

1-1-2013

## Simple and convenient preparation of novel 6,8-disubstituted quinoline derivatives and their promising anticancer activities

SALİH ÖKTEN

OSMAN ÇAKMAK

RAMAZAN ERENLER

ÖNEM YÜCE ŞAHİN

ŞABAN TEKİN

Follow this and additional works at: <https://journals.tubitak.gov.tr/chem>

 Part of the [Chemistry Commons](#)

---

### Recommended Citation

ÖKTEN, SALİH; ÇAKMAK, OSMAN; ERENLER, RAMAZAN; ŞAHİN, ÖNEM YÜCE; and TEKİN, ŞABAN (2013) "Simple and convenient preparation of novel 6,8-disubstituted quinoline derivatives and their promising anticancer activities," *Turkish Journal of Chemistry*. Vol. 37: No. 6, Article 5. <https://doi.org/10.3906/kim-1301-30>

Available at: <https://journals.tubitak.gov.tr/chem/vol37/iss6/5>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Chemistry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact [academic.publications@tubitak.gov.tr](mailto:academic.publications@tubitak.gov.tr).

## Simple and convenient preparation of novel 6,8-disubstituted quinoline derivatives and their promising anticancer activities

Salih ÖKTEN,<sup>1</sup> Osman ÇAKMAK,<sup>2,\*</sup> Ramazan ERENLER,<sup>3</sup> Önem YÜCE,<sup>4</sup> Şaban TEKİN<sup>4</sup>

<sup>1</sup>Department of Primary Education, Division of Science Education, Faculty of Education, Kırıkkale University, Kırıkkale, Turkey

<sup>2</sup>Department of Chemistry, Faculty of Arts and Science, Yıldız Technical University, Davutpaşa, İstanbul, Turkey

<sup>3</sup>Department of Chemistry, Faculty of Arts and Science, Gaziosmanpaşa University, Tokat, Turkey

<sup>4</sup>Department of Biology, Faculty of Arts and Science, Gaziosmanpaşa University, Tokat, Turkey

Received: 10.01.2013 • Accepted: 13.05.2013 • Published Online: 04.11.2013 • Printed: 29.11.2013

**Abstract:** A short and easy route is described for 6,8-disubstituted derivatives of quinoline and 1,2,3,4-tetrahydroquinoline from 6,8-dibromoquinolines **2** and **7** by various substitution reactions. While copper-promoted substitution of 6,8-dibromide **2** produced monomethoxides **3** and **4**, a prolonged reaction time mainly afforded dimethoxide **6** instead of **5**, whose aromatization with DDQ and substitution reaction of dibromide **7** with NaOMe in the presence of CuI also gave rise to dimethoxide **6**. Several 6,8-disubstituted quinolines were obtained by treatment of 6,8-dibromoquinoline (**7**) with *n*-BuLi followed by trapping with an electrophile [Si(Me)<sub>3</sub>Cl, S<sub>2</sub>(Me)<sub>2</sub>, and DMF]. Furthermore, **7** was also converted to mono and dicyano derivatives. The anticancer activities of compounds **2**, **7**, **6**, **12**, **13**, **15**, and **16** against HeLa, HT29, and C6 tumor cell lines were tested, and 6,8-dibromo-1,2,3,4-tetrahydroquinoline (**2**) and 6,8-dimethoxyquinoline (**6**) showed significant anticancer activities against the tumor cell lines.

**Key words:** Anticancer effect, bromoquinoline, cyanoquinoline, lithium–bromine exchange, methoxyquinoline, quinoline derivatives

### 1. Introduction

The quinoline and 1,2,3,4-tetrahydroquinoline skeletons are often used in the designs of many synthetic compounds with diverse pharmacological properties. Quinoline bromides specifically contain a key structural component of numerous compounds, which can undergo metal–halogen exchanges<sup>1</sup> and couplings;<sup>2</sup> therefore, they are useful fine chemicals. However, their prices tend to be quite high because of the complex and difficult synthetic methods involved in the production of these quinoline bromides.<sup>3</sup> Therefore, the development of simple and cheap synthetic methods for the syntheses of quinoline derivatives is important. Although different methods have been described in the literature, efficient, large-scale, cheap technologies are still needed. Current strategies for the synthesis of quinoline derivatives include cyclization reactions starting from benzene (or cyclohexane) derivatives with N-functionalities, but these compounds have also been prepared by various conventionally named reactions, such as Skraup,<sup>4</sup> Friedländer,<sup>5</sup> Doebner-von Miller,<sup>6</sup> Pfitzinger,<sup>7</sup> Conrad-Limpach,<sup>8</sup> and Combes.<sup>9</sup> Unfortunately, these methods for quinoline synthesis often do not allow for adequate diversity and substitution on the quinoline ring system.

\*Correspondence: cakmak.osman@gmail.com

Dedicated to Professor Metin Balcı on the occasion of his 65th birthday

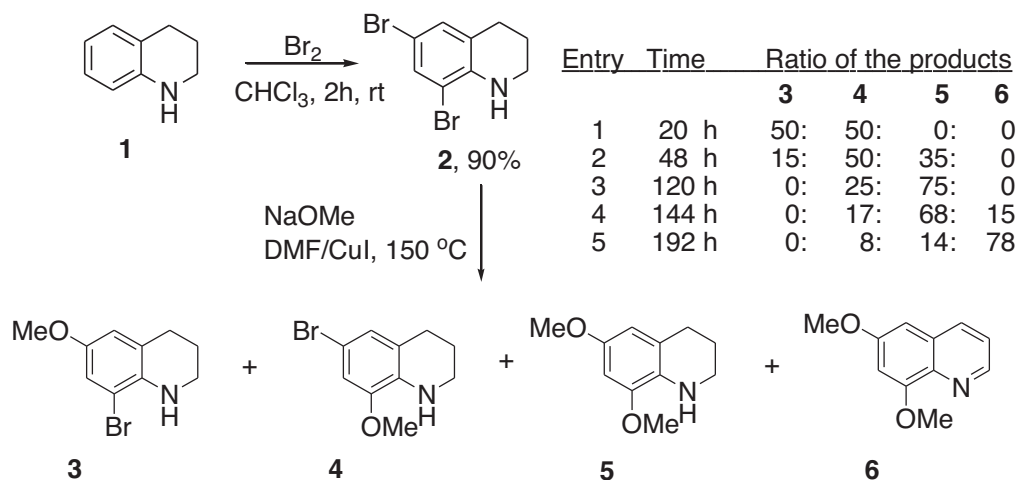
For instance, a bromoquinoline-based derivative synthesis is often restricted due to the difficulty in the preparation of bromoquinolines. However, recently, we developed an efficient synthesis method for 6,8-dibromoquinolines **2** and **7** based on the bromination of 1,2,3,4-tetrahydroquinoline (**1**),<sup>10</sup> and we synthesized a series of trisubstituted quinoline derivative from a corresponding tribromoquinoline. As an extension of that study, we report here a convenient synthesis for 6,8-disubstituted quinoline derivatives from brominated quinolines **2** and **7** via a metal–bromine exchange, and the values for the brominated products **2** and **7**, as precursors to the corresponding disubstituted quinolines, are presented. In addition, because many quinoline derivatives demonstrate impressive anticancer activities,<sup>11–14</sup> we studied the anticancer activities of the 6,8-disubstituted quinoline derivatives against several tumor cell lines; overall, 6,8-dibromide **2** and 6,8-dimethoxide **6** showed promising anticancer activities.

