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Research Article

Synthesis, Antibacterial, Antioxidant, and Molecular Modeling Studies of Novel [2,3′-Biquinoline]-4-Carboxylic Acid and Quinoline-3-Carbaldehyde Analogs

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Currently, it has been common to see people being affected and dying from untreatable infections caused by multidrug-resistant (MDR) germs. To tackle this problem, developing new effective chemotropic agents is urgently needed. Hence, this project aims to design, synthesize, and evaluate their antibacterial and antioxidant activities of new series of [2,3′-biquinoline]-4-carboxylic acid and quinoline-3-carbaldehyde analogs. The molecular docking analysis of the compounds against *E. coli* DNA gyrase was computed to investigate the binding mode of the compounds within the active site of the enzyme. In this regard, a new series of [2,3′-biquinoline]-4-carboxylic acid and quinoline-3-carbaldehyde analogs were synthesized by utilization of Vilsmeier–Haack, Doebner, nucleophilic substitution, and hydrolysis reactions. The structures of the synthesized compounds were determined using UV-Vis, FT-IR, and NMR. The synthesized compounds were screened for their antibacterial activity against four bacterial strains using disc diffusion methods. The findings of the study revealed that seven of synthetic compounds possess good antibacterial activity compared to ciprofloxacin which was used as a positive control in the experiment. Among them, compounds 4, 9, and 10 displayed the highest mean inhibition zone of 13.7 ± 0.58, 16.0 ± 1.7, and 20.7 ± 1.5 mm, respectively, at 0.1 μg/μL. The radical scavenging property of these compounds was evaluated using DPPH radical assay where compounds 9 and 20 showed the strongest activity with IC_{50} values of 1.25 and 1.75 μg/mL, respectively. At the same concentration, the IC_{50} value of ascorbic acid was 4.5 μg/mL. The synthesized compounds were also assessed for their in silico molecular docking analysis. Compounds 4 (−6.9 kcal/mol), 9 (−6.9 kcal/mol), and 10 (−7.9 kcal/mol) showed the maximum binding affinity close to ciprofloxacin (−7.2 kcal/mol) used as a positive control. Thus, compounds 4, 9, and 10 showed the best antibacterial activities in both *in vitro* and molecular docking analyses among the synthetic compounds. The results of in silico molecular docking evaluation of the synthetic compounds against *E. coli* DNA gyrase B were in good agreement with the *in vitro* antibacterial analysis. Therefore, the antibacterial activity displayed by these compounds is encouraging for further investigation to improve the activities of [2,3′-biquinoline]-4-carboxylic acid by incorporating various bioisosteric groups in either of the quinoline rings.

1. Introduction

The introduction of antibiotics into clinical use was the greatest medical achievement of the twentieth century [1]. The discovery of antibiotics undeniably improved human and animal health significantly. However, antibiotic resistance becomes one of the serious global public health challenges of the twenty-first century [2]. Currently, it has been common to see people being affected and dying from untreatable infections caused by multidrug resistance (MDR) germs globally including the developed nations [3]. The widespread and incorrect use of antibiotics is thought to be the main causes of various resistance mechanisms in bacteria that lead to MDR [4]. Antimicrobial resistance mechanisms fall into four main categories: limiting uptake of a drug, modifying a drug target, inactivating a drug, and

active drug efflux [5]. Because of the differences in their morphology, there is variation in the types of mechanisms used by Gram-negative bacteria versus Gram-positive bacteria. Gram-negative bacteria make use of all four main mechanisms, whereas Gram-positive bacteria less commonly use limiting the uptake of a drug [5]. One or more of the four drug resistance mechanisms are employed depending on the types of drug administered. Fluoroquinolone drugs target mainly DNA gyrase and DNA topoisomerase IV. Resistance to fluoroquinolones typically is caused by alterations in the target enzymes (DNA gyrase and topoisomerase IV) and modification in drug entry and efflux [6]. Gram-negative bacteria are the cause of more than 30% of hospital-acquired infections, and the majority of urinary tract infections were caused by *E. coli* [7]. The development of new antibiotic agents against MDR is one of the strategies to overcome the challenges of the treatment of MDR diseases [8]. However, declining private investment and lack of innovation in the development of new antibiotics are undermining efforts to combat drug-resistant infections [9, 10].

