

Synthesis of 2-substituted 8-propargyloxyquinoline derivatives and determination of their antioxidant, antibacterial, and DNA binding activities

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Abstract: 2-Substituted 8-propargyloxyquinoline derivatives have been synthesized from 2-substituted-8-hydroxyquinolines by O-propargylation method (30%–98% yields). The newly synthesized compounds were tested for in vitro antioxidant activities such as DPPH radical scavenging, ferrous chelating, and reducing power activities. The maximum radical scavenging (46.5%) and reducing power activities were obtained from **1** and maximum ferrous chelating effect was obtained from **6** (72.1%) at the concentration of 500 $\mu\text{g/mL}$. To indicate DNA binding activity of the complexes calf thymus DNA was used. The compounds were also evaluated for their antimicrobial activity against three gram-positive and three gram-negative bacteria. Compounds **3** and **5** exhibited antibacterial activity against all tested gram-positive bacteria.

Key words: Quinoline, propargyloxyquinoline, antioxidant, antibacterial, DNA binding activity

1. Introduction

Heterocyclic compounds play an important role in designing new classes of structural entities of medicinal importance with potentially new mechanisms of action. These heterocyclic compounds are well known to possess diverse pharmacological activities including antimicrobial, anticancer, anticonvulsant, and antimalarial activities.^{1–3} Among the important heterocyclic moieties of biological and pharmacological interest, the quinoline ring is endowed with various activities, such as antituberculosis,⁴ antimalarial,⁵ antiinflammatory,⁶ anticancer,⁷ antibiotic,⁸ antihypertensive,⁹ tyrosine kinase (PDGF-RTK) inhibition,¹⁰ and anti-HIV^{11,12} (Figure 1). The quinoline ring plays an important role in the development of new anticancer agents as their derivatives have shown excellent results through different mechanisms of action such as growth inhibitors by cell cycle arrest, apoptosis, inhibition of angiogenesis, disruption of cell migration, and modulation of nuclear receptor responsiveness.^{13–15} They also find application in agrochemicals and affect chemicals such as dye stuffs and corrosion inhibitors.¹⁶

Alkynes or acetylenic compounds play an important role as building blocks in many synthetic transformations and new materials. In addition, an acetylenic group is a common structural motif found in various natural products and also attracts great interest in medicinal chemistry and pharmaceutical industry.¹⁷ It moreover functions as a key pharmacophoric unit in acetylenic antibiotics¹⁸ and its presence in anticancer¹⁹ and antitubercular²⁰ agents is noteworthy.

Molecular conjugation has been known for the rational design of new biologically active entities by

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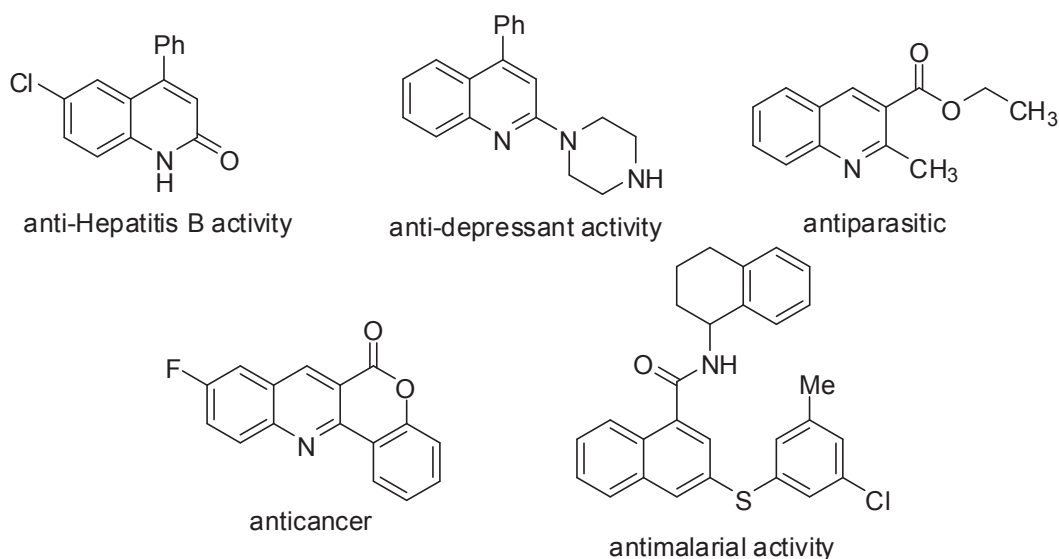


Figure 1. Biologically active quinoline derivatives.

fusion of compounds and/or pharmacophoric units recognized and derived from known bioactive molecules. Inspired by the biological profile of the quinoline nucleus and the increasing importance of acetylenic groups in pharmaceutical and biological fields, here we aimed to synthesize 2-substituted 8-propargyloxyquinoline derivatives to obtain certain new chemical entities having the potential to show various biological activities.