## 2. Results and discussion

### 2.1. Synthesis and structural assignment

Dibromides **2** and **7** were synthesized according to previously reported procedures starting from 1,2,3,4-tetrahydroquinoline (**1**) (Schemes 1 and 2).<sup>10</sup>

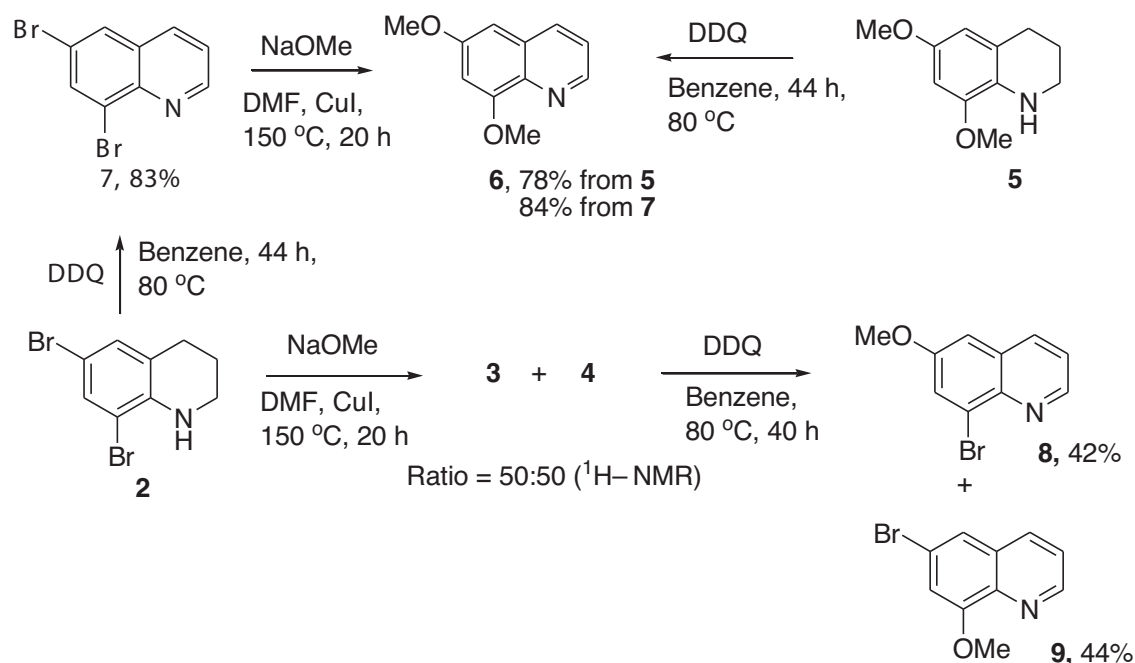
In the first step, dibromide **2** was treated with MeONa in the presence of CuI in boiling DMF to give a mixture of methoxyquinolines **3** and **4**, and the proportion of the products depended on the reaction time. In the 20-h reaction period (entry 1), monomethoxides **3** and **4** were obtained in a ratio of 1:1, whereas prolonged reaction times up to 120 h raised the amount of monomethoxide **4** and dimethoxide **5** formed to a ratio of 1:3 (entry 3). Surprisingly, the longer reaction times (entries 4 and 5) also mainly resulted in the formation of aromatized product **6**. We assume that the high temperature conditions in the presence of CuI with longer reaction times induced aromatization of the dimethoxy compound **5** to give **6**.



**Scheme 1.** Copper-assisted methoxylation of **2**.

The reaction mixture in entry 2 was separated by column chromatography using SiO<sub>2</sub> with hexane/AcOEt. The solvent polarity was gradually increased from 9:1 to 9:3, and the first fraction was found to be a mixture of 6-methoxide **3** and 8-methoxide **4**. Methoxide **4** was isolated as a pure compound in a yield of 14% in the second fraction, and the following fraction contained **4** and 6,8-dimethoxide **5** as a mixture. Lastly, **5** was isolated in pure form (18% yield) in the final fraction.

DDQ aromatization of compound **5** in benzene at reflux for 40 h provided 6,8-dimethoxyquinoline (**6**). The reaction mixture of **3** and **4** (entry 1) was also aromatized, and products **8** and **9** were easily isolated by column chromatography (Scheme 2).



**Scheme 2.** Synthesis of **6**, **8**, and **9**.

To obtain pure dimethoxide **6**, the reaction with **2** was repeated for 144 h (entry 4) and 192 h (entry 5). However, the aromatized product **6** along with products **4** and **5** were produced instead of pure dimethoxide **5** (Scheme 2). We achieved pure dimethoxide **6** by direct treatment of 6,8-dibromoquinoline **7** with  $\text{NaOCH}_3$  in the presence of  $\text{CuI}$ . 6,8-Dimethoxyquinoline **6** was obtained as the sole product in a yield of 84% (Scheme 2).

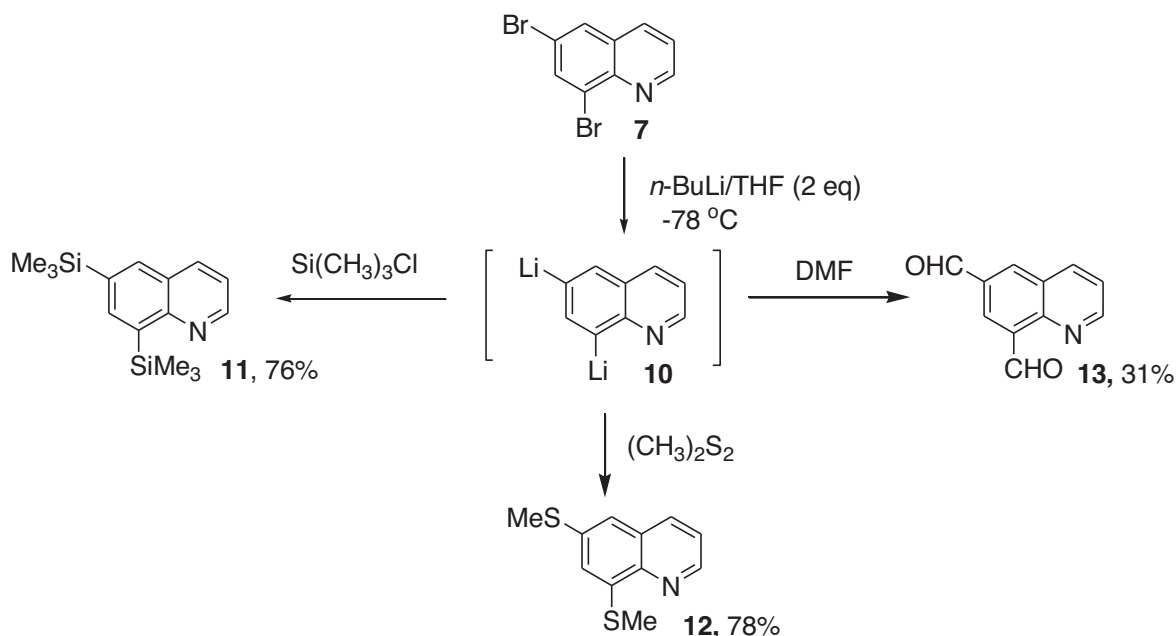
It is reported that Alfonsi et al. carried out many reactions for synthesis of 5,8-dimethoxy quinoline (**5**). One of the best yields of the sequential alkylation/gold-catalyzed annulation reactions of anilines with propargylic bromide was only 28%.<sup>15</sup> Therefore, we developed an alternative, more efficient and simple preparation method for compound **5**.

The  $^1\text{H}$  NMR spectra of dimethoxide **6** are quite similar to those of the starting material, dibromide **3**, and consist of the same signal systems except for the higher shifted aryl protons present due to the 2 MeO donor groups in **6**.

Structural characterizations of all of the methoxide compounds were further confirmed by mass spectroscopy and other 2-dimensional NMR spectra. Compound **4** provided  $^2J_{\text{CH}}$  couplings through an HMBC correlation, and clear evidence for the positions of the MeO group (8- or 6-methoxide) and the aromatic protons H-5 and H-7 was observed. The fact that C-8 (146.8 ppm) correlated with H-70 (6.72 ppm) but not with H-5 (6.77 ppm) confirmed that the MeO group was attached to C-8. Furthermore, H-5 (6.77 ppm) and H-7 (6.72 ppm) correlate with C-6 (133.6 ppm), which is in agreement with the suggested structure for **4**.

