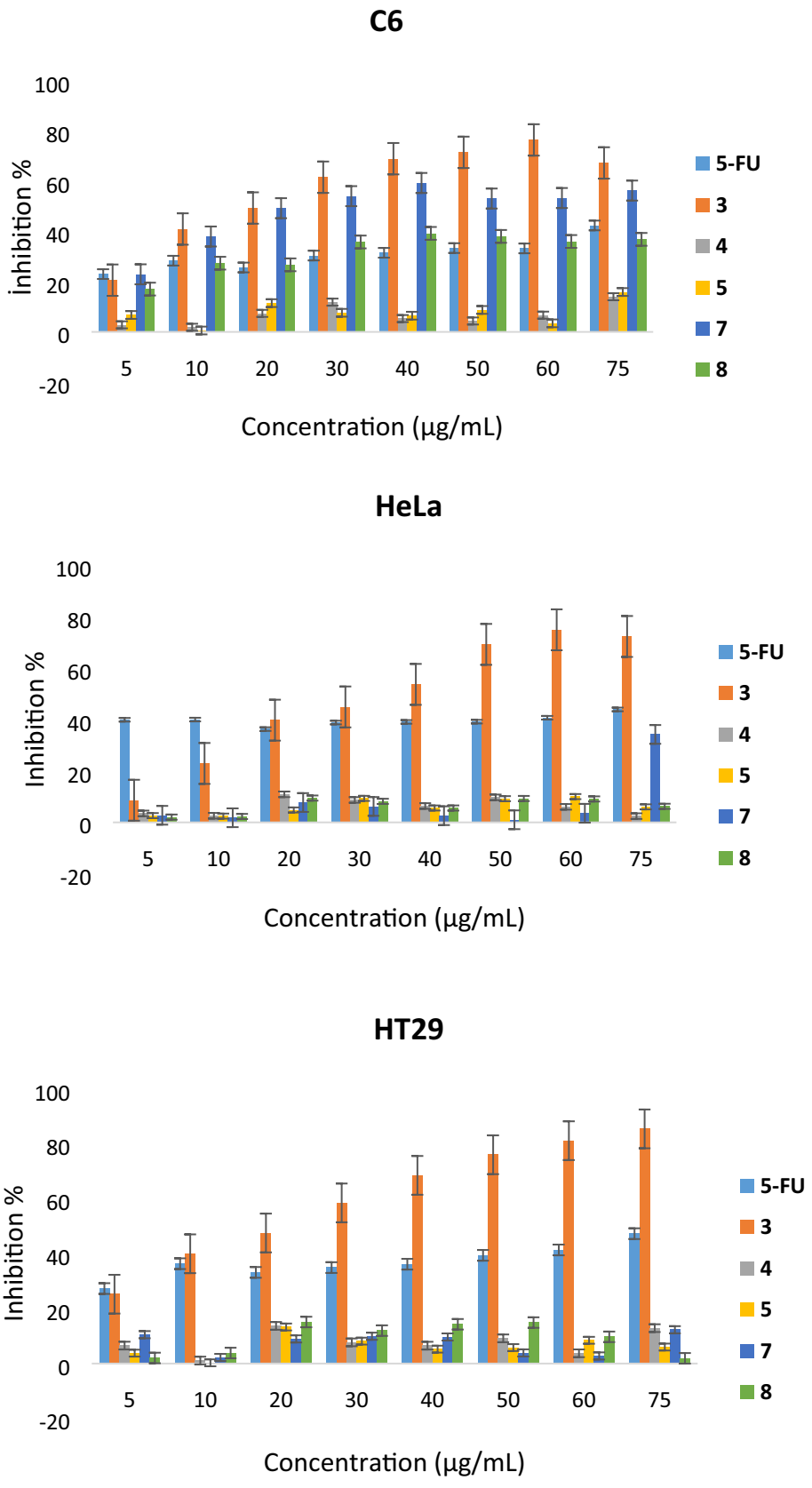



**Fig. 2** The antiproliferative activity of quinolines against C6, HeLa, and HT29 cancer cell lines in vitro using the BCPE assay. The cells were treated with eight different concentrations of quinoline compounds ($P < 0.05$)