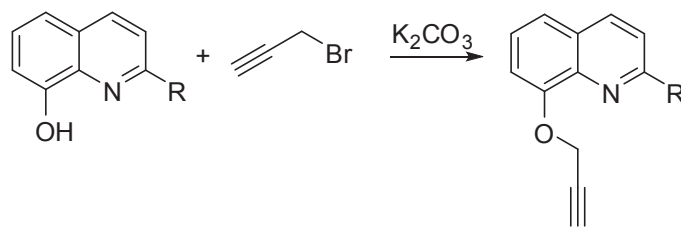
2. Results and discussion

2.1. Chemistry

The terminal alkyne moiety on 8-(prop-2-ynyloxy)quinoline skeletons **1–9** makes them valuable precursors for the synthesis of triazole derivatives via the Huisgen dipolar cycloaddition method using click chemistry. In the literature, there are various examples of quinoline-based triazole compounds derived from 8-(prop-2-ynyloxy)quinoline **1**, **2**, and **5**, which have been used as fluorescence metal sensors and antibacterial agents.^{21–29}

Propargyl-substituted quinoline derivatives **1–9** were synthesized by O-propargylation method with the addition of propargyl bromide in the presence of K_2CO_3 to commercially available 2-substituted 8-hydroxyquinoline derivatives (Scheme).

For the construction of bis(prop-2-ynyloxy) quinoline derivatives **7** and **8**, quinoline-2,8-diol was subjected to a propargylation process and bis-propargylated quinoline **7** was obtained. In addition, O,N-propargylated quinoline derivative **8** was obtained as a tautomerization product (Table 1).



Scheme. Synthesis of 8-(prop-2-ynyloxy)quinoline derivatives.

8-Hydroxyquinoline-2-carboxylic acid was reacted with propargyl bromide following the same procedure and bis-propargylated quinoline carboxylate **9** was obtained with good yield (Table 1).

Tautomeric equilibrium should be responsible for the relatively low yield during the synthesis of **7** from dihydroxy derivative (quinoline-2,8-diol). Indeed, **8** was obtained with a better yield. In order to find the major tautomeric component, computational calculations were performed. 3-Dimensional structures of compounds **3**, **3-T**, **7**, and **8** were obtained by geometry optimization using density functional theory at B3LYP/6-311++G(d,p) level with no symmetry restrictions (Figure 2). Full optimization of all bond lengths, bond angles, and torsional angles was achieved by using the aforementioned method successfully. The Gaussian 09W package program was used to perform all computational calculations. For each quinoline derivative, vibrational analyses were done using the same basis set employed in the corresponding geometry optimizations. The frequency analysis did not yield any imaginary frequencies, indicating that the structure of each molecule corresponds to at least a local minimum on the potential energy surface. The normal mode analysis was performed for $3N-6$ vibrational degrees of freedom, N being the number of atoms forming the corresponding molecule.

The results of the computations indicate that **3** and its corresponding propargylated derivative **7** are less stable than **3-T** (N-H tautomer) and its corresponding propargylated derivative **8**, respectively. The ground state energy value difference between **3** and **3-T** and that of **7** and **8** is 5.2 J/mol and 7.8 J/mol, respectively.

