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RESEARCH ARTICLE

A SAR Study: Evaluation of Bromo Derivatives of 8-Substituted Quinolines as Novel Anticancer Agents

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Abstract: Brominated 8-hydroxy, 8-methoxy, 8-amino quinolines **5**, **6**, **8**, **9** and novel cyano 8-hydroxyquinolines **11**, **12** were evaluated *in vitro* for their anticancer effects on various cell lines. 5,7-Dibromo- **5**, 7-bromo- **6**, 7-cyano- **11** and 5,7-dicyano-**12** 8-hydroxyquinolines were shown to have strong antiproliferative activity against various tumor cell lines, including C6 (rat brain tumor), HeLa (human cervix carcinoma), and HT29 (human colon carcinoma) with IC_{50} values ranged from 6.7 to 25.6 µg/mL. A structure activity relationship (SAR) was conducted that quinoline core containing hydroxly group at C-8 positon led to more anti cancer potentials. The results of Lactate Dehydrogenase (LDH) cytotoxic, DNA laddering and inhibition assays indicated that **5**, **6**, **11** and **12** have high cytotoxic effects and appototic potentials. Furthermore, **5** and **12** have inhibitory effects on relaxation of supercoiled plazmid DNA by supressed the Topoisomerase I enzyme. As a result, **5**, **6**, **11** and **12** may have promising anticancer drug potential and **5** and **12** may be novel topoisomerase inhibitors.

Keywords: SAR, bromination, hydroxyquinoline, methoxyquinoline, cyanoquinoline, anticancer effect, cytotoxicity, antitopoisomerase.

1. INTRODUCTION

In drug design and development, a great many studies have been reported evaluating biologically important heterocyclic "privileged structures" as promising drugs or candidates [1-4]. 8-hydroxyquinoline (**1**, 8-OHQ) and its derivatives represent excellent scaffolds with a wide spectrum of pharmacological and biological activities, such as metal-chelators for neuroprotection [5], anti-HIV agent [6], antifungal agent [7], antibacterial agent [8], anticancer agent [9-11], antimalarial agent [12], antiparasitic amoebic dysentery agent [13] and many others. Due to the indispensable role of quinoline and its analogs in pharmacology, great number of methods were developed for their synthesis, especially 8-substituted quinolines **2-4** (Fig. **1**), such as hydroxy-, methoxy-, amino-, and halogen-substituted quinolines.

Selective bromination of organic molecules, especially aromatics, has gained significant commercial importance in the last few decades. The bromo-substituted quinolines possess a variety of biological activities and also serve as precursors and starting materials for numerous compounds used as pharmaceuticals, organochemicals, and various specialty industrial compounds [14, 15]. Bromoquinolines can undergo easily metal-halogen exchange, couplings and metal assisted substitutions to convert into corresponding quinoline derivatives having medicinal importance. In our previous works, we studied anticancer activity of quinoline analogs and found that 6,8-dibromo-1,2,3,4-tatrahydroquinoline (6,8-DiBrTHQ) has remarkably potent antiproliferative activity

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2 X= OH
3 X= OCH_3
4 X= NH_2

Fig. (1). Structures of quinoline and 8-substituted quinolines.

against HeLa, HT29 and C6 cell lines [14, 16]. Then we synthesized brominated methoxy, hydroxy derivatives (SO18, SO22 and SO29) [17-18] starting with 6,8-DiBrTHQ and determined their high anticancer and cytotoxic activities with concentrations at about 5 µg/mL in each cell lines. Thus, our interests focused 8-substituted (OH, OCH_3, and NH_2) (**2-4**) quinolines based bromoquinolines due to their diverse pharmacological profiles. Although many experimental works have examined the bromination of these derivatives (**2-4**), it still remains an area of active research.

Recently, we have reinvestigated the bromination of 8-methoxy, 8-amino- and 8-hydroxyquinoline with different equivalents of molecular bromine. Also, we demonstrated regioselectivity on bromination of 8-substituted quinolines [19]. The work presented here is the continuation of our ongoing research [19]. In the present study, we describe the results of the structure activity relationship (SAR) of 8-substituted quinoline derivatives (**5**-**6**, **8**-**9**) with bromo groups as novel anticancer drug candidates and demonstrate both novel anticancer activity and mechanism of actions of several brominated 8-substituted quinolines (**5**-**6**, **8**-**9**). Moreover, novel cyano 8-hydroxyquinolines **11**, **12** were synthesized with copper assisted substitution reactions to estimate the effect of different substituents on biolological activity. To achieve these goals, first we prescreened antiproliferative activities of the compounds (**5**, **6**, **8**, **9**, **11** and **12**) against HeLa (human cervix carcinoma), HT29 (human colon carcinoma), C6 (rat brain tumor cells) cell lines by sulforhodamine B (SRB) cell proliferation assay. Then, BrdU cell proliferation assay against HeLa, HT29 and C6 cancer cell lines and L929 (Mouse fibroblast cell line) healthy cell line was performed to active compounds in SRB assay. Furthermore, LDH cytotoxic activity, DNA laddering effect and Topoisomerase I inhibitory activities were evaluated to understand the mechanism of actions of active compounds. Eventhough 7-bromo **6**, 5-bromo **8** and 5,7-dibromo **5**, **9** 8-substituted quinolines are known compounds, this is the first investigation revealing antiproliferative potential of **5** and **6** on several cancer cell lines *in vitro*.