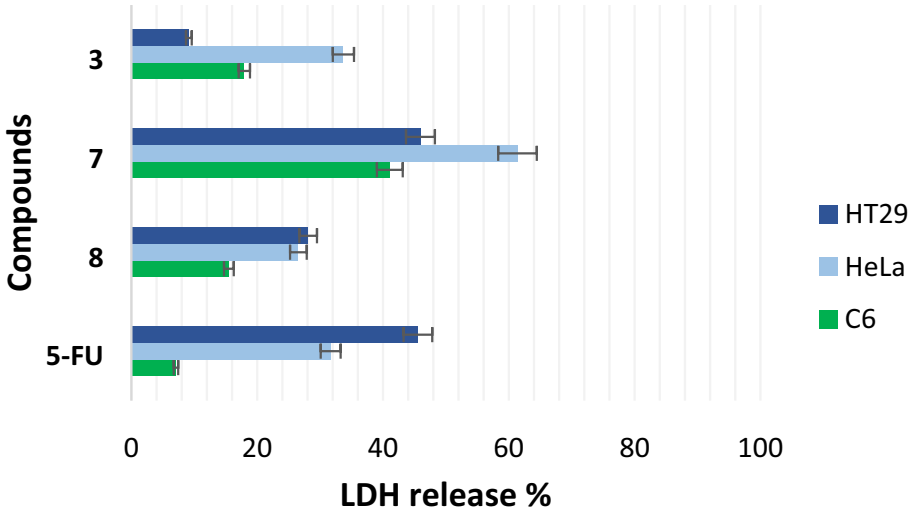



**Fig. 3** The cytotoxic activity of **3**, **7**, and **8** at their $IC_{50}$ concentrations on C6, HeLa, and HT29. The LDH cytotoxicity test showed a significant increase ($P < 0.05$) in the cytotoxicity of cells. Percent cytotoxicity was reported as mean values ± SD of three independent assays

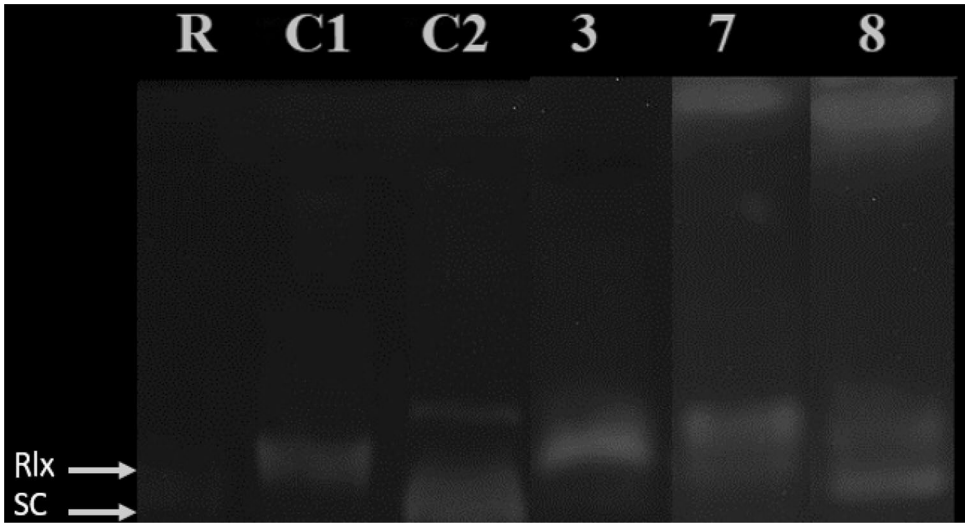



**Fig. 4** The inhibition of human topoisomerase I (topo I) by **3**, **7**, and **8**. C1, supercoiled DNA + Topo I; C2, supercoiled DNA + Topo I + CPT; CPT: camptothecin; Lane 3, DNA + Topo I + compound **3**; Lane 7, DNA + Topo I + compound **7**; Lane 8, DNA + Topo I + compound **8**; R, relaxed pHOT-1 DNA marker; Rlx, relaxed DNA; SC, supercoiled DNA

percentage (9–33%), especially in the HT29 cell line (9%) compared with 5-FU (45%). According to these results, it can be concluded that phenyl groups bounded to 1,2,3,4-tetrahydroquinoline ring at C-6 or C-6 and C-8 dramatically decrease the antiproliferative activity. On the other hand, the phenyl derivative **5** can probably show high inhibition of cell proliferation if this compound is aromatized.

The results of DNA laddering, Topo I and cell migration assays show that 6,8-dibromoTHQ **3** caused DNA fragmentation while it did not inhibit the Topo I enzyme and cell migration. On the contrary, its aromatized from **9** bearing phenyl groups at C-6 and C-8 positions could not induce the apoptosis detected by DNA laddering assay but it inhibited Topo I enzyme [2]. According to these results, 6,8-dibromoTHQ **3** stimulates apoptosis on the C6 cell line while 6,8-diphenylquinoline (**9**) inhibits the Topo I enzyme to cause antiproliferative activity.

In the LDH assay, **7** and **8** exhibited high cytotoxic effect against C6, HeLa, and HT29 cancer cell lines (Fig. 3). The results showed that **7** and **8**, especially **7**, significantly inhibited C6 cells by BCPE assay through induction of necrotic cell death.