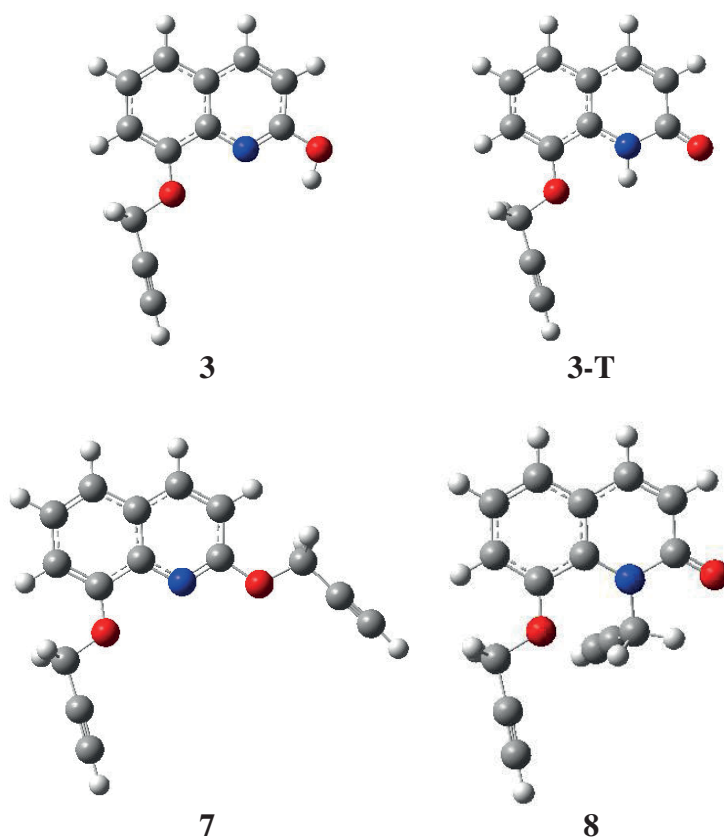
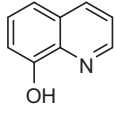
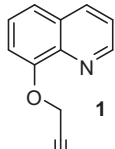
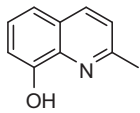
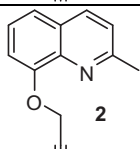
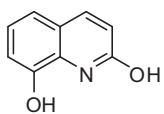
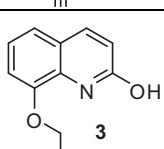
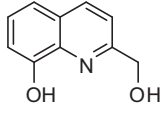
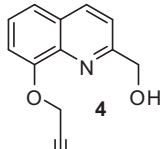
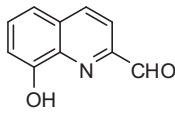
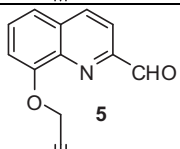
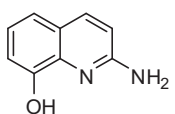
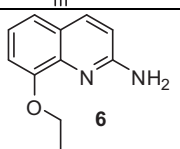
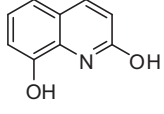
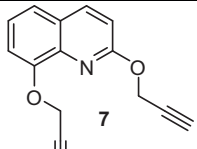
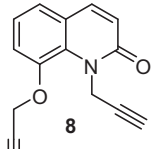
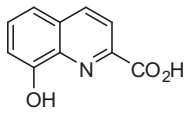
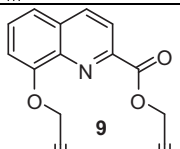


Figure 2. Geometry optimized structures of **3**, **3-T**, **7**, and **8**.

Table 1. 2-Substituted 8-propargyloxyquinoline derivatives.

Entry	starting	product	Yield (%)
1			95
2			93
3			82
4			85
5			86
6			85
7			30
			45
8			85

2.2. Antioxidant activity

2.2.1. DPPH radical scavenging activity

The DPPH method is known as a quick, easy, and practical application for screening many compounds for radical scavenging activity.³⁰ The decrease in absorbance caused by the reduction of DPPH in the medium is used to assess antioxidant activity. The radical scavenging activities for the compounds are shown in Figure 3. It can be seen from the results that scavenging activity increased with the increasing concentration. Compounds **1** (46.5%) and **7** (24.4%) exhibited remarkable activity at a concentration of 500 $\mu\text{g}/\text{mL}$, while other compounds did not show significant free radical scavenging activity. Trolox showed 99.4% free radical scavenging activity at 500 $\mu\text{g}/\text{mL}$.

2.2.2. Chelating activity

It is well known that chelating agents are important for delaying radical degradation. The main strategy of preventing ROS formation associated with redox active metal catalysis involves chelating metal ions.³¹ As shown in Figure 4, the maximum chelating activities of **6**, **1**, **3**, **5**, **7**, and **4** were 72.1%, 7.6%, 6.5%, 4.8%, 4.6%, and 3.7% at a concentration of 500 $\mu\text{g}/\text{mL}$, respectively. The percentage chelating activity of compound **6** increased from 8.7% to 72.1% with an increase in concentration from 25 to 500 $\mu\text{g}/\text{mL}$. EDTA exhibited higher chelating activity than the test compounds, showing 99.5% chelating activity at a concentration of 500 $\mu\text{g}/\text{mL}$.