Generally, quinolines are a structurally varied group of compounds, mainly comprising quinoline nucleus, found in various natural and synthetic products which exhibited a broad range of biological activities [11]. Quinolines and their derivatives exhibited anti-inflammatory [9, 10], antibacterial [11–13], antifungal [14, 15], antimalarial [16, 17], antiallergy [18], antitumor [19, 20], antituberculosis [14], and antihypertensive activities [18, 21].

The occurrence of the quinoline scaffold in a huge range of medical and industrial settings can be attributed mainly to its versatility and broad potential for functionalization [22]. Many synthetic methods have been developed for the preparation of quinolines, and improvements in the synthesis methodology are still an active research issue [23].

Quinoline scaffold has been used to develop antimalarial, antibacterial, and anticancer marked drugs. The investigation of new bioactive molecules among derivatives of quinoline-carboxylic acid is highly promising. Since 1962, 4-quinolone-3-carboxylic acid derivatives have been clinically used as antibacterial agents worldwide. Several research results revealed that various quinolone and quinoline-carboxylic acid motifs exhibited better antibacterial activities than the aldehyde or amide derivatives [22–24]. Therefore, we described herein the synthesis and antibacterial and antioxidant evaluation of novel [2,3′-biquinoline]-4-carboxylic acid and quinoline-3-carbaldehyde analogs. We also computed the *in silico* molecular docking analysis of the synthesized analogs to investigate the binding mode of the synthetic compounds within the active size site of the enzyme.

2. Materials and Methods

2.1. General. Commercially available chemicals were purchased from Lova Chemie PVT LTD and used without further purification. Melting points were determined using capillary tubes with Japson analytical melting point apparatus and are uncorrected. The progress of the reactions was monitored with TLC. The NMR spectra of the synthesized compounds were measured using NMR Bruker Avance 400 spectrometer operating at 400 MHz. The IR spectra of compounds were recorded using KBr pellets with a Perkin-Elmer BX IR Spectrometer (400–4000 cm^{-1}). UV-Vis spectra were determined using a double beam UV-Vis spectrophotometer (SM-1600 Spectrophotometer) using methanol as a solvent. Analytical thin-layer chromatography was conducted on a 0.2 mm thick layer of silica gel GF254 (Merck) on an aluminum plate, and spots were visualized with 254 nm and 366 nm wavelength UV light. Silica gel gravity column chromatography was carried out using 100 mesh silica gel.

2.2. Synthesis. 2-Chloroquinoline-3-carbaldehyde and 2-chloro-8-methylquinoline-3-carbaldehyde were prepared by the literature report method [23, 25].

Synthesis of 2′-Chloro-[2,3′-Biquinoline]-4-Carboxylic acid (4). 2-Chloroquinoline-3-carbaldehyde (0.38 g, 0.002 mol) and pyruvic acid (0.15 mL, 0. 002 mol) were added to 15 mL glacial acetic acid in a 100 mL round bottom flask. The mass was mounted on a magnetic stirrer and refluxed for an hour while being stirred. Aniline (0.17 mL, 0.002 mol) was added to the reaction mixture and then refluxed for additional 8 hours. The mixture was cooled to room temperature, and the precipitate was separated by suction filtration. The crude yield (569 mg, 85%) was purified over silica gel column chromatography with CH_2Cl_2 : MeOH (9 : 1) as eluent. The yield was 54%; Yellow powder; mp 204–206°C : IR(υ cm^{-1}, KBr): 3423–2521 (*br*, acid-OH.), 2979 (C-H str.), 2838 (C-H str.), 1675 (acidic C=O), 1584 (aromatic C=C); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 7.26 (1H, *t*, J = 7.21 Hz, H-6′), 7.45 (1H, *m*, J = 7.25 Hz, H-6), 7.60 (H, *m*, H-7′), 7.73 (H, *m*, H-7), 7.86 (1H, *m*, H-5′), 7.92 (1H, *d*, J = 7.14 Hz, H-8′), 8.19 (1H, *d*, J = 7.4 Hz, H-8′), 7.87 (1H, *d*, J = 7.4 Hz, H-8), 8.89 (1H, *s*, H-4′), 8.98 (1H, *s*, H-3) and 12.26 (1H, *s*, O-H); ^{13}C NMR (100 MHz, DMSO-d6): δ_C 115.5 (C-3), 119.6 (C-5), 122.8 (C-4a), 123.4 (C-6′), 124.3 (C-8′), 125.9 (C-6), 128.4 (C-3′), 129.1 (C-4′a), 129.6 (C-5′), 130.1 (C-8), 130.4(C-7), 132.0 (C-7), 136.2(C-4), 139.8(C-4′), 141.2(C-8′a), 148.7 (C-8a),154.3 (C-2′), 161.7 (C-2), and 168.2 (C-9).