2. MATERIALS AND METHODS

2.1. Materials

All the reagents and solvents for synthesis were commercially available. Thin layer chromatography was carried out on Merck silica F_{254} 0.255-mm plates, and spots were visualized by UV fluorescence at 254 nm. To isolate the products classic column chromatography was performed using Merck 60 (70-230 mesh) silica gel. Melting points were recorded on a Thomas-Hoover capillary melting point apparatus. Solvents were evaporated at reduced pressure. IR and NMR spectra were recorded on a JASCO 430 FT/IR instrument and a Bruker 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, respectively.

2.2. Chemistry

Genaral Procedure for Bromination of 8-Substituted Quinolines (2-4)

To synthesize brominated 8-substituted quinolines (**5**-**10**) experimental procedures were repeated in literature [19]. In brief, to a solution of 8-substituted quinoline **2**-**4** (2 mmol, 1 eq) in distilled $CHCl_3$ (15 mL) was added a solution of molecular bromine (different eqivalents) in $CHCl_3$ over 10 min in the dark at ambient temperature and stirred for 2 days. The reaction was monitored by TLC; after completion of the reaction, the organic layer was washed with 5% $NaHCO_3$ (3 × 20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The products was isolated by alumina column, eluting with AcOEt/hexane (1:5, 150 mL).

5,7-Dibromo-8-hydroxyquinoline (5)

Compound **2** (2 mmol, 1 eq) was reacted with molecular bromine (4 mmol, 2 eq) according to general bromination procedure for **2**-**4**. Yellow needles, yield: 90%; M.p.196-198 °C; ^{1}H NMR (400 MHz, $CDCl_3$) (δ/ppm): 8.89 (dd, J_{23}= 4.4 Hz, J_{24}= 1.2 Hz, 1H, H-2), 8.54 (dd, J_{42}= 1.2 Hz, J_{43}= 8.4 Hz, 1H, H-4), 7.96 (s, 1H, H-5), 7.65 (dd, J_{32}= 4.4 Hz, J_{34}= 8.4 Hz, 1H, H-3), 3.3 (s, 1H, -*OH*); ^{13}C NMR (100 MHz, $CDCl_3$) (δ/ppm): 149.7, 148.9, 138.6, 136.9, 134.1, 126.8, 123.0, 110.3, 104.8; IR (ν/cm^{-1}): 3066, 2921, 1581, 1563, 1490, 1457, 1396, 1365, 1332, 1270, 1201, 1133, 933, 871, 806, 784, 723, 649.

7-Bromo-8-hydroxyquinoline (6)

Compound **2** (2 mmol, 1 eq) was reacted with molecular bromine (3 mmol, 1.5 eq) according to general bromination procedure for **2**-**4**. Yield: 51%; M.p. 138-139 °C; ^{1}H NMR (400 MHz, $CDCl_3$) (δ/ppm): 8.83 (dd, J_{23}= 4.4 Hz 1H, H-2, J_{24}= 1.6 Hz,), 8.51 (dd, 1H, H-4, J_{42}= 1.2 Hz, J_{43}= 8.4 Hz), 7.73 (d, 1H, H-6, J_{65}= 8.4 Hz), 7.59 (dd, 1H, H-3, J_{32}= 4.4 Hz, J_{34}= 8.4 Hz,), 7.10 (d1 H, H-5, J_{56}= 8.4 Hz,), 3.3 (s, 1H, -*OH*).

5,7-Dibromo-8-methoxyquinoline (7)

Compound **4** (2 mmol, 1 eq) was reacted with molecular bromine (excessive eq) according to general bromination procedure for **2**-**4**. White needles, yield: 45%; M.p. 99-102 °C; ^{1}H NMR (400 MHz, $CDCl_3$): (δ/ppm): 9.00 (dd, J_{23}= 3.2 Hz, J_{24}= 1.6 Hz, 1H, H-2), 8.52 (dd, 1H, H-4, J_{43}= 8 Hz, J_{42}= 1.6 Hz), 8.02 (s, 1H, H-6) 7.58 (dd, 1H, H-3, J_{34}= 8.4 Hz, J_{32}= 3.2 Hz), 4.19 (s, 3H, *OCH_3*); ^{13}C NMR (100 MHz, $CDCl_3$) (δ/ppm): 153.3, 150.9, 143.8, 136.1, 133.7, 128.3, 122.5, 116.3, 116.5, 62.1 (*OCH_3*); IR (ν/cm^{-1}): 2919, 2850, 1733, 1600, 1578, 1490, 1462, 1383, 1370, 1353, 1086.

5-Bromo-8-methoxyquinoline (8)

Compound **3** (2 mmol, 1 eq) was reacted with molecular bromine (2 mmol, 1 eq) according to general bromination procedure for **2**-**4**. Brown solid, M.p. 80-82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) (δ/ppm): 8.89 8.91 (dd, 1H, H-2, J_{23}=

4.0 Hz, J_{24}= 1.2 Hz), 8.43 (dd, 1H, H-4, J_{43}= 8.4 Hz, J_{42}= 1.6 Hz), 7.66 (d, 1H, H-6, J_{67}= 8.4 Hz), 7.49 (dd, 1H, H-3, J_{32}= 4.0 Hz, J_{34}= 8.4 Hz), 6.87 (d, 1H, H-7, J_{76}= 8.4 Hz), 4.04 (s, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) (δ/ppm): 152.2, 149.7, 140.8, 135.5, 130.0, 128.1, 122.8, 111.8, 108.1, 56.2 (OCH_3); IR (ν/cm^{-1}): 2915, 2848, 1600, 1588, 1500, 1460, 1352, 1300.