To demonstrate the value of 6,8-dibromide **7** as a precursor for other useful compounds, we investigated the metal-halogen exchange reaction of **7**. As a result, 6,8-bis(methylsilyl)quinoline **11** (76%) and 6,8-

bis(methylthio)quinoline **12** (78%) were obtained in moderate yields. In the NMR spectra, the 2 silyl signals in **11** and 2 methylthiol signals in **12** were clearly observed, and these results confirmed the formation of the compounds. Moreover, the similarities in the signal systems for compounds **11** and **12** with that of **7** were quite helpful for the identification of the structures (Scheme 3).



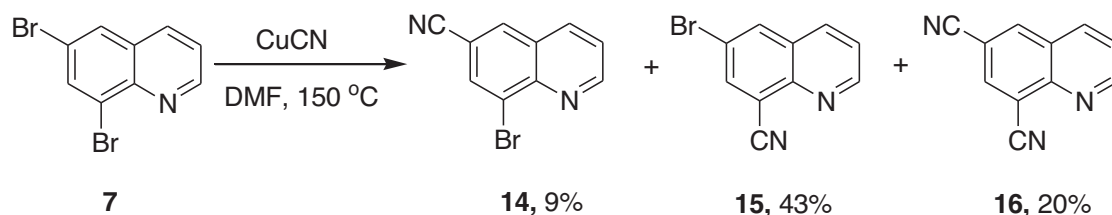
**Scheme 3.** Preparations of 6,8-disubstituted quinoline derivatives **11–13**.

Lejejs et al. obtained methylthioquinoline **12** with a long sequential reaction procedure (10 steps) starting from 6-nitro-8-aminoquinoline, in which synthesis and physicochemical properties of 6-methylthio-8-mercaptoquinoline (precursor of **12**) were reported.<sup>16</sup> The authors claimed that the earlier method<sup>17</sup> was more complicated and their new method reduced the number of reaction stages and increased the yield (overall yield: 20%). Thus, we developed an effective and simple method for the synthesis of valuable compound **12** starting from 1,2,3,4-tetrahydroquinoline using just 3 sequential, precisely selective and simple reactions that all proceed in high yields.

Preparation of quinoline-6,8-dicarbaldehyde **13** led to a lower yield (31%) than expected, as the dilithiated quinoline in THF at  $-78\text{ }^\circ\text{C}$  was trapped by DMF. The  $^1\text{H}$  NMR spectra of **13**, which consisted of 2 aldehyde singlet signals ( $\delta$  11.48 and 10.30) and 5 signals of aromatic H-atoms, ( $\delta$  9.21, dd, H-2), 7.67 (dd, H-3), 8.46 (d, H-4), 8.67 (d, H-5), and 8.80 (d, H-7), matched fairly well with the proposed structure. In addition, the  $^{13}\text{C}$  NMR spectrum of **13**, consisting of 11 signals with 2 C = O signals ( $\delta$  191.8 and 190.7), was also in good agreement with the suggested structure (Scheme 3).

Finally, dibromide **7** was treated with CuCN in boiling DMF. The nucleophilic substitutions of **7** resulted in the formation of a mixture of cyanoquinolines **14**, **15**, and **16** (Scheme 4). According to the  $^1\text{H}$  NMR and mass spectral data, 3 cyanoquinolines were formed. The conversion and product ratio were 83% and 47:12:22 for **14–16**, respectively, as assigned by  $^1\text{H}$  NMR. Furthermore, the products were easily isolated by silica gel column ( $\text{SiO}_2$ , AcOEt/hexane); this process yielded 9%, 43%, and 20% 8-bromo-6-cyanoquinoline **14**, 6-bromo-8-cyanoquinoline **15**, and 6,8-dicyanoquinoline **16**, respectively. However, when the reaction time was

prolonged, polymerization occurred instead of the formation of dicyanide **16** as the sole product. In the case of the lower reaction temperature and longer reaction time (100 °C and 2 days), no conversion occurred.

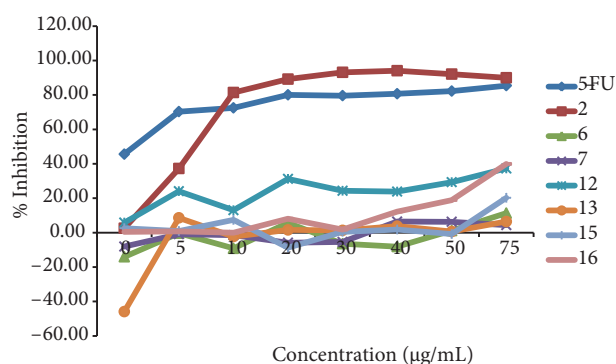


**Scheme 4.** Synthesis of the cyanoquinoline derivatives **14–16**.

In the  $^1\text{H}$  NMR spectrum, 6,8-dicyanide **16** displays the same signal system as dibromide **7**, except with higher field shifting. Furthermore, the 2-dimensional NMR spectra provided information about the position of the cyanide group. For example, in the HBMN spectra of **14**, the characteristic cyanide C-atom (119.0 ppm) correlated with H-5 and H-7 ( $\delta$  8.22 and 8.25, respectively), which confirmed that the cyanide group was bonded to C-6 in compound **14**.

## 2.2. Antiproliferative activities of quinoline compounds against HeLa cells

In the present study, the antiproliferative activities of **2**, **6**, **7**, **12**, **13**, **15**, and **16** were tested against HeLa cells in vitro at 5, 10, 20, 30, 40, 50, 75, and 100  $\mu\text{g}/\text{mL}$  concentrations.<sup>18</sup> The results showed that only 6,8-dibromo-1,2,3,4-tetrahydroquinoline (**2**) significantly inhibited proliferation of HeLa cells at 10  $\mu\text{g}/\text{mL}$  and higher concentrations ( $P \leq 0.05$ ) tested (Figure 1). It was the most potent antiproliferative compound against HeLa cells among the compounds tested in this study.

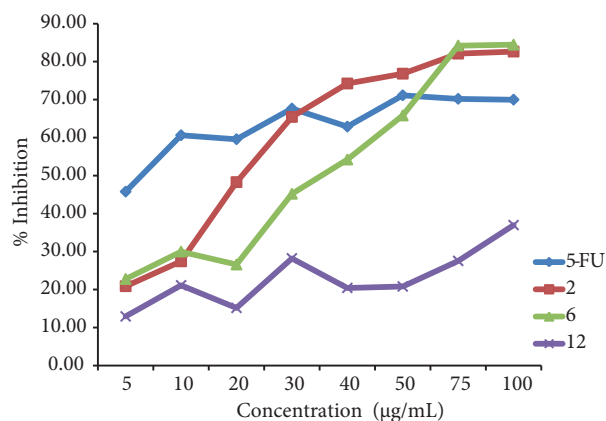


**Figure 1.** Antiproliferative activities of quinoline derivatives **2**, **6**, **7**, **12**, **13**, **15**, and **16** on the proliferation of HeLa cells in vitro. All compounds were tested at 5, 10, 20, 30, 40, 50, 75, and 100  $\mu\text{g}/\text{mL}$  concentrations. Compound **2** significantly inhibited proliferation of HeLa cells at 10  $\mu\text{g}/\text{mL}$  and higher concentrations tested ( $P \leq 0.05$ ). The data show the averages of 2 individual experiments. The DMSO and 5-FU were used as the negative and positive control respectively in all experiments.