Synthesis of 2′-Chloro-8,8′-Dimethyl-[2,3′-Biquinoline]-4-Carboxylic Acid (7). 2-Chloro-8-methylquinoline-3-carbaldehyde (0.41 g, 0.002 mol) and pyruvic acid (0.15 mL, 0.0026 mol) were added to 15 mL glacial acetic acid in a 100 mL round bottom flask. The mixture was refluxed for an hour on stirring by a magnetic stirrer. To the mixture, *o*-toluidine (0.18 mL, 0.002 mol) was added and refluxed for additional 8 hours. The precipitate formed after cooled overnight was separated by suction filtration. The product was 557 mg (76.6%) which was purified by silica gel column chromatography using CH_2Cl_2 : MeOH (18 : 1) as eluent. Dark powder; mp 182–184°C; IR(υ cm−1, KBr): 3569–2577 (*br*, acid-OH.), 3141 (aromatic C-H) 2904 (C-H str.), 2848 (C-H str.), 1663 (acidic C=O), 1630 (imine C=N), 1562 (aromatic C=C); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 2.20 (3H, *s*, H-10), 2.43 (3H, *s*,H-11), 6.37 (1H, *d*, J = 7.96 Hz,

H-6′), 6.49 (1H, *m*, *J* = 6.88 Hz, H-6), 6.91 (H, *m*, H-7′), 7.01 (2H, *m*,H-5′, H-7), 7.26 (1H, *d*, *J* = 7.96 Hz, H-5), 7.38 (1H, *d*, *J* = 6.88 Hz, H-5′), 7.67 (1H, *s*, H-3), and 11.01 (1H, *s*, O-H); ^{13}C NMR (100 MHz, DMSO-d6): δ_C 17.8 (C-10), 18.6 (C-11), 109.8(C-3), 116.4(C-5), 119.5 (C-6′), 122.1 (C-4′a), 122.4 (C-6), 123.6 (C-3′), 126.1(C-5′), 127.1 (C-4′a), 127.2 (C-7′), 128.2 (C-7), 129.6 (C-8), 130.3 (C-8′), 130.9(C-4), 131.3 (C-4′), 135.9 (C-8′a), 136.6(C-8a), 146.5(C-2), 162.8(C-2′), and 170.3(C-9).