5,7-Dibromo-8-aminoquinoline (9)

Compound **4** (2 mmol, 1 eq) was reacted with molecular bromine (2 mmol, 2 eq) according to general bromination procedure for **2-4**. Brown solid, yield: 99%; M.p. 118-120 °C; ^{1}H NMR (400 MHz, $CDC1_3$) (δ/ppm): 8.77(dd, 1H, H-2, J_{23}= 4.0 Hz, J_{24}= 1.2 Hz), 8.40 (dd, 1H, H-4, J_{42}= 1.2 Hz, J_{43}= 8.4 Hz), 7.80 (s, 1H, H-6), 7.51 (dd, 1H, H-3, J_{32}= 4.4 Hz, J_{34}= 8.4 Hz) 5.48 (bs, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) (δ/ppm): 148.4, 142.1, 138.4, 135.7, 133.2, 126.7, 122.5, 106.9, 103.2.

5-Bromo-8-aminoquinoline (10)

Compound **4** (2 mmol, 1 eq) was reacted with molecular bromine (3 mmol, 1.5 eq) according to general bromination procedure for **2-4**. ^{1}H NMR (400 MHz, $CDC1_3$) (δ/ppm): 8.78 (dd, 1H, H-2, J_{23}= 4.0 Hz, J_{24}= 1.2 Hz), 8.44 (dd, 1H, H-4, J_{42}= 1.2 Hz, J_{43}= 8.4 Hz), 7.60 (d, 1H, H-6, J_{67}= 8.0 Hz), 7.52 (dd, 1H, H-3, J_{32}= 4.0 Hz, J_{34}= 8.4 Hz), 6.83 (d, 1H, H-7, J_{76}= 8.0 Hz), 5.07 (bs, 2H, NH_2).

Genaral Procedure for Treatment of Bromo 8-Hydroxyquinolines (5-6) with CuCN

The similar procedure was applied in literature [20]. In brief, 7-bromo-8-hydroxyquinoline (**6**) and 5,7-dibromo-8-hydroxyquinoline (**5**) (1 eq) were dissolved individually in freshly distilled DMF (50 mL) and mixed with copper cyanide (2 eq for **6**, 4 eq for **5**). The reaction mixture was stirred at reflux (*ca*. 150 °C) under argon for 6 h. The resulting mixture was poured while still hot into a solution of hydrated ferric chloride (4 g) and concentrated hydrochloric acid (1 mL) in water (10 mL). The reaction mixture was maintained at 60-70 °C for 20 min to decompose the complex. The aqueous layer was extracted with warm toluene (4 × 50 mL), and the extracts were combined with the organic layer, washed with dilute hydrochloric acid (1:1, 25 mL), water (10 mL) and 10% aqueous sodium hydroxide (15 mL). The remaining organic layer was filtered to remove the insoluble matter and dried over Na_2SO_4. After evaporation of the solvent, NMR analysis of the residue indicated the formation of 7-cyano-8-hydroxyquinoline (**11**) and 5,7-dicyano-8-hydroxyquinoline (**12**).

7-Cyano-8-hydroxyquinoline (11)

Yield: 36%; M.p. 151-153 °C; ^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 10.14 (dd, J_{23}= 4.0 Hz 1H, H-2, J_{24}= 1.2 Hz,), 9.83 (dd, 1H, H-4, J_{42}= 1.2 Hz, J_{43}= 8.0 Hz), 9.14 (d, 1H, H-6, J_{65}= 8.2 Hz), 8.90 (dd, 1H, H-3, J_{32}= 4.0 Hz, J_{34}= 8.0 Hz,), 8.67 (d, 1H, H-5, J_{56}= 8.2 Hz,), 6.0 (s, 1H, -OH). IR (ν/cm^{-1}): 3428, 2931, 2254, 1733, 1652, 1452, 1401, 1043, 1022, 997, 823.

5,7-Dicyano-8-hydroxyquinoline (12)

Yield: 23%; M.p. 202-204 °C; ^{1}H NMR (400 MHz, DMSO-d_6): (δ/ppm): 9.38 (dd, J_{23}= 3.0 Hz, J_{24}= 1.1 Hz, 1H, H-2), 8.82 (d, 1H, H-4, J_{43}= 7.6 Hz), 8.74 (dd, 1H, H-3, J_{34}= 7.6 Hz, J_{32}= 3.0 Hz), 7.90 (s, 1H, H-5), 4.90 (s, 1H, -*OH*); IR (ν/cm^{-1}): 33979, 3060, 2208, 1600, 1652, 1494, 1454, 1386, 1247, 756.

2.2. Anticancer Studies

2.2.1. Cell Culture

C6 (rat brain tumor cells), HT29 (human colon cancer cells), HeLa (human cervical cancer cells) and L929 (Mouse fibroblast cells) cell lines were maintained in Dulbecco's Modified Eagle's medium (DMEM, Sigma), supplemented with 10% (v/v) fetal bovine serum (Sigma, Germany) and PenStrep solution (Sigma, Germany). Upon confluence, cells were detached from the flasks using 4 mL of trypsin-EDTA (Sigma, Germany), centrifuged, and the cell pellet was re-suspended with 4 mL of supplemented DMEM.