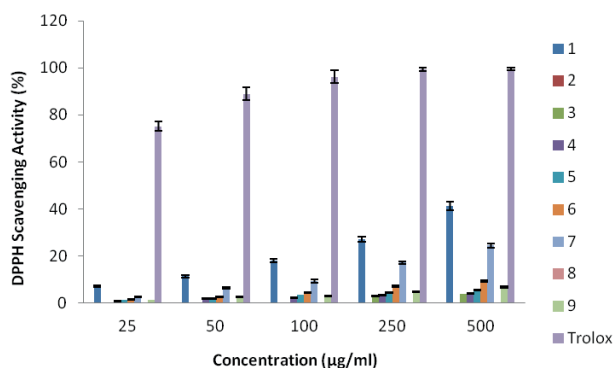


Figure 3. Radical scavenging activity percentages of the compounds.

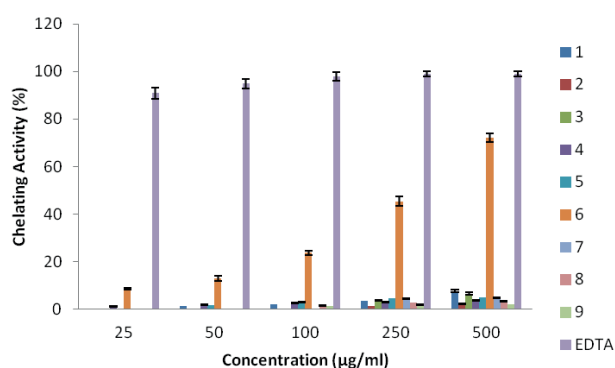


Figure 4. Chelating effect of synthesized compounds on ferrous ion.

2.2.3. Reducing power

Reducing power may provide important information on antioxidant activity. Compounds having reducing power are electron donors and can reduce oxidized intermediates of lipid peroxidation processes so that they can be used as primary and secondary antioxidants.³² High absorbance value indicates high reducing power capacity of the studied compound. Figure 5 showed that reducing power activity increases with increasing sample concentration in the test range. According to the results, the compound with highest activity was **1** with an absorbance value of 0.512 at a concentration of 500 $\mu\text{g}/\text{mL}$. At this concentration value, **1** was followed by **7** at 0.372, **5** at 0.236, **4** at 0.179, **9** at 0.158, and **2** at 0.144. Test compounds showed lower reducing power activity than α -tocopherol at all concentration.

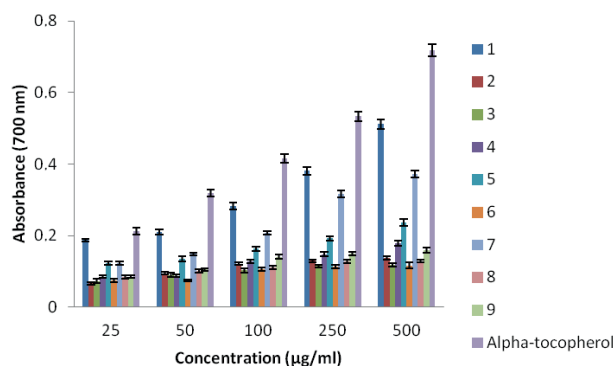


Figure 5. Reducing power of compounds.

2.3. Antimicrobial activity

The inhibition zones of the newly synthesized complexes against bacteria at the end of the incubation time at 37 °C are given in Table 2. According to results, compounds **3** and **5** were observed to have antibacterial activity against all tested gram-positive bacteria and compounds **3**, **5**, and **2** showed antimicrobial activity against gram-negative *Legionella pneumophila*. On the other hand, compounds **7**, **4**, **6**, and **9** did not exhibit antibacterial activity against any bacteria. Compounds **3** and **5** showed highest activity (12 mm) against *Staphylococcus aureus* and *Enterococcus hirae*, respectively. All compounds showed lower activity against test bacteria than standard antibiotics. Further modification can be done on the structure of the compounds (especially **5**) for better drug candidates in the future.

Table 2. Antimicrobial effect of synthesized compounds.

Bacteria	Compounds and standard antibiotic disks ^a										
	1	2	3	4	5	6	7	8	9	S	TE
<i>S. aureus</i>	0	0	12	0	12	0	0	0	0	16	24
<i>B. cereus</i>	8	0	7	0	9	0	0	7	0	14	20
<i>E. hirae</i>	0	0	10	0	12	0	0	0	0	19	22
<i>E. coli</i>	0	0	0	0	0	0	0	0	0	24	23
<i>P. aeruginosa</i>	0	0	0	0	0	0	0	0	0	22	14
<i>L. pneumophila</i>	0	11	10	0	11	0	0	0	0	15	21

^a Inhibition diameter in millimeters. S = Streptomycin (10 µg) and TE = tetracycline (30 µg).