Synthesis of 2′-Methoxy-8′-Methyl-[2,3′-Biquinoline]-4-Carboxylic Acid (9). 2-Chloro-8-methylquinoline-3-carbaldehyde (1.70 g, 0.008 mol) was refluxed for 4 hours in a mixture of methanol (15 mL), potassium bicarbonate (1.10 g, 0.008 mol), and DMF (10 mL). The methanol was removed by distillation, and the residue was added to 100 mL crushed ice water. The precipitate was separated by suction filtration and was washed with excess cold water. The purity of 2-methoxy-8-methylquinoline-3-carbaldehyde was checked with TLC. The yield was 1.4 g (82.4%). 2-Methoxy-8-methylquinoline-3-carbaldehyde (0.4 g, 0.002 mol) was added to pyruvic acid (0.15 mL, 0.002 mol) in 15 mL glacial acetic acid. The mixture was refluxed for an hour while being stirred. Aniline (0.17 mL, 0.002 mol) was added to the reaction mixture and refluxed for 12 hours while the progress of the reaction was monitored occasionally with TLC. The product was then collected by filtration after cooling to room temperature. The crude product (650 mg, 72.5%) was purified over silica gel column chromatography using CH_2Cl_2 : MeOH (8 : 1) as eluent. The yield was 160 mg (53.3%); Gray powder; mp 202–204°C; IR(υ cm^{-1}, KBr): 3456–2644 (*br*, acid-OH.), 2938 (C-H str.), 2848 (C-H str.), 1709 (acidic C=O), 1630 (imine C=N), 1585 (aromatic C=C); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 2.68 (3H, s,H-10), 4.11 (3H, *s*, H-11), 7.37 (1H, *t*, *J* = 7.53 Hz, H-6′), 7.59 (1H, *d*, *J* = 6.72 Hz, H-7′), 7.74 (H, *m*, H-6′), 7.86 (2H, *m*,H-5′, H-7), 8.20 (1H, *d*, *J* = 7.53 Hz, H-8), 8.51 (1H, *s*, *J* = 6.88 Hz, H-3), and 8.74 (2H, *m*, H-4′, H-5); ^{13}C NMR (100 MHz, DMSO-d6): δ_C 17.8 (C-10), 53.9 (C-11), 123.6(C-3′), 123.8(C-3), 124.0 (C-4′a), 124.8 (C-6′) 125.2 (C-5), 125.9 (C-4a), 126.8 (C-5′), 128.5(C-6), 130.2 (C-8), 130.5 (C-7′), 131.5 (C-7), 134.6 (C-4), 136.7 (C-8′), 140.9(C-4′), 145.2 (C-8′a), 149.0(C-8a), 154.6(C-2), 158.6(C-2′), and 168.2(C-9).

Synthesis of 2′-Methoxy-8,8′-Dimethyl-[2,3′-Biquinoline]-4-Carboxylic Acid (10). 2-Methoxy-8-methylquinoline-3-carbaldehyde (0.40 g, 0.002 mol) was mixed with pyruvic acid (0.15 mL, 0.002 mol) in 15 mL glacial acetic acid and refluxed for an hour with stirring. Then, *o*-toluidine (0.15 mL, 0.002 mol) was added to the reaction mixture, and the reflux was continued for additional 10 hours. It was cooled to room temperature, and the precipitate was separated by suction filtration. The crude yield (579.7 mg, 80.7%) was purified over silica gel chromatography using CH_2Cl_2 : MeOH (18 : 1) as eluent. The yield was 154 mg (51.3%); Gray powder; mp 97–99°C; IR(υ cm^{-1}, KBr): 3377.9–2531.8 (*br*, acid-OH.), 2982.0(C-H str.), 2903.7 (C-H str.), 1721.2 (acidic C=O), 1641.0 (imine C=N), 1584.7 (aromatic C=C); ^{1}H NMR (400 MHz, DMSO-d6): δ_H 2.69 (3H, s,H-10), 2.83 (3H, *s*, H-11), 4.13 (3H, *s*, H-12), 7.39 (1H, *m*, H-6′), 7.60 (2H, *m*, H-6, H-7′), 7.71(1H, *m*, H-7), 7.742 (1H, *m*, H-5′), 8.51(2H, *d*, H-4′, H-5, *J* = 8.28 Hz) and 8.81 (1H, *s*, H-3); ^{13}C NMR (100 MHz, DMSO-d6): δ_C 17.8 (C-10), 18.6 (H-11), 53.9 (C-12), 123.0(C-3′), 123.7(C-3), 123.9 (C-5), 124.1 (C-4′a), 124.8 (C-6′), 125.3 (C-4a), 126.8 (C-6), 128.2(C-5′), 130.4 (C-7), 131.1 (C-7), 134.6 (C-8), 137.1(C-8′), 137.6 (C-4), 141.0(C-4′), 145.2 (C-8′a), 147.8 (C-8a), 153.0C-2), 158.7(C-2′), and 168.4 (C-9).