## 2.3. Antiproliferative activities of quinoline compounds against HT-29 cells

Quinoline compounds **2**, **6**, and **12** were tested against proliferation of HT-29 cells at 5, 10, 20, 30, 40, 50, 75, and 100  $\mu\text{g}/\text{mL}$  concentrations. The results showed that compound **2** more significantly inhibited the proliferation

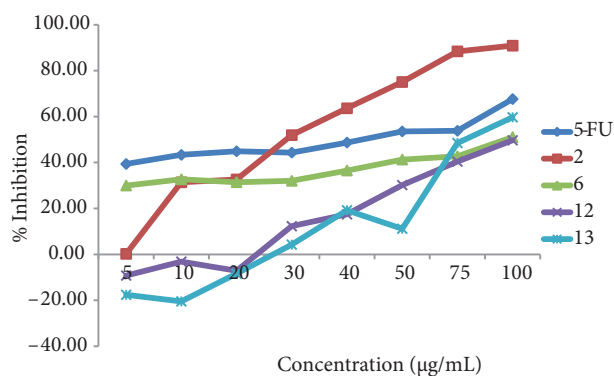
of HT-29 cells than control compound 5-FU at 30  $\mu\text{g}/\text{mL}$  and higher concentrations ( $P \leq 0.05$ ) (Figure 2). Compound **6** was more inhibitory against HT-29 cells than 5-FU at 70  $\mu\text{g}/\text{mL}$  and higher concentrations ( $P \leq 0.05$ ) (Figure 2).



**Figure 2.** Antiproliferative activities of quinoline derivatives **2**, **6**, and **12** on the proliferation of HT-29 cells in vitro. All compounds were tested at 5, 10, 20, 30, 40, 50, 75, and 100  $\mu\text{g}/\text{mL}$  concentrations. Compounds **2** and **6** significantly inhibited ( $P \leq 0.05$ ) the proliferation of HT-29 cells at 30  $\mu\text{g}/\text{mL}$  and 70  $\mu\text{g}/\text{mL}$  concentrations and higher concentrations, respectively. The data show the averages of 2 individual experiments. The DMSO and 5-FU were used as the negative and positive control respectively in all experiments.

#### 2.4. Antiproliferative activities of quinoline compounds against C6 cells

The antiproliferative activities of **2**, **6**, **12**, and **13** on C6 cells were also tested at 5, 10, 20, 30, 40, 50, 75, and 100  $\mu\text{g}/\text{mL}$  concentrations. Compound **2** was the most antiproliferative compound tested at 30  $\mu\text{g}/\text{mL}$  and higher concentrations ( $P \leq 0.05$ ) (Figure 3).



**Figure 3.** The antiproliferative activities of **2**, **6**, **12**, and **13** on C6 cells in vitro. All compounds were tested at 5, 10, 20, 30, 40, 50, 75, and 100  $\mu\text{g}/\text{mL}$  concentrations. Compound **2** was the most antiproliferative compound tested at 30  $\mu\text{g}/\text{mL}$  and higher concentrations ( $P \leq 0.05$ ). The data show the averages of 2 individual experiments. The DMSO and 5-FU were used as the negative and positive control respectively in all experiments.

### 3. Conclusion

We developed a simple and convenient route to a variety of 6,8-disubstituted quinolines, which are difficult to prepare by traditional methodologies, by starting with a commercially available, cheap starting material,

tetrahydroquinoline **1**. Our suggested methods are more convenient and efficient for known compounds **6**,<sup>15</sup> **8**,<sup>19</sup> and **9**,<sup>20</sup> which valuable fine chemicals, and **12**,<sup>16</sup> and they are fully characterized. The prepared compounds are often the starting points for other polyfunctionalized quinoline derivatives. For instance, the products can be easily brominated at the 3-position of the quinoline ring, which could give 3,6,8-trisubstituted quinoline derivatives, in the presence of Br<sub>2</sub> and pyridine according to the Eisch procedure.<sup>21</sup> On the other hand, the bromomethoxide compounds **3**, **4**, **8**, and **9** and the bromocyanides **14** and **15** can also be converted to other disubstituted quinolines due to their bromine groups. Compounds **3**<sup>22</sup> and **4**<sup>23</sup> are known but they are protected by patents. We are currently working on bromination of the methoxytetrahydroquinolines **3–5** to further study their functionalization and to investigate their anticancer activities.

In addition, 6,8-dibromo-1,2,3,4-tetrahydroquinoline (**2**) significantly inhibited the proliferation of HeLa, HT-29, and C6 cells in vitro at concentrations of 10 µg/mL, 30 µg/mL, and 30 µg/mL and higher concentrations, respectively, as compared to a control cancer drug, 5-Fluorouracil (Figures 1–3). However, the compound 6,8-dimethoxyquinoline (**6**) selectively inhibited the proliferation of HT-29 cells only (Figure 2) at concentrations of 70 µg/mL and higher. In contrast, compound **6** and the others tested did not exert any antiproliferative activity against the cell lines used (Figures 1–3). Therefore, compound **2** was the most potent antiproliferative compound tested in this study. The results suggest that this compound may be a novel anticancer drug candidate.

The 6,8-dibromide of the 1,2,3,4-tetrahydroquinoline **2** structure (but not the 6,8-dibromide functionality of quinoline **7**) and the 6,8-dimethoxy groups of quinoline **6** could be responsible for the antiproliferative potentials of the compounds because of the possible reactivities of both groups. Therefore, we propose that compounds with both groups might exert stronger anticancer activities. Although the results show anticancer potentials for 6,8-dibromide **2** and 6,8-dimethoxide **6**, the anticancer potentials of other proposed, substituted quinolines and tetrahydroquinolines and their mechanisms of action need to be determined. The selective and potent anticancer activities of compounds **2** and **6** need to be tested in further pharmacological studies. Furthermore, variations in these substituents on the lead compounds, along with their mechanisms of action for their anticancer activities, are being studied and will be reported in due course.

## 4. Experimental

### 4.1. General

Thin layer chromatography was performed on silica F<sub>254</sub> 0.255 mm plates, and spots were visualized by UV at 254 nm. Classic column chromatography was performed using (70–230 mesh) silica gel. Melting points were determined on a capillary melting point apparatus. Solvents were concentrated at reduced pressure. IR spectra were recorded on an IR instrument. Mass spectra were recorded on a spectrometer under electron-impact (EI) and chemical ionization conditions. NMR analyses were recorded on a NMR instrument for the <sup>1</sup>H NMR (400 MHz) and for the <sup>13</sup>C NMR (100 MHz) spectra.

### 4.2. Synthesis of the methoxy derivatives of 1,2,3,4-tetrahydroquinoline (Scheme 2) (entry 2)

Freshly cut Na (1.5 g, 65.2 mmol) was added to dry MeOH (40 mL) under an Ar atmosphere. After complete dissolution, the warm solution was diluted with dry DMF, and vacuum-dried CuI (1.78 g, 3.44 mmol) was added. Next, 6,8-dibromo-1,2,3,4-tetrahydroquinoline (**2**) (1 g, 3.44 mmol) in dry DMF (25 mL) was added to the mixture, which was stirred magnetically under an Ar atmosphere at reflux (ca. 150 °C) for 48 h. The



reaction progress was monitored by TLC. After cooling to rt, H<sub>2</sub>O (50 mL) was added to the mixture, and the aq layer was extracted with CHCl<sub>3</sub> (3 × 50 mL). The organic layers were combined, washed with H<sub>2</sub>O (3 × 25 mL), and dried (Na<sub>2</sub>SO<sub>4</sub>). After filtration and removing the solvent, the residue was filtered through a short silica gel column (5.0 g). A mixture (400 mg) of 8-bromo-6-methoxyquinoline (**3**), 6-bromo-8-methoxyquinoline (**4**), and 6,8-dimethoxyquinoline (**5**) was obtained in a ratio of 15:50:35, respectively, as assigned by <sup>1</sup>H NMR. The mixture was purified by silica gel (SiO<sub>2</sub>, 60 g) column chromatography, eluting with AcOEt/hexane, (600 mL, 1:9). Compounds **5** and **6** were collected as a mixture in the first eluent (320 mL). However, after the solvent polarity was increased to 2:9 AcOEt/hexane, compound **4** (112 mg, 14%) was isolated in pure form (second eluent 150 mL). After the solvent polarity was increased to 3:9 (AcOEt/hexane), the third eluent (100 mL) was a mixture of products **4** and **5**. Lastly, 6,8-dimethoxide **5** (120 mg, 18%) was isolated as a pure substance, as the fourth eluent (240 mL). The reaction was repeated for 20 (entry 1) and 120 h (entry 3) under the same reaction conditions. In the 20-h reaction, the product ratio of 6-bromo-8-methoxide **4** and 8-bromo-6-methoxide **3** was 50:50, respectively. In the 120-h reaction, 6-bromo-8-methoxide **4** and 6,8-dimethoxide **5** were isolated in a ratio of 25:75, respectively (entry 3). The reactions were also repeated for 144 h and 192 h using the same reaction conditions. The ratios of the products are shown in Scheme 1 (entries 4 and 5). The reaction was also repeated under the same reaction conditions with CuI but not MeONa for 3 days. However, no conversion (aromatization) was observed.