2.2.2. Sulforhodamine B (SRB) Cell Proliferation Assay

Prescreening of the compounds were performed by sulforhodamine B (SRB) cell proliferation assay according to the literature [21], with some modifications. Cell suspensions containing 3 × 10^3 cells in 100 mL were pipetted into the wells of 96-well cell culture plates (COSTAR, Corning, USA). Test compounds (**3**, **5**, **6**, **8** and **9**) and 5-FU were dissolved in sterile DMSO. The DMSO amount was adjusted to 0.5%. The cells were treated with **3**, **5**, **6**, **8**, **9** and 5-FU individualy at final concentrations of 5, 10, 20, 30, 40, 50, and 75 µg/mL. Cell controls and solvent controls were treated with DMEM+10% FBS and sterile DMSO, respectively. The final volume of the wells was adjusted to 200 mL with DMEM+10% FBS. Cells were incubated overnight and fixed with 300 µL of TCA (trichloroacetic acid), washed three times with 300 µL of ddH_2O, and dried at 37 °C for 2 h. Then, cells were stained with 0.4% (wt/vol) SRB dissolved in 1% acetic acid at 25 °C for 90 min, washed four times with 300 µL of 1% acetic acid to remove unbound dye, and dried completely. Bound dye was solubilized with 100 µL of 10 mM unbuffered Tris base (pH 10.5) for 5 min on a shaker. Absorbance was read at 492 nm using a microtiter plate reader (Ryto, China).

2.2.3. BrdU Cell Proliferation Assay

Cell suspensions containing 3 × 10^3 cells in 100 mL were pipetted into the wells of 96-well cell culture plates (COSTAR, Corning, USA). The cells were treated with **5**, **6**, **11**, **12** and 5-FU dissolved in DMSO at final concentrations of 5, 10, 20, 30, 40, 50, and 75 µg/mL. Cell controls and solvent controls were treated with supplemented DMEM and sterile DMSO, respectively. The final volume of the wells was adjusted to 200 mL by supplemented DMEM.

The cells were incubated at 37 °C with 5% CO_2 overnight. The antiproliferative activity of the compounds was determined using a BrdU cell proliferation ELISA Kit

(Roche, USA) and a calorimetric immunoassay based on BrdU incorporation into the cellular DNA, as described in the literature [22]. Briefly, cells were exposed to a BrdU labeling reagent for 4 h, followed by fixation in FixDenat solution for 30 min at room temperature. Afterwards, cells were cultured with 1:100 diluted anti-BrdU-POD for 1.5 h at room temperature, substrate solution was added to each well, and BrdU incorporation was measured at 450–650 nm using a microplate reader (Rayto, China). Each experiment was repeated at least three times for each cell line.

2.2.4. Calculation of % Inhibition and IC_{50}

BCPA assay results were reported as the percent inhibition of test and control substances. The percent inhibition was calculated according to the following formula: % inhibitions [1-(Absorbance of Treatments/Absorbance of DMSO)/ 100]. The half-maximal inhibitory concentration (IC_{50}) of the test and control compounds was calculated using XLfit5 software (IDBS) and expressed as $\mu g\ mL^{-1}$ with 95% confidence intervals.

2.2.5. Cytotoxic Activity Assay

The cytotoxicity of **5**, **6**, **11**, **12** and 5-FU on C6 cells was determined with a LDH cytotoxicity detection kit (Roche), based on the measurement of LDH activity released from the cytosol of damaged cells into the supernatant, according to the manufacturer's instructions. Briefly, 3×10^4 cells in 100 µL were seeded into 96-well microtiter plates as triplicates and treated with IC_{50} concentrations of **5**, **6**, **11** and **12** as described above, at 37 °C with 5% CO_2 overnight. LDH activity was determined by measuring the absorbance at 492–630 nm using a microplate reader.

2.2.6. Analysis of DNA Laddering

The DNA laddering effect of the test compounds was measured according to the method in literature [23], with some modifications. Briefly, 7.5×10^5 cells were seeded into 25 cm^2 culture flasks and treated with IC_{50} concentrations of **5**, **6**, **11** and **12** at 37 °C with 5% CO_2 overnight. Treated cells were harvested using a sterile plastic scraper, transferred to a 15-mL sterile Falcon tube, washed with 1 mL of sterile DPBS, and pelleted by spinning at 1500 × g for 5 min. The cell pellet was resuspended with 200 mL of ice-cold DPBS by gentle pipetting, fixed with 5 mL of ice-cold 70% ethanol, vortexed, and incubated at -20 °C for 24 hours. The cells were centrifuged at 1500 × g for 5 min, the supernatant was removed, and the remaining ethanol was removed by air drying. The cell pellet was resuspended in 50 mL of phosphate–citrate buffer (consisting of 192 parts of 0.2 M Na_2HPO_4 and 8 parts of 0.1 M citric acid, pH 7.8), incubated at 37 °C for 30 min in a shaker incubator, and centrifuged at 1500 × g for 5 min. Forty mL of supernatant was transferred to a 1.5 mL microcentrifuge tube, mixed with 5 mL of Tween-20 solution (0.25% in ddH_2O) and 5 mL of RNase A solution, and incubated at 37 °C for 30 min in a shaker incubator. Afterwards, 5 mL of proteinase K was added to each tube and incubated at 37 °C for 10 min. Finally, the entire content of the microcentrifuge tube was mixed with 4 mL of 6 × loading buffer, loaded into a 1.5% agarose gel containing 0.5 µg/mL ethidium bromide, and electrophoresed at 200 mA for 40 min. DNA laddering in the gels was visualized using the gel documentation system (UVP, England).