The morpholine and piperazine substituted at C-3 quinoline derivatives (**7** and **8**) displayed an antiproliferative effect against only the C6 cell line ($IC_{50}$ values 125.2 and 128.7 µg/mL, respectively). The LDH assay displayed that **8** causes approximately 17% membrane damage at its $IC_{50}$ concentration. However, morpholine substituted quinoline led to cytotoxicity (42%, LDH release) at the C6 cell line. If **7** and **8** are compared with 5-FU, the toxicity of **8** is very close to the cytotoxicity value of 5-FU.

According to Table 1, the most interacted residues in the active site of the PLCγ1 are VAL495, ALA515, LYS517, GLU534, VAL564, TRY566, and LEU633, on the contrary MET538 aminoacid did not show enough interactions

with the molecules. On the other side, while the best binding energy was obtained for **3** with an energy value of 8.1 kcal $mol^{-1}$, the weakest energy was obtained for **7** with an energy value of 7.7 kcal $mol^{-1}$ compound **7** gave low binding energy due to weak interactions with all amino acids except MET538. The binding of **3**, **7**, and **8** are highly likely to prevent LEU484, VAL595, LYS517, GLU534, VAL564, TRY566, ALA567, LEU533, and ALA643 the residues FGFR2 to conduct its kinase activity on PLCγ1 (Table 1). Therefore, **3**, **7**, and **8** could represent a potential drug candidate which should be further investigated for metastatic cancer treatment.

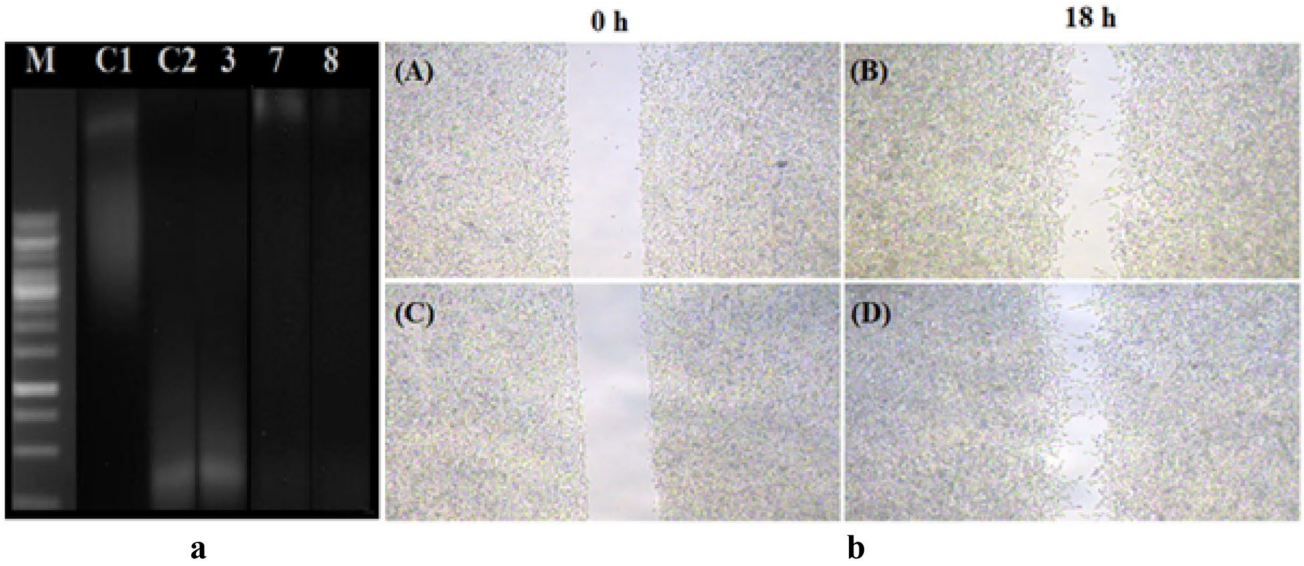



**Fig. 5** **a** Agarose gel electrophoresis for detecting DNA fragmentation in C6 cell line (right). M: 1 kb DNA marker, C1 (control, untreated C6); C2 (positive control, C6 + CPT); Lane 3 (C6 + compound **3**), Lane 7 (C6 + compound **7**), Lane 8 (C6 + compound **8**), **b** Representation of the migration effect of compound **3** (left). (A) Control, untreated C6 cells; (B) untreated C6 cells after 18 h (C) C6 cells + compound **3** (D) C6 cells + compound **3** after 18 h