**8-Bromo-6-methoxy-1,2,3,4-tetrahydroquinoline (3).** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 6.90 (d, 1H, *J*<sub>75</sub> = 2.4 Hz, 1H, H-7), 6.57 (d, *J*<sub>57</sub> = 2.4 Hz, 1H, H-5), 4.1 (s, 1H, NH), 3.73 (s, 3H, -OMe), 3.32 (t, *J*<sub>23</sub> = 5.6 Hz, 2H), 2.74 (t, *J*<sub>43</sub> = 6.4 Hz, 2H), 1.94 (m, 2H).

**6-Bromo-8-methoxy-1,2,3,4-tetrahydroquinoline (4).** Yellow oil. IR (KBr, cm<sup>-1</sup>): 3424 (NH), 2931, 2836, 1579, 1500, 1463, 1413, 1359, 1328, 1247, 1191, 1108, 1012, 867, 829, 732, 567; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 6.77 (d, *J*<sub>57</sub> = 1.6 Hz, 1H, H-5), 6.72 (d, *J*<sub>75</sub> = 1.6 Hz, 1H, H-7), 4.1 (s, 1H, NH), 3.82 (s, 3H, -OCH<sub>3</sub>), 3.32 (t, *J*<sub>23</sub> = 5.5 Hz, 2H), 2.74 (t, *J*<sub>43</sub> = 6.4 Hz, 2H), 1.94 (m, *J*<sub>32</sub> = 5.5 Hz, *J*<sub>34</sub> = 6.3 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 146.8, 133.6, 125.3, 124.1, 110.8, 107.1, 55.6 (-OMe), 41.3, 26.5, 22.0; MS *m/z* (rel. int.%): 240 (50, M<sup>+</sup>), 242 (48), 225 (52), 226 (50), 145 (100), 146 (34), 129 (14), 117 (18), 101 (9), 90 (14), 76 (9), 62 (10); Anal. Calcd for C<sub>10</sub>H<sub>12</sub>BrNO (242.11): C, 49.61%; H, 5.00%; found: C 49.57%; H 4.95%.

**6,8-Dimethoxy-1,2,3,4-tetrahydroquinoline (5).** Pale brown oil. IR (KBr, cm<sup>-1</sup>): 3411 (NH), 2931, 2836, 2358, 2331, 1602, 1504, 1461, 1374, 1274, 1251, 1215, 1197, 1149, 1112, 1053, 1016, 935, 829; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 6.2 (d, *J*<sub>57</sub> = 2.1 Hz, 1H, H<sub>5</sub>), 6.3 (d, *J*<sub>75</sub> = 2.1 Hz, 1H, H-7), 3.29 (t, *J*<sub>23</sub> = 5.1 Hz, 2H, H-2), 1.97 (m, *J*<sub>32</sub> = 5.4 Hz, *J*<sub>34</sub> = 6.3 Hz, 2H, H-3), 2.76 (t, *J*<sub>43</sub> = 6.4 Hz, 2H, H-4), 3.8 (s, 3H, OMe), 3.75 (s, 3H, OCH<sub>3</sub>) 4.1 (s, NH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 151.6, 147.7, 128.5, 122.0, 104.8, 96.8, 55.8, 55.4, 41.8, 27.0, 22.5; MS *m/z* (rel. int.%): 192 (50, M<sup>+</sup>), 177 (100), 134 (24), 117 (6), 106 (7), 90 (6), 76 (5), 64 (4); Anal. Calcd for C<sub>11</sub>H<sub>15</sub>NO<sub>2</sub> (193.24): C, 68.37%; H, 7.82%; found: C 68.32%; H 7.78%.

### 4.3. Synthesis of 6,8-dimethoxyquinoline (6)

DDQ (504 mg, 1.76 mmol) in dry benzene (20 mL) was added to a solution of 6,8-dimethoxide **5** (165 mg, 0.84 mmol) in dry benzene (20 mL). The reaction mixture was stirred at 80 °C for 40 h under an Ar atmosphere. Reaction progress was monitored by TLC. After the mixture had cooled, the dark green solid was filtered,

and the solvent was removed under reduced pressure. The residue was purified by a short silica column (1:9, AcOEt/hexane). 6,8-Dimethoxyquinoline (**6**) was obtained in a yield of 78% (145 mg), as a yellowish oil: (**6**). IR (KBr,  $\text{cm}^{-1}$ ): 2958, 2937, 1620, 1596, 1575, 1504, 1450, 1423, 1380, 1332, 1261, 1216, 1190, 1167, 1116, 1051, 1130, 995, 941, 835, 788, 770, 665, 620;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.57 (d,  $J_{23} = 4.0$  Hz, 1H, H-2), 7.75 (d,  $J_{43} = 8.2$  Hz, 1H, H-4), 7.14 (dd,  $J_{32} = 4.1$  Hz,  $J_{34} = 8.2$  Hz, 1H, H-3), 6.5 (d, 1H), 6.4 (d, 1H), 3.85 (s, 3H, OMe), 3.68 (s, 3H, OMe);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  158.1, 156.1, 146.5, 136.7, 134.5, 129.8, 121.9, 101.0, 96.7, 55.8 ( $\text{OCH}_3$ ), 55.3 ( $\text{OCH}_3$ ); MS  $m/z$  (rel. int.%): 187 (100,  $\text{M}^+$ ), 188 (70), 189 (10), 159 (59), 158 (42), 157 (44), 144 (21), 143 (22), 129 (35), 115 (46), 116 (27), 102 (22), 88 (29), 75 (22), 62 (22). Anal. Calcd for  $\text{C}_{11}\text{H}_{11}\text{NO}_2$  (189.21): C, 69.83%; H, 5.86%; found: C 69.74%; H 5.81%.

#### 4.4. Synthesis of 8-bromo-6-methoxyquinoline (**8**) and 6-bromo-8-methoxyquinoline (**9**)

A solution of DDQ (504 mg, 1.76 mmol, 2.1 eq) in dry benzene (20 mL) was added to a mixture of 8-bromo-6-methoxide **3** and 6-bromo-8-methoxide **4** mixture (50:50, 200 mg, 0.83 mmol) in dry benzene (20 mL). The mixture was refluxed at 80 °C for 2 days under an Ar atmosphere. Reaction progress was monitored by TLC. After completing the reaction, filtering off the a dark green solid and removing the solvent under reduced pressure, the reaction mixture (200 mg) was chromatographed (silica gel, 25 g) by eluting with AcOEt/hexane (600 mL, 1:9). 6-Bromo-8-methoxyquinoline **9** (78 mg, 44%) and 8-bromo-6-methoxyquinoline **8** (76 mg, 42%) were isolated in their pure forms and their  $R_f$  values were 0.65 and 0.40, respectively (AcOEt/hexane, 1:9).