2.2.7. DNA Topoisomerase I Inhibition Assay

The DNA topoisomerase I inhibitory activities of the compounds **5**, **6**, **11**, **12** in cell-free systems were evaluated by using a topoisomerase I assay kit (TopoGen, USA). The principle of the assay is to measure the conversion of supercoiled pHOT1 plasmid DNA into its relaxed form in the presence of DNA topoisomerase I alone and with test compounds. The supercoiled substrate (pHOT1 plasmid DNA) and its relaxed product can easily be distinguished in agarose gels because the relaxed isomers migrate more slowly than the supercoiled isomer. In brief, 20 mL of the reaction mixture containing 1 mL plasmid pHOT1 DNA in relaxation buffer was incubated with 2U recombinant human topoisomerase I in the presence of IC_{50} concentrations of **5**, **6**, **11**, **12** and camptothecin as the positive control. The reactions were carried out at 37 °C for 30 min and then terminated by the addition of stop solution. After the termination, the sample was analyzed using a 1% agarose gel at 4 V/cm for 60 min. After electrophoresis, DNA bands were stained with ethidium bromide solution (1 µg/mL) and photographed using a gel imaging system (UVP BioSpectrum, Germany).

3. RESULT AND DISCUSSION

3.1. Chemistry

In our recent article [19], one pot synthesis is described for bromination of 8-substituted quinolines **2**-**4** (Fig. **1**) using molecular bromine.

To functionalize 8-substituted quinolines **2**-**4** by bromination, 8-hydroxy **2**, 8-amino **3** and 8-methoxy **4** quinolines were converted corresponding to bromo derivatives. While the treatment of 8-hydroxyquinoline (**2**) with 1.5 equivalents of molecular bromine afforded a mixture of 7-bromo-8-hydroxyquinoline (**6**) and 5,7-dibromo-8-hydroxyquinoline (**5**), easily separated into its components based on their solu-

Scheme (1). Synthesis of bromo hydroxyquinoline derivatives (**5**, **6**).

bilities in 51% and 37% yields, respectively, using 2 equivalents of Br_2 furnished 5,7-dibromo-8-hydroxyquinoline (**5**) in 90% yield as a sole (Scheme **1**) [19].

Bromination of 8-aminoquinoline (**4**) afforded a mixture of 5-bromo-8-aminoquinoline (**10**) and 5,7-dibromo-8-aminoquinoline (**9**) with 1.5 equivalents of bromine (Scheme **2**). The mixture of **9** and **10** remained unseparated due to their close R*f* values. However, The treatment of 8-aminoquinoline (**4**) with 2 equivalents of Br_2 produced 5,7-dibromo-8-aminoquinoline (**9**) in excellent yield (99%) as a sole. Moreover, 5-bromo-8-methoxyquinoline (**10**) and 5,7-dibromo-8-methoxyquinoline (**9**) were synthesized by treatment of 8-methoxyquinoline (**3**) with excessive amount of molecular bromine. Then, **9** and **10** were isolated by applying column chromatography in 45% and 43% yields, respectively (Scheme **2**). The bromination of 8-methoxyquinoline (**3**) with 1 equivalent of Br_2 afforded 5-bromo-8-methoxyquinoline (**8**) in high yield (92%) as a sole product [19].

To demonstrate the value of **5** and **6** as starting materials for numerious useful compounds, we investigate copper induced reactions of bromo 8-hydroxquinolines **5** and **6**. 7-Bromo-8-hydroxyquinoline (**6**) and 5,7-dibromo-8-hydroxyquinoline (**5**) were individually reacted with CuCN in refluxing DMF to afford corresponding novel cyanoquinoline derivatives **11** and **12** according to reported procedure in literature [20]. Monocyanide **11** and dicyanide **12** were synthesized in low yields 36% and 23%, respectively.

The structures of cyanide containing compounds **11-12** were determined by ^{1}H NMR and IR. The preparation of novel cyano-8-hydroxyquinoline derivatives **11-12** was revealed by the appearance of the absorbance signal of the –C≡N stretching frequency at ± 2254 and 2208 cm^{-1}, respectively in the IR spectra. In the ^{1}H NMR spectrum of **11**, signals for the aromatic protons H-5 and H-6 (δ_H 8.67 and 9.14 respectively) were shifted downfield when compared to signals of the starting material **6** (H-5 and H-7; δ_H 7.10, 7.73 respectively). Similarly, the ^{1}H NMR spectrum of **12** consisted of four signals as expected. It was seen that the signals appeared in downfield after substitution when compared to signals of the starting material **5** which is evidence for the existence of cyanization at both C-5 and C-7 positions.

3.2. Anticancer Studies

3.2.1. Prescreening of 3, 5, 6, 8, 9, 11 and 12 for their Antiproliferative Activities Against HT29, HeLa and C6 Cells by the SRB Assay

The compounds **3**, **5**, **6**, **8** and **9** were screened for their antiproliferative potential against the HT29, HeLa, and C6 using the SRB cell proliferation assay. Compounds signifi-

Scheme (2). Synthesis of bromo methoxy and aminoquinoline derivatives (**7-9**).

Scheme (3). Synthesis of cyano 8-hydroxyquinoline derivatives (**11-12**).

cantly inhibited proliferation of HeLa ($p < 0.05$), C6 ($p < 0.05$), and HT-29 ($p < 0.05$) cells, as compared to 5-FU at 5 µg/mL and higher concentrations, while **3**, **8** and **9** did not inhibit proliferation as shown in Fig. **2**. These results indicated that 8-hydroxy compounds **5**, **6**, **11** and **12** are more ciritical against HT-29, HeLa, and C6 cell lines than 8-methoxy **3**, **8** and 8-amino **9** compounds. The substituent at C-8 position is important to show anticancer activity against tested cancer cell lines. Because both brominated 8-hydroxy derivatives **5**, **6** and cyano substitued 8-hydroxy quinolines **11**, **12** showed high antiproliferative activity.