2.4. DNA binding activity

In this study, the DNA binding activity was monitored by gel electrophoresis. It is stated that DNA has three important factors in the mobility of gel electrophoresis. These factors are the charge, flexibility, and size. The binding of DNA to the compounds causes it to move more slowly in the gel because the bound DNA turns into a larger structure than the free DNA.³³ Agarose gel electrophoresis results showed that **1** migrated more slowly than the control. However, the other compounds did not act on CT-DNA as there was not a difference in the bands compared to the control DNA (Figure 6).

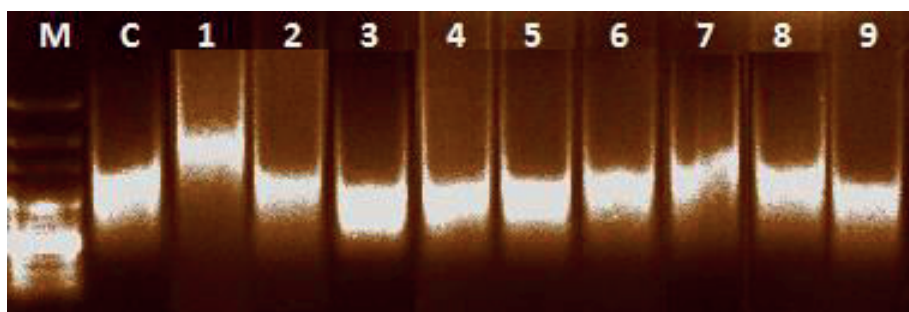


Figure 6. DNA binding of newly synthesized complexes. Lane M, DNA marker; Lane C, control, CT-DNA; Lane 1, CT-DNA + 500 $\mu\text{g/mL}$ of **1**; Lane 2, CT-DNA + 500 $\mu\text{g/mL}$ of **2**; Lane 3, CT-DNA + 500 $\mu\text{g/mL}$ of **3**; Lane 4, CT-DNA + 500 $\mu\text{g/mL}$ of **4**; Lane 5, CT-DNA + 500 $\mu\text{g/mL}$ of **5**; Lane 6, CT-DNA + 500 $\mu\text{g/mL}$ of **6**; Lane 7, CT-DNA + 500 $\mu\text{g/mL}$ of **7**; Lane 8, CT-DNA + 500 $\mu\text{g/mL}$ of **8**; Lane 9, CT-DNA + 500 $\mu\text{g/mL}$ of **9**.

2.5. Conclusions

We have described the synthesis of 2-substituted 8-propargyloxyquinoline compounds by O-propargylation of commercially available 2-substituted 8-hydroxyquinoline derivatives. In conclusion, the newly synthesized compounds were tested for biological assays such as free radical scavenging, metal chelating, reducing power, antibacterial, and DNA binding activities. Compound **1** exhibited the highest free radical scavenging and reducing power activity. Compound **6** exhibited maximum chelating activity. Compound **5** possessed significant growth inhibition activity against all tested gram-positive bacteria and showed the highest activity against *S. aureus* and *E. hirae*.

3. Experimental

3.1. General

All the chemicals used in the biological tests were purchased from Sigma. (Sigma-Aldrich GmbH, Steinheim, Germany). Antimicrobial test disks were obtained from Oxoid (7.0 mm, Oxoid Ltd., Basingstoke, UK). ^1H NMR and ^{13}C NMR spectra were recorded in CDCl_3 on a Bruker Spectrospin Avance DPX-400 spectrometer. ^1H (400 MHz) and ^{13}C NMR were recorded in CDCl_3 and the chemical shifts are expressed in ppm relative to CDCl_3 (δ 7.26 and 77.0 for ^1H and ^{13}C NMR, respectively) as the internal standard. LC/MS-MS spectra were recorded on a Thermo Scientific Q Exactive instrument. Melting points were measured by a Stuart SMP3 instrument.

Flash column chromatography was performed using thick-walled glass columns and silica gel (60-mesh; Merck). The reactions were monitored by thin-layer chromatography (TLC) using Merck 0.2-mm silica gel 60 F254 analytical aluminum plates, visualized by UV light. All extracts were dried over anhydrous magnesium sulfate and solutions were concentrated under reduced pressure by using a rotary evaporator.

3.2. General procedure for the synthesis of propargyl derivatives 1–9

To a solution of hydroxyquinoline (10 mmol) in dry acetone (25 mL) was added K_2CO_3 (25 mmol). The solution was refluxed for 30 min. After cooling, propargyl bromide (12 mmol, 25 mmol for dipropargyl derivatives) was added dropwise. The mixture was refluxed overnight. The reaction mixture was filtered and the filtrate was concentrated in vacuum. Crude product was purified by flash column chromatography using ethyl acetate/hexane (1:3) as the eluent.