**8-Bromo-6-methoxyquinoline (8)**. White solid; mp: 78-79 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.94 (d, 1H, H-2), 8.05 (d,  $J_{43} = 4.0$  Hz, 1H), 7.43 (dd,  $J_{32} = 4$  Hz, 1H, H-3), 7.15 (d,  $J_{57} = 1.6$  Hz, 1H, H-5), 7.58 (d,  $J_{75} = 1.6$  Hz, 1H, H-7), 4.10 (s, 1H, OMe);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  156.0, 149.4, 139.0, 135.0, 130.0, 125.3, 122.6, 121.6, 120.5, 56.4; Anal. Calcd for  $\text{C}_{10}\text{H}_8\text{BrNO}$  (238.08): C, 50.45%; H, 3.39%; found: C 50.40%; H 3.33%.

**6-Bromo-8-methoxyquinoline (9)**. White solid; mp: 75-76 °C. IR (KBr,  $\text{cm}^{-1}$ ): 3027, 2358, 2341, 1585, 1546, 1467, 1440, 1348, 1301, 1232, 1181, 1151, 1081, 1021, 962, 862, 808, 775, 603, 593;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.91 (dd,  $J_{24} = 2.8$  Hz,  $J_{23} = 4.0$  Hz, 1H, H-2), 8.07 (dd,  $J_{43} = 8.0$  Hz, 1H), 7.47 (dd,  $J_{32} = 4.0$  Hz,  $J_{34} = 8.0$  Hz, 1H, H-3), 7.10 (d,  $J_{57} = 2.8$  Hz, 1H, H-5), 7.78 (d,  $J_{75} = 2.8$  Hz, 1H, H-7), 3.94 (s, 1H, -OMe);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  157.5, 148.6, 141.3, 135.6, 130.9, 130.0, 125.9, 122.2, 105.4, 55.8; MS  $m/z$  (rel. int.%): 236 (6,  $\text{M}^+$ ), 238 (6), 205 (35), 208 (35), 126 (100), 127 (14), 98 (34), 97 (19), 73 (44), 74 (25), 63 (39), 49 (50); Anal. Calcd for  $\text{C}_{10}\text{H}_8\text{BrNO}$  (238.08): C, 50.45%; H, 3.39%; found: C 50.41%; H 3.32%.

#### 4.5. Synthesis of 6,8-dimethoxyquinoline (**6**)

Freshly cut sodium (0.7 g, 30 mmol) was added to dry methanol (25 mL) under nitrogen gas atmosphere. When dissolution was complete, the warm solution was diluted with dry dimethylformamide by addition of vacuum dried cuprous iodide (0.49 g, 1.72 mmol). After dissolution, 6,8-dibromoquinoline (**3**) (0.5 g, 1.72 mmol) was added to dry DMF (15 mL). The reaction mixture was stirred magnetically under a nitrogen gas atmosphere at reflux (ca. 150 °C) for 20 h. The reaction's progress was monitored by TLC until the starting material was all consumed. After cooling to room temperature,  $\text{H}_2\text{O}$  (50 mL) and chloroform (70 mL) were added to the reaction mixture. The organic layers were separated, washed with  $\text{H}_2\text{O}$  ( $2 \times 20$  mL), and dried over sodium

sulfate. The solvent was removed and the crude product was passed through a short column packed with silica gel (5 g). After filtration and purification, the resultant product was 6,8-dimethoxyquinoline (**6**) in a yield of 84% (0.28 g), as a pure yellowish oil substance.

#### 4.6. Synthesis of 6,8-bis(trimethylsilyl)quinoline (**11**)

*n*-Butyl lithium (2.65 mL, 1.6 M, 4.26 mmol, 4.1 eq) was added to a vacuum-dried flask containing dibromide **7** (0.3 g, 1.04 mmol) in THF (20 mL) at  $-78$  °C and stirred for 90 min. Next, chlorotrimethylsilane ( $\text{Me}_3\text{SiCl}$ , 0.24 g, 2.2 mmol) was added to the mixture at  $-78$  °C, and the mixture was stirred at  $-78$  °C for 90 min and then at room temperature for 1 h. After quenching with water (30 mL), the aqueous layer was extracted with diethyl ether ( $3 \times 25$  mL). The combined organic layers were washed sequentially with water ( $3 \times 25$  mL) and dried over sodium sulfate. The solvent was removed by reduced pressure, and the crude product was purified using short silica gel column chromatography (hexane,  $R_f = 0.5$ ). Recrystallization from  $\text{CH}_2\text{Cl}_2$ /hexane (2:1, 6 mL) gave product **11** (240 mg, 76%). Pale yellow solid; mp:  $70$ – $72$  °C. IR (KBr,  $\text{cm}^{-1}$ ): 3048, 3015, 2952, 2897, 1600, 1558, 1475, 1401, 1338, 1257, 1233, 1139, 1094, 998, 871, 833, 808, 772, 745, 688, 616;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.91 (dd,  $J_{23} = 4$  Hz,  $J_{24} = 1.6$  Hz, 1H, H-2), 7.37 (dd,  $J_{32} = 4$  Hz,  $J_{34} = 8$  Hz, 1H, H-3), 8.12 (dd,  $J_{42} = 1.6$  Hz,  $J_{43} = 8$  Hz, 1H, H-4), 8.0–7.98 (d,  $\text{H}_5$ ,  $\text{H}_7$ ), 0.48 (s, 9H,  $\text{Si}(\text{CH}_3)_3$ ), 0.38 (s, 9H,  $\text{Si}(\text{CH}_3)_3$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  152.9, 149.4, 140.0, 139.5, 138.0, 136.0, 135.1, 126.9, 120.7,  $-0.15$ ,  $-1.06$ ; MS  $m/z$  (rel. int.%): 271 (21,  $\text{M}^+$ ), 272 (14), 273 (6), 257 (100), 258 (22), 259 (10), 241 (7), 227 (8), 198 (10), 169 (7), 155 (8), 121 (20), 107 (7), 72 (13), 68 (13); Anal. Calcd for  $\text{C}_{15}\text{H}_{23}\text{NSi}_2$  (273.52): C, 65.87%; H, 8.48%. Found: C, 65.78%; H, 8.41%.

#### 4.7. Synthesis of 6,8-bis(methylthio)quinoline (**12**)

*n*-Butyl lithium (2.65 mL, 1.6 M, 4.26 mmol, 4.1 eq) was added to a vacuum-dried flask containing dibromide **7** (0.3 g, 1.04 mmol) in THF (20 mL) at  $-78$  °C. After stirring for 90 min, 1,2-dimethyldisulfate ( $(\text{CH}_3\text{S})_2$ , 1.96 mL, 2.08 mmol) was added to the mixture at  $-78$  °C. The mixture was stirred at  $-78$  °C for 90 min and then at room temperature for 1 h. After quenching the reaction with water (30 mL), the aqueous layer was extracted with diethyl ether ( $3 \times 25$  mL). The combined organic layers were washed sequentially with water ( $2 \times 25$  mL) and dried over sodium sulfate. The solvent was removed by reduced pressure, and the crude product was purified using short silica gel column chromatography (hexane,  $R_f = 0.55$ ). Recrystallization from  $\text{CH}_2\text{Cl}_2$ /hexane (2:1, 5 mL) gave compound **12** (180 mg, 78%). Pale green sphere; mp:  $92$ – $94$  °C. IR (KBr,  $\text{cm}^{-1}$ ): 3060, 3022, 2982, 2359, 2338, 1582, 1555, 1474, 1420, 1365, 1185, 995, 968, 865, 830, 787, 769, 607;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.83 (dd,  $J_{23} = 4.4$  Hz,  $J_{24} = 1.6$  Hz, 1H, H-2), 8.00 (dd,  $J_{42} = 1.6$  Hz,  $J_{43} = 8.4$  Hz, 1H, H-4), 7.4 (dd,  $J_{32} = 4.4$  Hz,  $J_{34} = 8.4$  Hz, 1H, H-3), 7.24 (d, 1H), 7.22 (d, 1H), 2.59 (s, 3H,  $\text{SCH}_3$ ), 2.57 (s, 3H,  $\text{SCH}_3$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  148.2, 143.8, 140.6, 137.8, 135.0, 128.4, 122.2, 122.0, 118.0, 15.6, 14.3; MS  $m/z$  (rel. int.%): 220 (100,  $\text{M}^+$ ), 221 (15), 222 (10), 187 (64), 188 (8), 175 (15), 174 (29), 173 (35), 159 (18), 128 (15), 129 (15), 115 (20), 102 (18), 86 (15), 80 (5); Anal. Calcd for  $\text{C}_{11}\text{H}_{11}\text{NS}_2$  (221.34): C, 59.69%; H, 5.01%; found: C 59.60%; H 4.92%.