Synthesis of 2-Phenylquinoline-4-Carboxylic Acid (12). Benzaldehyde (2 mL, 0.020 mol) and pyruvic acid (1.5 mL, 0.020 mol) were added to glacial acetic acid (15 mL), and the mixture was refluxed for an hour with stirring. To the mixture, aniline (1.9 mL, 0.02 mol) was added and refluxed for additional 10 hours. It was cooled to room temperature and basified with 5% aqueous NaOH. Then, it was filtered by gravity filtration, and the filtrate was acidified with 5% aqueous HCl. It was cooled in an ice bath. The precipitate was collected by suction filtration. The crude product was recrystallized from methanol. The yield was 3.10 g (62.2%); Yellow powder; mp 212–214°C : IR(υ cm^{-1}, KBr): 3389 (*br*, acid-OH.), 3040 (aromatic C-H), 2927(C-H str.), 2848 (C-H str.), 1709(acidic C=O), 1607 (aromatic C=C), 1584; ^{1}H NMR (400 MHz, DMSO-d6): δ_H 7.56 (3H, *m*, H-3′, H-4′, H-5′), 7.72 (1H, *t*, *J* = 7.02 Hz, H-6), 7.87(1H, *t*, *J* = 7.02 Hz, H-7), 8.25 (3H, *m*, H-8, H-2′, H-6′), 8.44(1H, *s*, H-3) and 8.63 (1H, *d*, *J* = 7.91 Hz, H-5); ^{13}C NMR (100 MHz, DMSO-d6): δ_C 119.9 (C-3), 123.9(C-5), 125.9(C-4a), 128.0 (C-2′, C-6′), 128.6 (C-6), 129.3 (C-8) 129.5 (C-3′, C-5′), 130.8 (C-4′), 131.2(C-7), 137.5 (C-4), 131.1 (C-7), 139.0 (C-1′), 148.8(C-8a), 156.2(C-2), and 167.8(C-9).

Synthesis of 2-(o-Tolylimino)propanoic Acid (15). 2-Thiocyanatoquinoline-3-carbaldehyde (0.43 g, 0.002 mol) and pyruvic acid (0.15 mL, 0.002 mol) were added to glacial acetic acid (15 mL), and the mixture was refluxed for an hour with stirring. Then, to the mixture, *o*-toluidine (0.18 mL, 0.002 mol) was added and refluxed for additional 10 hours. The mixture was allowed to cool to room temperature and basified with 5% aqueous NaOH. Then, it was filtered by gravity filtration, and the filtrate was acidified with 5% aqueous HCl. It was cooled in an ice bath. The precipitate was collected by suction filtration and purified over silica gel column chromatography using CH_2Cl_2 : MeOH (9.5; 0.5) as an eluent to furnish not the desired product 14 but the undesired product 15. The yield was 125 mg (48.5%); Yellow powder; mp 285–287°C : IR(υ cm^{-1}, KBr): 3385 (*br*, acid-OH), 3042 (aromatic C-H), 2927(C-H str.), 2848 (C-H str.), 1720 (carboxylic C=O), 1674 (imine C=N), 1607 (aromatic C=C), 1584; ^{1}H NMR (400 MHz, DMSO-d6): δ_H 2.06 (3H, *s*, H-3), 2.22 (3H, *s*, H-7), 7.06 (1H, *t*, *J* = 7.90 Hz, H-4′), 7.14 (1H, *t*, *J* = 7.90 Hz, H-5′), 7.19(1H, *d*, *J* = 8.41 Hz, H-3), 7.39 (1H, *d*, *J* = 8.41 H-6), and 9.29 (1H, *s*, H-N =); ^{13}C NMR (100 MHz, DMSO-d6): δ_C 18.2 (C-3), 23.7 (C-7), 125.4 (C-5′, C-6′), 126.3(C-4′), 130.7 (C-3′), 132.0 (C-2′), 136.0(C-1′), and 168.6(C-1, C-2).