3.2.2. Antiproliferative Activities of 5, 6, 11 and 12 Against HT29, HeLa, C6 and L929 Cells

To support the anticancer effects of **5**, **6**, **11** and **12**, showing higher inhibition on SRB assay, their antiproliferative activities against the HT29, HeLa, C6 and L929 cell lines were further investigated and confirmed using a BrdU cell proliferation ELISA, a more sensitive proliferation assay. In contrast to 5-FU, all compounds **5**, **6**, **11** and **12** significantly inhibited proliferation of HeLa ($p < 0.05$), C6 ($p < 0.05$), and HT-29 ($p < 0.05$) cells at 5 to 75 µg/mL concentrations (Fig. **3**). These results show that hydroxy group at C-

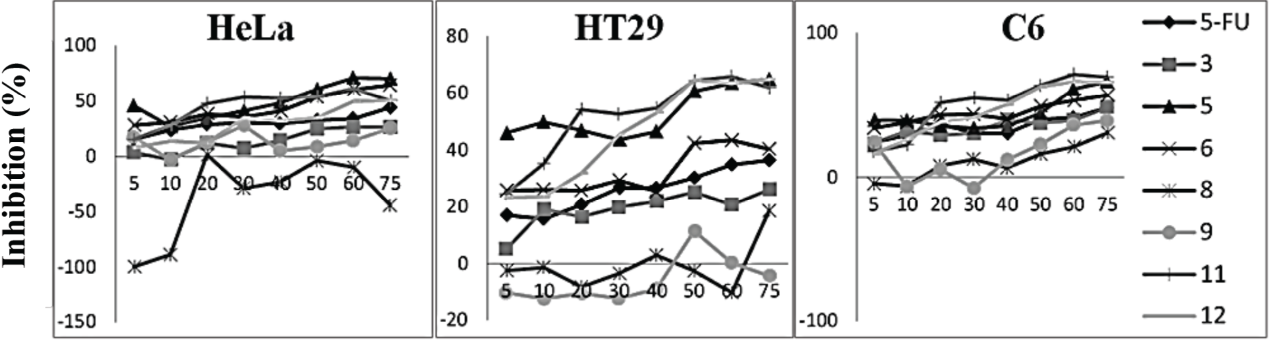

Fig. (2). Prescreening of compounds **3**, **5**, **6**, **8**, **9**, **11** and **12** for their antiproliferative potential on the HeLa, HT29, and C6 tumor cell lines by the SRB assay. Percentage inhibition was reported as mean value ± SEM of three independent assays ($p < 0.05$).

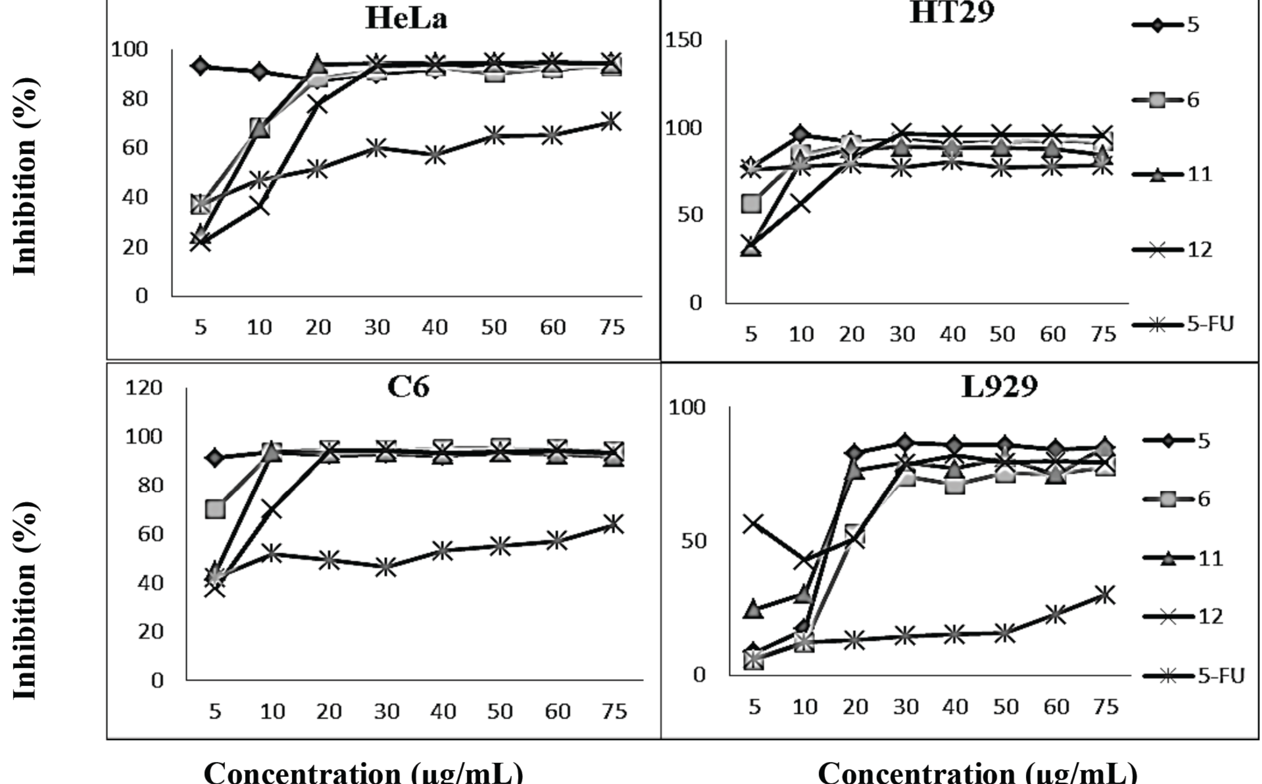

Fig. (3). Antiproliferative activity of **5**, **6**, **11** and **12** against HeLa, HT29, C6 and L929 cell lines. Cell proliferation was measured using a BrdU cell ELISA assay. Percent inhibition was reported as the mean value ± SEM of three independent assays ($p < 0.05$).