3.2.1. 8-(Prop-2-ynyloxy)quinoline, 1

Brown oil (1.74 g, 95% yield); ^1H NMR (CDCl_3 , 400 MHz): δ 8.91–8.89 (m, 1H), 8.10–8.07 (m, 1H), 7.46–7.36 (m, 3H), 7.24–7.21 (m, 1H), 5.00–4.99 (m, 2H), 2.50–2.49 (m, 1H); ^{13}C NMR (CDCl_3 , 400 MHz): δ 153.0, 149.4, 140.3, 135.9, 129.4, 126.4, 121.7, 120.6, 109.9, 78.3, 76.0, 56.5. The characterization data are consisted with the literature data.^{21,22}

3.2.2. 2-Methyl-8-(prop-2-ynyloxy)quinoline, 2

White solid (1.83 g, 93% yield); mp 98–100 °C; ^1H NMR (CDCl_3 , 400 MHz): δ 7.98 (d, $J = 8.4$ Hz, 1H), 7.38 (d, $J = 2.1$ Hz, 1H), 7.37 (s, 1H), 7.27 (d, $J = 8.4$ Hz, 1H), 7.24–7.22 (m, 1H), 5.01 (d, $J = 2.4$ Hz, 2H), 2.76 (s, 3H), 2.50 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR (CDCl_3 , 400 MHz): δ 158.3, 152.5, 139.8, 136.1, 127.7, 125.4, 122.6, 120.5, 110.2, 78.5, 76.0, 56.6, 25.7. LC-MS/MS. Anal. Calcd. for $\text{C}_{13}\text{H}_{11}\text{NO}$ $[\text{M}+\text{H}]^+$: m/z 198.0913. Found: m/z 198.09120. The characterization data are consisted with the literature data.²⁶

3.2.3. 8-(Prop-2-ynyloxy)quinolin-2-ol, 3

Light yellow solid (1.63 g, 82% yield); mp 148–150 °C; ^1H NMR (CDCl_3 , 400 MHz): δ 9.34 (bs, 1H), 7.69 (dd, $J = 2.1$ and 9.6 Hz, 1H), 7.18–7.06 (m, 3H), 6.64 (dd, $J = 1.7$ and 9.6 Hz, 1H), 4.82 (dd, $J = 1.4$ and 2.4 Hz, 2H), 2.57 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR (CDCl_3 , 400 MHz): δ 161.9, 143.3, 140.3, 128.8, 122.7, 122.0, 120.6, 120.2, 111.9, 77.5, 76.7, 56.8. LC-MS/MS. Anal. Calcd. for $\text{C}_{12}\text{H}_9\text{NO}_2$ $[\text{M}+\text{H}]^+$: m/z 200.0706. Found: m/z 200.0712.

3.2.4. (8-(Prop-2-ynyloxy)quinolin-2-yl)methanol, 4

Light yellow solid (1.81 g, 85% yield); mp 101–103 °C; ^1H NMR (CDCl_3 , 400 MHz): δ 8.11 (d, $J = 8.5$ Hz, 1H), 7.46 (d, $J = 1.2$ Hz, 1H), 7.44 (s, 1H), 7.38 (d, $J = 8.5$ Hz, 1H), 7.28–7.26 (m, 1H), 4.99 (d, $J = 2.4$ Hz, 2H), 4.94 (s, 2H), 2.54 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR (CDCl_3 , 400 MHz): δ 158.6, 152.7, 138.9, 136.9, 128.8, 126.2, 120.8, 119.1, 111.5, 78.4, 76.1, 64.8, 57.0. LC-MS/MS. Anal. Calcd. for $\text{C}_{13}\text{H}_{11}\text{NO}_2$ $[\text{M}+\text{H}]^+$: m/z 214.0863. Found: m/z 214.0869.

3.2.5. 8-(Prop-2-ynyloxy)quinoline-2-carbaldehyde, 5

White solid (1.81 g, 86% yield); mp 150–152 °C; ^1H NMR (CDCl_3 , 400 MHz): δ 10.28 (s, 1H), 8.27 (dd, $J = 0.7$ and 8.5 Hz, 1H), 8.04 (d, $J = 8.5$ Hz, 1H), 7.63–7.59 (m, 1H), 7.50 (dd, $J = 1.2$ and 8.3 Hz, 1H), 7.34 (dd, $J = 1.2$ and 7.8 Hz, 1H), 5.08 (d, $J = 2.4$ Hz, 2H), 2.57 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR (CDCl_3 , 400 MHz): δ 193.6, 153.7, 151.6, 140.0, 137.3, 131.4, 129.4, 120.6, 117.9, 111.0, 77.8, 76.6, 56.9. LC-MS/MS. Anal. Calcd. for $\text{C}_{13}\text{H}_9\text{NO}_2$ $[\text{M}+\text{H}]^+$: m/z 212.0706. Found: m/z 212.0704.