Synthesis of 2-((2-Hydroxyethyl)amino)quinoline-3-Carbaldehyde (17). 2-Chloroquinoline-3-carbaldehyde (0.5 g, 2.6 mmol) was added to ethanolamine (10 mL) in a 100 mL round bottom flask and heated in an oil bath at 100°C for 2 hours. After cooling to ambient temperature, it was poured into 100 mL crushed ice water. The precipitate was collected

by suction filtration and allowed to dry in the air (0.62 g, 2.4 mmol). The dried product was refluxed in 20% H_2SO_4 (10 mL) for 2 hours. It was cooled to room temperature and added to crushed ice water (50 mL). The precipitate was collected by suction filtration. The yield was 0.46 g (89%). It was a yellow powder and decomposed without melting at 130°C. IR(υ cm^{-1}, KBr): 3367(alcohol-OH), 2938 (C-H-str.), 2870 (C-H str.), 1652 (aldehyde C=O), 1630 (imine C=N) 1562(aromatic C=C) : ^{1}H NMR (400 MHz, DMSO-d_6): δ_H, 3.65 (4H, H-10, H-11), 4.96 (1H, *s*, O-H), 7.26 (1H, *s*, H-8), 7.55–7.84 (3H, *m*, H-6, H-7, H-5), 8.24 (1H, *s,* H-4), 8.68(1H, *s*, H-9), and 10.02 (1H, *s*, N-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 43.1 (C-10), 60.1 (C-11), 117.7 (C-3), 122.1(C-4a), 122.8(C-8), 126.2 (C-6), 130.2 (C-7), 134.0 (C-5), 149.7 (C-4), 151.6(C-8), 154.7 (C-2), and 194.8(C-9).

Synthesis of 3-Chloro-3-(2-Chloro-6-Iodoquinolin-3-yl) acrylaldehyde (20). 4-Iodoaniline (3 g, 0.014 mol) was refluxed in a mixture of acetic anhydride (10 mL) and acetic acid (10 mL) for two hours in a 250 mL round bottom flask. After cooling to room temperature, it was added to 100 mL crushed ice water, and the precipitate was collected by suction filtration. The yield was 3.3 g. Then, a Vilsmeier reagent was prepared by adding *N,N*-dimethylformamide (5 mL, 0.09 mol) to a 100 mL round bottom flask guarded with a drying tube; it was cooled to 0°C using an ice bath. Then, phosphorus oxychloride (59 mL, 0.63 mol) was added dropwise to it from the dropping funnel guarded by the drying tube while being stirred by a magnetic stirrer. This addition was done for 30 minutes. Then, *N-(*4-iodophenyl) acetamide (3.3 g, 0.013 mol) was added to it. After 5 minutes, the dropper funnel was replaced by an air condenser with guarding tube at its end, and the mixture was heated for 24 hours on an oil bath at 85–90°C. Then, it was cooled to room temperature, poured into a beaker containing 150 mL crushed ice water, and stirred for 20 minutes. The brown solid product was collected by suction filtration and washed with 50 mL cold water. TLC analysis revealed that the product (1.29 g) was a mixture of two compounds. The product was suspended in hot EtOAc, and the soluble part was found to be 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl) acrylaldehyde (125 mg, 0.48 mmol, 3.7%) whereas the insoluble part was identified as compound **21** (1.16 g, 96.3%). Brown crystal; mp 154–156°C; IR(υ cm^{-1}, KBr): 2927 (C-H-str.), 2848 (C-H str.), 1686(aldehyde C=O), 1608(imine C=N) 1573(aromatic C=C) : ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 7.77 (1H, *d*, J = 8.67 Hz, H-8), 7.92 (1H, *m,* H-5), 8.10 (1H, *dd*, H-7, J = 1.67, 1.94 Hz), 8.35 (1H, *s*, H-4 J = 1.94 Hz), 8.61 (H-61, *s*, H-9) and 10.53 (1H, *s*, H-9); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 93.7 (C-6), 124.9 (C-3), 126.8 (C-4a), 128.1(C-8), 130.0(C-10), 138.2 (C-4), 138.7(C-5), 138.9 (C-9), 142.2 (C-7), 148.4 (C-8a) 150.6 (C-2), and 188.8(C-11).