#### 4.8. Synthesis of quinoline-6,8-dicarbaldehyde (**13**)

*n*-Butyl lithium (2.65 mL, 1.6 M, 4.26 mmol, 4.1 eq) was added to a solution of dibromide **7** (0.3 g, 1.04 mmol) in THF (20 mL) in a vacuum-dried flask at  $-78$  °C. The mixture was stirred for 90 min, and then

dimethylformamide (DMF, 0.15 g, 2.08 mmol) was added to the mixture at  $-78\text{ }^{\circ}\text{C}$ . The mixture was stirred at  $-78\text{ }^{\circ}\text{C}$  for 90 min and then at room temperature for 1 h. After quenching with water (30 mL), the aqueous layer was extracted with diethyl ether ( $3 \times 25\text{ mL}$ ). The combined organic layers were washed sequentially with water ( $3 \times 25\text{ mL}$ ) and dried over sodium sulfate. The solvent was removed by reduced pressure, and the crude product was purified by short silica gel column chromatography (AcOEt/hexane, 2:1,  $R_f = 0.23$ ). Recrystallization of the product from  $\text{CH}_2\text{Cl}_2$ /hexane (2:1, 5 mL) gave pure **13** (57 mg, 31%). White sphere; mp: 193–195  $^{\circ}\text{C}$ . IR (KBr,  $\text{cm}^{-1}$ ): 3056, 3037, 2865, 1697, 1677, 1608, 1572, 1494, 1460, 1425, 1257, 1157, 977, 900, 786, 745, 640, 574;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.21 (dd,  $J_{23} = 4.1\text{ Hz}$ ,  $J_{24} = 1.8\text{ Hz}$ , 1H, H-2), 8.80 (d,  $J_{75} = 1.6\text{ Hz}$ , 1H, H-7), 8.67 (d,  $J_{57} = 1.6\text{ Hz}$ , 1H, H-5), 8.46 (d,  $J_{43} = 8.36\text{ Hz}$ , 1H, H-4), 7.67 (dd,  $J_{32} = 4.3\text{ Hz}$ ,  $J_{34} = 8.2\text{ Hz}$ , 1H, H-3), 11.48 (s, 1H, -CHO), 10.30 (s, 1H, -CHO);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  191.8, 190.7, 153.8, 149.7, 137.8, 136.8, 133.7, 132.7, 128.2, 127.8, 122.9; MS  $m/z$  (rel. int.%): 185 (100,  $\text{M}^+$ ), 186 (9.2), 156 (11.5), 127 (13.7), 118 (7.5), 101 (7.4), 86 (5), 76 (10), 56 (9); Anal. Calcd for  $\text{C}_{11}\text{H}_7\text{NO}_2$  (185.18): C, 71.35%; H, 3.81%; found: C 71.30%; H 3.74%.

#### 4.9. Synthesis of the cyanoquinolines

6,8-Dibromoquinoline (**7**) (582 mg, 2 mmol) was dissolved in freshly distilled DMF (50 mL) and mixed with cuprous cyanide (7.16 g, 8 mmol, 4 eq). The reaction mixture was stirred magnetically at reflux (ca. 150  $^{\circ}\text{C}$ ) under Ar for 6 h. The hot resulting brown mixture was poured into a solution of hydrated ferric chloride (4 g) and concentrated hydrochloric acid (1 mL) in water (10 mL) (any remaining residues were conveniently transferred with hot dimethylformamide). The reaction mixture was maintained at 60–70  $^{\circ}\text{C}$  for 20 min to decompose the complex.

The separatory funnel was lit with light to observe the layers because the interface was obscured by dark colors. The aqueous layer was extracted with warm toluene ( $4 \times 50\text{ mL}$ ), and the extracts were combined with the organic layer, washed with dilute hydrochloric acid (1:1, 25 mL), water, and 10% aqueous sodium hydroxide. The remaining organic layer was filtrated to remove the dark insoluble matter and dried over  $\text{Na}_2\text{SO}_4$ . After evaporation of the solvent, NMR analysis of the residue (0.38 g) indicated the formation of **14**, **15**, and **16** in a ratio of 12:47:22, respectively, and the reaction conversion was 83%.

The reaction mixture (380 mg) was applied to a short silica gel (60 g) chromatography column eluting with AcOEt/hexane (600 mL, 1:9). 6-Bromo-8-cyanoquinoline **15** was collected as the first eluent (190 mL, 46 mg, 9%), and then the solvent polarity was increased to a ratio of 2:9 (AcOEt/hexane). 8-Bromo-6-cyanoquinoline **14** (240 mL, 200 mg, 43%) was next obtained, and then the polarity of the solvent was increased to the ratios of 3:9 and 4:9 (AcOEt/hexane). 6,8-Dicyanoquinoline **16** (240 mL, 70 mg, 20%) was isolated in pure form.

The reaction was repeated for 16 h using the same reaction conditions. After the reaction, a complex reaction mixture was obtained; polymerization likely occurred.

The reaction was repeated at 100  $^{\circ}\text{C}$  for 2 days under the same reaction conditions. However, the reaction did not run at the reaction temperature.

**8-Bromo-6-cyanoquinoline (14)**. White needle crystalline; mp: 176–178  $^{\circ}\text{C}$ . IR (KBr,  $\text{cm}^{-1}$ ): 3076, 3046, 2229, 1581, 1567, 1483, 1361, 1307, 1238, 1200, 1135, 1093, 1032, 887, 840, 785, 723;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.13 (dd,  $J_{23} = 4\text{ Hz}$ ,  $J_{24} = 2\text{ Hz}$ , 1H, H-2), 7.60 (dd,  $J_{32} = 4\text{ Hz}$ ,  $J_{34} = 8\text{ Hz}$ , 1H, H-3), 8.19 (dd,  $J_{43} = 8\text{ Hz}$ ,  $J_{42} = 1.6\text{ Hz}$ , 1H, H-4), 8.22 (d,  $J_{57} = 2.0\text{ Hz}$ , 1H, H-5), 8.25 (d, 1H, H-7);  $^{13}\text{C}$  NMR (100

MHz, CDCl<sub>3</sub>):  $\delta$  152.8, 146.2, 138.3, 135.4, 134.9, 129.2, 123.6, 119.0, 115.7, 115.0; MS  $m/z$  (rel. int.%): 231 (100, M<sup>+</sup>), 233 (98), 152 (64), 125 (46), 98 (32), 75 (29), 78 (28), 62 (21), 50 (18), 49 (22); Anal. Calcd for C<sub>10</sub>H<sub>5</sub>BrN<sub>2</sub> (233.06): C, 51.53%; H, 2.16%. Found: C, 51.48%; H, 2.13%.