3.2.6. 8-(Prop-2-ynyloxy)quinolin-2-amine, 6

Light yellow solid (1.68 g, 85% yield); mp 152–154 °C; ^1H NMR (CDCl_3 , 400 MHz): δ 7.79 (d, $J = 8.8$ Hz, 1H), 7.23 (dd, $J = 1.9$ and 7.5 Hz, 1H), 7.16–7.09 (m, 2H), 6.69 (d, $J = 8.8$ Hz, 1H), 5.42 (bs, 2H), 4.92 (d, $J = 2.4$ Hz, 2H), 2.53 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR (CDCl_3 , 400 MHz): δ 156.9, 151.0, 139.2,

137.9, 124.5, 121.8, 120.8, 112.3, 110.9, 78.8, 75.8, 56.5. LC-MS/MS. Anal. Calcd. for $C_{12}H_{10}N_2O$ $[M+H]^+$: m/z 199.0866. Found: m/z 199.0873.

3.2.7. 2,8-Bis(prop-2-ynyloxy)quinoline, 7

White solid (0.71 g, 30% yield); mp 115–117 °C; 1H NMR ($CDCl_3$, 400 MHz): δ 8.00 (d, $J = 8.8$ Hz, 1H), 7.41 (dd, $J = 1.6$ and 7.8 Hz, 1H), 7.34–7.26 (m, 2H), 6.98 (d, $J = 8.8$ Hz, 1H), 5.19 (d, $J = 2.5$ Hz, 2H), 5.05 (d, $J = 2.4$ Hz, 2H), 2.53 (t, $J = 2.4$ Hz, 1H), 2.52 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 159.9, 151.9, 139.3, 138.3, 126.7, 124.2, 121.6, 114.9, 113.1, 79.2, 79.0, 75.7, 74.4, 58.1, 53.6. LC-MS/MS. Anal. Calcd. for $C_{15}H_{11}NO_2$ $[M+H]^+$: m/z 238.0863. Found: m/z 238.0869.

3.2.8. 1-(Prop-2-ynyl)-8-(prop-2-ynyloxy)quinolin-2(1H)-one, 8

Yellow solid (1.07 g, 45% yield); mp 123–125 °C; 1H NMR ($CDCl_3$, 400 MHz): δ 7.61 (d, $J = 9.4$ Hz, 1H), 7.29 (dd, $J = 3.2$ and 6.3 Hz, 1H), 7.19–7.18 (m, 2H), 6.69 (d, $J = 9.4$ Hz, 1H), 5.41 (d, $J = 2.4$ Hz, 2H), 4.85 (d, $J = 2.4$ Hz, 2H), 2.58 (t, $J = 2.4$ Hz, 1H), 2.20 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 162.6, 146.1, 139.8, 130.1, 123.2, 123.0, 122.9, 121.8, 116.3, 80.4, 78.0, 76.4, 70.6, 58.4, 36.3. LC-MS/MS. Anal. Calcd. for $C_{15}H_{11}NO_2$ $[M+H]^+$: m/z 238.0863. Found: m/z 238.0869.

3.2.9. Prop-2-ynyl 8-(prop-2-ynyloxy)quinoline-2-carboxylate, 9

Light yellow solid (2.25 g, 85% yield); mp 160–162 °C; 1H NMR ($CDCl_3$, 400 MHz): δ 8.28 (d, $J_{AB} = 8.5$ Hz, 1H), 8.22 (d, $J_{AB} = 8.5$ Hz, 1H), 7.61–7.57 (m, 1H), 7.50 (dd, $J = 1.2$ and 8.3 Hz, 1H), 7.33 (dd, $J = 1.2$ and 7.7 Hz, 1H), 5.06 (d, $J = 2.4$ Hz, 2H), 5.04 (d, $J = 2.5$ Hz, 2H), 2.54 (t, $J = 2.4$ Hz, 1H), 2.53 (t, $J = 2.5$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 164.6, 153.8, 146.2, 139.8, 137.2, 130.7, 129.0, 121.7, 120.2, 110.9, 78.1, 77.5, 76.5, 75.4, 56.8, 53.4. LC-MS/MS. Anal. Calcd. for $C_{16}H_{11}NO_3$ $[M+H]^+$: m/z 266.0812. Found: m/z 266.0820.