The NMR result of compound **21** (96.3%) revealed as it is N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium (**21**). A brown powder; mp, 128–130°C; IR(υ cm^{-1}, KBr): 2966.1 (C-H-str.), 2922.0 (C-H str.), 1709.3 (formamide C=N(Me)$_2$), 1607.5 (imine C=N) 1578.0(aromatic C=C) : ^{1}H NMR (400 MHz, DMSO-d6): δ_H 3.34 (3H, *s*, CH_3), 3.48 (3H, *s*, CH_3), 7.72(2H, *m*, H-2, H-6), 7.75 (2H, *d*, J = 7.20 Hz, H-3, H-5), and 8.65 (1H, *s*, H-8); ^{13}C NMR (100 MHz, DMSO-d6): δ_C 37.0 (CH_3), 43.6 (CH_3), 90.3 (C-4), 121.3 (C, C-2, C-6), 137.1 (C-1), 138.6(C, C-3, C-5), 130.0(C-10), 138.2 (C-4), 138.7(C-5), and 153.5(C-8).

2.3. Antibacterial Activity. The synthetic compounds were screened for their *in vitro* antibacterial activity against two Gram-positive bacteria *(Streptococcus pyogenes,* ATCC19615) and (*Staphylococcus aureus*, ATCC25923) and two Gram-negative bacteria (*Escherichia coli,* ATCC 25922) and (*Pseudomonas aeruginosa,* ATCC27853) which were provided by Adama Public Health Research and Referral Laboratory Center. The identity of the bacterial strains was recognized and confirmed by the morphology of colony and Gram staining and by standard biochemical tests following the methods of Bergey's Manual of Determinative Bacteriology (1994) [26, 27]. The bacterial strains were brought to the microbiology laboratory with nutrient agar and preserved at 4°C until they are used. The antibacterial efficacy of the compounds was tested by the disc diffusion method using ciprofloxacin as standard and DMSO as a negative control. The test compounds were dissolved in dimethylsulfoxide (DMSO) and adjusted at concentrations of 0.1 and 0.2 μg/μL. The microbial cultures were grown overnight at 37°C in nutrient broth, adjusted to 0.5 McFarland standard using distilled water, and lawn inoculated onto Mueller-Hinton agar (MHA) plates. Sterile filter paper discs of 6 mm diameter were soaked in DMSO solution of the compounds at 0.1 and 0.2 μg/μL concentration. Then, the saturated paper discs were placed on the center of each MHA plate. The plates were then inverted and incubated for 24 hours at 37°C, and the zone of inhibition was recorded. The results were articulated as the mean of three measurements (Table 1).

2.4. Radical Scavenging Activity. DPPH (4 mg) was dissolved in methanol (100 mL) to provide 40 μg/mL DPPH solution. Likewise, the stock solutions of the synthetic compounds were dissolved in methanol to furnish 10 mg/mL. Each of the synthetic samples and ascorbic acid were diluted in MeOH and DPPH solution to give 25, 20, 15, 10, and 5 μg/mL, and the mixture was kept in a dark oven at 37°C for 30 minutes. Absorbance (A) was measured at 517 nm using a double beam UV-Vis Spectrophotometer(SM-1600 Spectrophotometer)). Inhibition of the DPPH radical by the compounds was calculated according to the following formula:

$$\%\ \text{inhibition} = \frac{A_o - A_1}{A_1}, \quad (1)$$

where A_o is the absorbance of the control and A_1 is the absorbance of the sample. The results are averages of three measurements. The IC_{50} value, compound concentration to reduce 50% of the DPPH, was calculated using Excel 16 [27].