**6-Bromo-8-cyanoquinoline (15).** White needle crystalline; mp: 180–182 °C. IR (KBr, cm<sup>-1</sup>): 3073, 3046, 2231, 1583, 1563, 1483, 1363, 1311, 1236, 1098, 1141, 1095, 1033, 883, 842, 784, 725; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  9.08 (d,  $J_{23} = 4$  Hz, 1H, H-2), 7.58 (dd,  $J_{32} = 4$  Hz,  $J_{34} = 8$  Hz, 1H, H-3), 8.17–8.22 (m, 3H, H-4, H-5, H-7); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  152.8, 146.1, 138.2, 135.5, 134.9, 129.2, 123.6, 119.0, 115.8, 114.8; MS  $m/z$  (rel. int.%): 231 (100, M<sup>+</sup>), 233 (97), 232 (11), 152 (65), 125 (48), 98 (34), 75 (30), 74 (30), 62 (22), 49 (23), 50 (20); Anal. Calcd for C<sub>10</sub>H<sub>5</sub>BrN<sub>2</sub> (233.06): C, 51.53%; H, 2.16%. Found: C, 51.49%; H, 2.11%.

**6,8-Dicyanoquinoline (16).** Yellow sphere; decomposed at melting point (260 °C). IR (KBr, cm<sup>-1</sup>): 3062, 2362, 2229, 1590, 1569, 1488, 1452, 1375, 1321, 1163, 1031, 930, 902, 881, 785; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  9.27 (dd,  $J_{23} = 4$  Hz,  $J_{24} = 1.6$  Hz, 1H, H-2), 7.74 (dd,  $J_{32} = 4$  Hz,  $J_{34} = 8$  Hz, 1H, H-3), 8.36 (dd,  $J_{43} = 8.4$  Hz,  $J_{42} = 1.6$  Hz, 1H, H-4), 8.5 (d,  $J_{57} = 1.6$  Hz, 1H, H-5), 8.3 (d,  $J_{75} = 1.6$  Hz, 1H, H-7); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  155.3, 148.2, 138.2, 136.8, 135.8, 127.8, 124.4, 116.5, 115.5, 115.2, 110.5; MS  $m/z$  (rel. int.%): 178 (100, M<sup>+</sup>), 179 (13), 151 (35), 124 (10), 98 (9), 74 (17), 61 (6); Anal. Calcd for C<sub>11</sub>H<sub>5</sub>N<sub>3</sub> (179.18): C, 73.74%; H, 2.81%. Found: C, 73.70%; H, 2.77%.

#### 4.10. Cell culture and cell proliferation assay

HeLa cells (human cervix carcinoma), HT29 (human colon carcinoma) cells, and C6 (rat brain tumor cells) were grown in Dulbecco's modified eagle's medium (DMEM, Sigma) and supplemented with 10% (v/v) fetal bovine serum (Sigma, Germany) and PenStrep solution (Sigma, Germany) at 37 °C in a 5% CO<sub>2</sub> humidified atmosphere. For the proliferation assay, cells were plated in 96-well culture plates (COSTAR, Corning, USA) at a density of 30,000 cells per well. Vehicle (DMSO), 5-Fluorouracil (5-FU), and various quinoline compounds at the concentrations of (5, 10, 20, 30, 40, 50, 75, and 100  $\mu$ g/mL) were added to each well. The concentration of the DMSO was less than 0.5% in all assays. The antiproliferative activities of the compounds were tested by using a BrdU Cell Proliferation ELISA kit (Roche, Germany) according to the manufacturer's procedure. Briefly, the cells were incubated with the compounds overnight before applying the BrdU Cell Proliferation ELISA reagent. Then the cells were pulsed with a BrdU labeling reagent for 4 h, followed by fixation in a FixDenat solution for 30 min at room temperature. Thereafter, cells were incubated with a 1:100 dilution of anti-BrdU-POD for 90 min at rt. The amount of cell proliferation was assessed by determining the A450 nm of the culture media after the addition of the substrate solution using a microplate reader (Ryto, China). The results were reported as percentages based on the inhibition of cell proliferation, where the optical density measured for the vehicle-treated cells was considered to be 100% proliferated. The experiments were repeated at least twice. The percent inhibition of cell proliferation was calculated as follows:  $[1 - (A_{treatments}/A_{vehicle\ control})] \times 100$ .

#### 4.11. Statistical analysis

The data are presented as the means  $\pm$  SEM of the 6 measurements for each cell type. The differences between the treatment groups were tested using ANOVA and P values, where values of  $<0.05$  were considered significant.

## 5. Acknowledgments

This study was supported by grants from the Scientific and Technological Research Council of Turkey (TÜBİTAK, Project number: 112T394).

## References

1. Lindley, J. *Tetrahedron* **1984**, *40*, 1433–1456.
2. Vladímir, V.; Kouznetsov, V. V.; Vargas Méndez, L. Y.; Carlos, C. M.; Meléndez Gómez, M. *Curr. Org. Chem.* **2005**, *9*, 141–16.
3. Company of a Chemistry Partner of Drug Discovery "BioBloks inc.", **2002**, San Diego, CA, USA. <http://www.bioblocks.com/quinolines.html>.
4. Manske, R. H. F.; Kulka, M. *Org. React.* **1953**, *7*, 59–98.
5. Cheng, C. C.; Yan, S. J. *Org. React.* **1982**, *28*, 37–201.
6. Bergstrom, F. W. *Chem. Rev.* **1944**, *35*, 153.
7. Jones, G. *In The Chemistry of Heterocyclic Compounds*, Weissberger, A.; Taylor, E. C.; Eds.; John Wiley and Sons, Chichester, 1977.
8. Reitsema, R. H. *Chem. Rev.* **1948**, *43*, 47.
9. Bergstrom, F. W. *Chem. Rev.* **1944**, *35*, 156.
10. Sahin, A.; Cakmak, O.; Demirtas, I.; Okten, S.; Tutar, A. *Tetrahedron* **2008**, *64*, 10068–10074.
11. Heiniger, B.; Gakhar, G.; Prasain, K.; Hua, D. H.; Nguyen, T. A. *Anticancer Res.* **2010**, *30*, 3927–3932.
12. Perchellet, E. M.; Crow, K. R.; Gakhar, G.; Nguyen, T. A.; Shi, A.; Hua, D. H.; Perchellet, J. P. *Int. J. Oncol.* **2010**, *36*, 673–688.
13. Shi, A.; Nguyen, T. A.; Battina, S. K.; Rana, S.; Takemoto, D. J.; Chiang, P. K.; Hua, D. H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3364–3368.
14. Solomon, R. V.; Lee, H. *Curr. Med. Chem.* **2011**, *18*, 1488–1508.
15. Alfonsi, M.; Arcadi, A.; Chiarini, M.; Marinelli, F.; *Tetrahedron Lett.* **2011**, *52*, 5145–5148.
16. Lejejs, J.; Bankovskii, Y.; Brovere, A.; Brusilovskii, P.; Zikmund, M. *Chem. Papers* **1991**, *23*, 657–666.
17. Gilman, H.; Benkeser, R. A.; Gainer, Q. S.; Lindbad, A. E.; Marshall, F. I. *J. Am. Chem. Soc.* **1946**, *68*, 1577.
18. Sahin, O. Y.; Okten, S.; Tekin, S.; Cakmak, O. *J. Biotechnol.* **2012**, *161*, 24.
19. Berkenheim, A. M.; Antik, L. V. *Zh. Obshch. Khim.* **1941**, *11*, 537–540.
20. Irving, H.; Pinnington, A. R. *J. Chem. Soc.* **1957**, 290.
21. Eisch, J. J. *J. Org. Chem.* **1962**, *27*, 1318.
22. Arvanitis, A. G.; Gilligan, P. J.; Hartz, R. A. *Patent: US2003/171380 A1*, **2003**.
23. Merch Sharp and Dohme Corp; Coburn, C. A.; Rosenblum, S. B.; Kozlowski, J. A.; Soll, R.; Wu, H.; Hu, B.; Zhong, B. Wang, D.; Shen, C.; Sun, F. *Patent:WO2012/125926 A2*, **2012**.