3.3. Antioxidant activity

3.3.1. DPPH radical scavenging activity

DPPH assays of the compounds were tested by the method of Dundar et al.³⁴ with minor modifications. Methanol solution of 1.0 mL of DPPH was added to 0.25 mL of test compounds in dimethyl sulfoxide (DMSO) at different concentrations (25, 50, 100, 250, and 500 μ g/mL) while 1.0 mL of DPPH solution was added to 0.25 mL of DMSO for the control reaction tube. The solution mixture was shaken and incubated at 25 °C in the dark for 60 min. After the incubation time, the absorbance of the solution was measured at 517 nm. Trolox was utilized as a positive control. The DPPH radical scavenger effect was measured using the following equation:

$$\text{Scavenging activity (\%)} = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100.$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The tests were applied in triplicate.

3.3.2. Metal chelating activity

Various concentrations (25–500 $\mu\text{g}/\text{mL}$) of 0.5 mL of DMSO solution of the test compounds were added to 1.85 mL of methanol and mixed with 50 μL of 2 mM FeCl_2 and reaction was started with the addition of 100 μL of 5 mM ferrozine. FeCl_2 and ferrozine were dissolved in DMSO, while 0.5 mL of DMSO without test complexes was used for the control tube. After 10 min of incubation at 25 °C, the absorbance of the test compound was measured at 562 nm. EDTA was utilized as a positive control. The result of metal chelating activity of the test samples was calculated by using the following formula:

$$\text{Metal chelating activity (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100,$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} represents the absorbance obtained in the presence of extracts or EDTA. The percentage of inhibition of the ferrozine/ Fe^{2+} complex formation was evaluated.³⁵

3.3.3. Reducing power

In this study, the assay of reducing power capacity was based on the reduction of $\text{Fe}^{3+}/$ ferricyanide complex to the ferrous form in the presence of reductants (antioxidants) in the tested compounds. The Fe^{2+} was then monitored by measuring the formation of Perl's Prussian blue at 700 nm.³⁶ All the compounds were prepared (25–500 $\mu\text{g}/\text{mL}$) in 0.25 mL of DMSO. Sodium phosphate buffer (0.25 mL, 200 mM, pH 6.6) and 0.25 mL of potassium ferricyanide (1%) were added and the solution mixture was incubated for 20 min at 50 °C. Then 0.25 mL of trichloroacetic acid (10%) was mixed in, and the solution mixture was centrifuged at 1000 rpm for 10 min. Sterilized water (0.5 mL) and 0.2 mL of freshly prepared ferric chloride (0.1%) were mixed with the supernatant (0.5 mL). Finally, the absorbance was calculated at 700 nm against a blank. As a positive control α -tocopherol was used. Increase in the absorbance value indicates high reducing power capacity of the studied compound.

3.4. Antibacterial activity

Antibacterial activities of the newly synthesized compounds were tested by disk diffusion method using nutrient agar medium.³⁷ *Escherichia coli* (ATCC 10536), *Legionella pneumophila* subsp. *pneumophila* (ATCC 33152), and *Pseudomonas aeruginosa* (ATCC 9027) were used as gram-negative bacteria and *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus*, and *Enterococcus hirae* (ATCC 10541) were used as gram-positive bacteria for testing the antibacterial activity of compounds. Sterile antibiotic disks were spread with 15 μL of the compounds and each disk was placed in a petri dish with nutrient agar inoculated with the bacteria. Tetracycline (30 μg) and streptomycin (10 μg) were used as standard antibiotic disks (positive control) and a DMSO-poured disk was used as a negative control. The petri dishes were incubated with bacterial cultures for 24 h at 37 °C. After incubation time, the antibacterial effect of the compounds was evaluated by measuring the inhibition zone diameters in millimeters.

3.5. DNA binding activity

The binding of compounds with CT-DNA were studied by agarose gel electrophoresis. For this aim, CT-DNA (concentration: 20 $\mu\text{g}/\text{mL}$) diluted to a 1:5 ratio with sterile distilled water and 5 μL of diluted DNA were treated with 500 $\mu\text{g}/\text{mL}$ complexes in DMSO (8 μL). The mixture of solution was incubated at 37 °C for 4

h in the dark and then 3 μ L of DNA loading dye was added. The samples were loaded on 0.8% agarose gel containing 7 μ L of 0.05% ethidium bromide. Electrophoresis was performed for 1 h at 80 V in TAE buffer (50 mM Tris base, 50 mM acetic acid, 2 mM EDTA, pH 7.8). The gel was displayed under UV light and photographed.³⁸

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