Table 1. IC_{50} (µg/mL) values of 5, 6, 11, 12 and 5-FU.

Compound	HeLa	HT29	C6	L929
5	9.3	17.9	16.3	30.2
6	7.5	25.3	18.6	40.1
11	8.4	13.2	20.3	27.6
12	6.7	12.6	25.6	31.4
5-FU	24.8	20.8	21.2	-

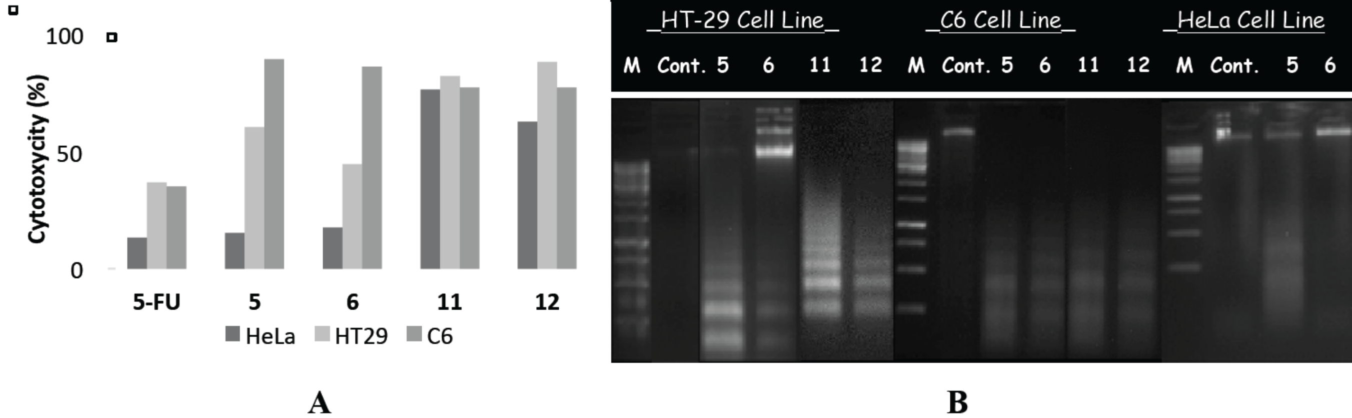

Fig. (4). Cytotoxic activity (**A**) and apoptotic potential (**B**) on HT-29, C6, and HeLa cell lines of **5**, **6**, **11** and **12**. Exponentially growing cells were incubated with IC_{50} concentrations of **5**, **6**, **11** and **12** and cytotoxicity was determined using the LDH cytotoxicity assay. The cytotoxicities of **5**, **6**, **11** and **12** were significantly ($p < 0.05$) higher than that of 5-FU. Percentage cytotoxicity was reported as the mean values ± SEM of three independent assays. In the apoptosis experiment, M: DNA Marker (1 kb), Cont: DNA from DMSO-treated cells. DNA was isolated and electrophoresed in 1.5% agarose gels, visualised under UV light on a transilluminator, and photographed.

8 position of quinoline core is more important to demonstrate the anticancer activity than bromo or cyano substituents at C-5 or C-7. In addition to determine the antiproliferative activities of 8-hydroxyquinoline derivatives **5**, **6**, **11**, **12** against normal cell lines, L929 cells, were treated with several concentrations of these compounds. In contrast to control compound. 5-FU, substituted 8-hydroxy quinolines inhibited the proliferation of L929. As shown in Fig. **3**, compounds **5**, **6**, **11** and **12** significantly inhibited the proliferation of HT29, HeLa and C6 at 5 µg/mL. However, they showed the antiproliferative activity against L929 cell lines at 20 µg/mL. Furthermore, in comparison to IC_{50} concentrations, **5**, **6**, **11** and **12** have higher values ranged from 27.6 to 40.1 µg/mL at L929 than values ranged from 6.7 to 9.3 µg/mL at especially HeLa cell lines in Table **1**.

Fig. (5). Anti-Topoisomerase I activity of **5**, **6**, **11** and **12**. Lane 1: Relaks DNA, Lane 2: (-) Control (SC DNA + Topo I), Lane 3: (+) Control (pHOT1 DNA + Camptothesin + Topo I), Lane 4: Compound **5** + pHOT1 DNA + Topo I, Lane 5: Compound **6** + pHOT1 DNA + Topo I, Lane 6: Compound **11** + pHOT1 DNA + Topo I, Lane 7: Compound **12** + pHOT1 DNA + Topo I. (TOPO I: Topoisomerase I, SC DNA: Supercoiled Plasmid DNA).