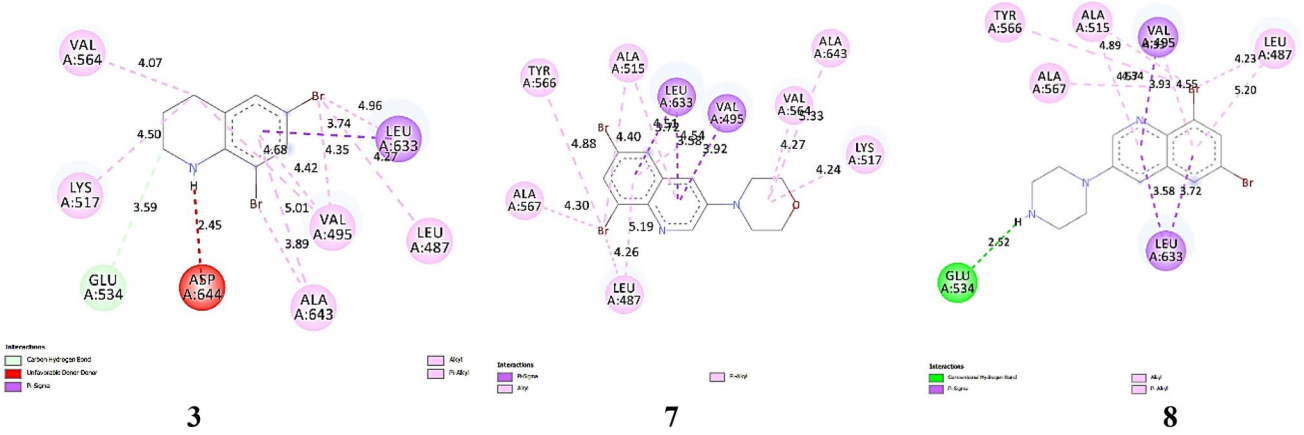



**Fig. 6** Docking poses of the active inhibitors of the target protein

**Table 1** Binding Energy Values (BE) (Kcal $mol^{-1}$) and interacted aminoacid with the new synthesized five molecules

| Compound | BE | LEU 487 | VAL 495 | ALA 515 | LYS 517 | GLU 534 | MET 538 | VAL 564 | TYR 566 | ALA 567 | LEU 633 | ALA 643 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **3** | − 8.1 | + | + | | + | + | | + | | | + | + |
| **7** | − 7.7 | + | + | + | + | + | | + | + | + | + | + |
| **8** | 7.9 | + | + | + | | + | | | + | + | + | |

**Table 2** $IC_{50}$ (μM) values of compounds **3**, **7**, and **8**

| Compound | C6 | HeLa | HT29 |
|---|---|---|---|
| **3** | 100.0 ± 7.8 | 144.8 ± 12.7 | 117.6 ± 8.2 |
| **7** | 125.2 ± 10.9 | > 1000 | > 1000 |
| **8** | 128.7 ± 12.1 | > 1000 | > 1000 |
| **5-FU** | 163.0 ± 11.3 | 469.6 ± 37.6 | 501.2 ± 45.4 |

## Conclusion

Recently synthesized phenyl, morpholine, and piperazine substituted quinoline and tetrahydroquinoline analogs were tested for their antitumor activities and their mechanism of action in vitro against three types of cancer cell lines. The piperazine and morpholine substituted quinoline bromides **7** and **8** have selective antiproliferative effect against the C6 cell line with $IC_{50}$ values 125.2 and 128.7 μM, respectively (Table 2). While monophenyl- **4** and diphenyl- **5** tetrahydroquinoline derivatives did not show any anticancer activity. However, the starting material of diphenyl tetrahydroquinoline (**5**), 6,8-dibromotetrahydroquinoline **3** ($IC_{50}$ values ranging 100–144 μM in Table 2), and its aromatized form **9** have both significant antiproliferative effect and lower cytotoxicities. Moreover, the docking study for anticancer activity suggested that especially **3** represents a potential antitumor drug candidate for metastatic cancer treatment due to its potential to suppress the PLCγ1.

**Author contributions** All authors were involved in research design and data collection. Köprülü and Ökten conducted data analysis and drafted manuscripts. Tekin and Çakmak revised the manuscript.

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## Authors and Affiliations

**Tuğba Kul Köprülü[1] · Salih Ökten[2] · Vildan Enisoğlu Atalay[3] · Şaban Tekin[4] · Osman Çakmak[5]**

[1] Hamidiye Vocational School of Health Services, Department of Medical Services and Techniques, Medical Laboratory Techniques, University of Health Sciences, Üsküdar, Istanbul, Turkey

[2] Faculty of Education, Department of Maths and Science Education, Division of Science Education, Kırıkkale University, Yahşihan, Kırıkkale, Turkey

[3] Health Services Vocational School, Department and Plant and Animal Production, Medicinal and Aromatic Plants, Üsküdar University, Üsküdar, Istanbul, Turkey

[4] Hamidiye Faculty of Medicine, Department of Basic Medical Sciences, Medical Biology, University of Health Sciences, Üsküdar, Istanbul, Turkey

[5] Faculty of Arts and Design, Department of Gastronomy, Istanbul Rumeli University, Silivri, 34570 Istanbul, Turkey