3.2.3. Cytotoxic Activity of 5, 6, 11 and 12 on HeLa, HT29 and C6 Cell Line and Detection of the Apoptotic Potential of 5, 6, 11 and 12 using DNA Laddering Assay

In the present study, we determined the DNA laddering potential of **5**, **6**, **11** and **12** on the HeLa, HT29, and C6 cell lines to test whether the mechanism of antiproliferative and cytotoxic activity of **5** and **6** involved apoptosis or not. The DNA laddering assay results showed that both **5**, **6**, **11** and **12** caused DNA fragmentation, indicating that these compounds act by inducing apoptosis on C6 and HT29 cell lines (Fig. **4B**); however, only compound **5** caused fragmentation of HeLa cell DNA. To determine the cytotoxic profiles of **5**, **6**, **11** and **12**, LDH assay was performed. The results showed that the cytotoxicity of all compounds were significantly higher than 5-FU at their IC_{50} concentrations against each cell lines. In Fig. **4A**, cyano substituted 8-hydroxyquinolines **11** and **12** showed high cytotoxicity (ranged from 60% and 90%). On the other hand, the cytotoxicities of **5** and **6** were significantly higher (%92 and 90%, respectively) at only C6 cell lines. Also, these compounds modaretely showed cytotoxicity (60% and 43%, respectively) against the HT29 cell line.

3.2.4. Detection of DNA Topoisomerase I Inhibitory Activity of 5 and 6

DNA topoisomerase I is a nuclear enzyme that plays essential roles in controlling the topological state of DNA to facilitate and remove barriers for vital cellular functions, including DNA replication and repair. Therefore, DNA topoisomerase I is an important target for anti-cancer agents. The DNA topoisomerase I inhibitory activity of **5**, **6**, **11** and **12** was investigated using a topoisomerase inhibition assay. At IC_{50} concentrations, **5**, **12** and camptothecin (positive control) inhibited the activity of recombinant human DNA topoisomerase I, whereas **6**, **11** did not (Fig. **5**).

CONCLUSION

The antiproliferative activity of some substituted quinoline derivatives against several cancer cells has been reported [14, 24, 25]. Eventhough, brominated 8-hydroxy (**5**, **6**), 8-methoxy (**8**) and 8-amino (**9**) quinolines were known compounds, this is the first investigation revealing their antiproliferative, Topoisomerase I inhibitory potentials and cytotoxic profiles *in vitro*. The antiproliferative activity of these compounds was concentration-dependent.

Compunds **5**, **6**, **11** and **12** significantly inhibited the proliferation of cancer cell lines and also healthy normal cell line (L929, mouse fibroblast cells) in cantrast to control compound, 5-FU [26]. However, in comparison to IC_{50} concentrations of cancer cells and normal cell, IC_{50} values (ranged from 27.6 to 40.1 μg/mL) in L929 are higher than values in cancer cell lines (Table **1**). These results showed inhition effects of these compounds were dose dependent. Furthermore, it suggests if **5**, **6**, **11** and **12** are used in effective lower concentrations (~ 5-10 μg/mL) in cancer cell lines, their antiproliferative and cytotoxic effects against healty cells (L929) can be decreased.

The cytotoxicities of **5**, **6**, **11** and **12** on tested cell lines, especiall on C6 cells, were confirmed to their antiproliferative activies compared with control, 5-FU. Compounds **5**, **6**, **11** and **12** showed about 80% cytotoxicity ($p < 0.05$) against the C6 cell line at their IC_{50} concentrations (Fig. **4A**), suggesting that these compounds may have anticancer potential, with significant antiproliferative and cytotoxic activities.

According to two antiproliferative assays, SRB and BrDU Eliza and cytotoxic assay, the SAR indicated that the kinds of substituent at C-8 of quinoline ring are affecting anti cancer activity. Because exchange of hydroxyl group with methoxy and amino groups caused a complete loss of antiproliferative activitiy (Fig. **2**). On the other hand, newly synthesized 7-cyano (**11**) and 5,7-dicyano-8-hydroxyquinolines (**12**) by substitution increased the antiproliferative activity in contrast to compounds **8** and **9**. These results confirmed that the presence of the bromide and OH groups (**5** and **6**) in these quinoline derivatives generate significant antiproliferative activity and these main groups must be present in the quinoline skeleton for better activity in any novel derivatives synthesized in the future.

Moreover, the DNA laddering assay results showed that both bromo (**5**, **6**) and cyano derivatives (**11**, **12**) induced DNA fragmentation in cancer cell lines. These results indicate that each compound act through induction of apoptosis. In addition, compounds **5** and **12** inhibited the DNA relaxation activity of topoisomerase I, suggesting that **5** and **12** inhibit cell proliferation by the suppression of DNA topoisomerase I during replication. Since DNA topoisomerase I is an important enzyme for bacterial replication, it is proposed that **5** and **12** may have significant antibacterial activity and be novel topoisomerase I inhibitors.

In conclusion, our present work demonstrated that bromo and cyano substituted 8-hydroxyquinolines have anticancer drug potential with antiproliferative, cytotoxic, and apoptotic activities; however, further *in vitro* and *in vivo* investigations are needed to reveal the anticancer drug potential and mechanism of action of compounds **5** and **6**. Furthermore, we investigated the possible role for 5,7-dibromo-8-hydroxyquinoline showing high inhibitory effect to behave as intermadiate for possible 5,7-disubstituted 8-hydroxyquinoline based drugs, for instance 5,7-dicyano-8-hydroxyquinoline **12** because 5,7-dibromo-8-hydroxyquinoline **5** moiety can induce through chemical reactivity the formation of 5,7-disubstituted derivatives *via* substitutution or coupling reactions.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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