2.5. Molecular Docking Studies. To study the interactions and binding affinity between the bacterial proteins and synthetic compounds in a 3D fashion, the compounds were

Table 1: The antibacterial activity of the synthetic compounds.

Compounds	Concentration (μg/μL)	Zone of inhibition in mm			
		Staphylococcus aureus	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*
4	0.10	11.0 ± 1.0^{Aa}	11.0 ± 1.0^{Bb}	12.7 ± 2.5^{Ca}	15.7 ± 1.5^{Da}
	0.20	12.3 ± 0.58^{A}	13.7 ± 0.58^{Bb}	13.0 ± 2.0^{C}	19.3 ± 3.1^{D}
7	0.10	10.3 ± 0.58^{Aa}	10.0 ± 0.0^{Bc}	9.7 ± 2.5^{C}	11.3 ± 2.3^{Db}
	0.20	13.0 ± 1.7^{Aa}	13.3 ± 2.1^{B}	12.7 ± 1.2^{C}	19.7 ± 1.5^{D}
9	0.10	11.0 ± 3.0^{Aa}	10.3 ± 1.2^{BD}	14.3 ± 1.5^{Cb}	12.0 ± 2.0^{D}
	0.20	14.3 ± 1.5^{Aa}	12.3 ± 1.2^{B}	14.0 ± 1.5^{C}	20.7 ± 1.5^{D}
10	0.10	11.0 ± 1.0^{A}	11.0 ± 1.0^{B}	13.7 ± 2.51^{C}	14.0 ± 1.0^{D}
	0.20	15.7 ± 0.58^{A}	11.0 ± 1.7^{B}	16.0 ± 1.7^{C}	19.7 ± 1.5^{D}
12	0.10	0.0 ± 0.0^{a}	0^{bc}	0^{ab}	0^{ab}
	0.20	7.0 ± 0.0^{a}	0^{bc}	7.0 ± 0.0^{ab}	0^{ab}
15	0.10	8.33 ± 0.58^{A}	7.0 ± 0.0^{bD}	7.0 ± 0.58^{C}	7.33 ± 0.58^{ab}
	0.20	9.67 ± 0.58^{A}	8.33 ± 0.58^{bD}	8.67 ± 0.58^{C}	9.67 ± 0.58^{ab}
17	0.10	9.7 ± 2.3^{A}	0_{bc}	9.0 ± 1.0^{C}	0^{ab}
	0.20	10.7 ± 2.1^{A}	0^{bc}	9.7 ± 2.9^{C}	9.3 ± 2.1^{ab}
20	0.10	12.7 ± 0.58^{A}	8.7 ± 2.1^{B}	13.7 ± 2.5^{C}	13.3 ± 1.5^{D}
	0.20	13.3 ± 1.5^{A}	11.0 ± 0.0^{B}	14.7 ± 2.1^{C}	19.7 ± 1.5^{D}
21	0.10	0^{a}	0^{bc}	0^{b}	0^{ab}
	0.20	0^{a}	0^{bc}	7.7 ± 0.58^{C}	0^{ab}
Ciprofloxacin	0.10	14.7 ± 0.58^{A}	14.7 ± 0.58^{Bc}	14.3 ± 0.58^{Cb}	19.0 ± 0.0^{Da}
	0.20	16.33 ± 0.58^{A}	16.0 ± 0.0^{Bb}	14.3 ± 2.1^{Cc}	22.0 ± 1.0^{Db}
DMSO	0	0	0	0	0

0 = inhibition zone was not observed. Mean zone of inhibition in mm (mean ± S.D) $n = 3$.

docked within the binding site of the protein. AutoDock Vina with our recently reported protocol was used to dock the proteins (PDB ID : 6F86, and PDB ID : 2INR) and compounds (**4**–**21**) into the active site of proteins [23, 28, 29]. The chemical structures of the compounds were drawn using the Chem Office tool (Chem Draw 16.0) assigned with proper orientation followed by the energy minimization of each molecule using ChemBio3D. The energy minimized ligand molecules were then used as input for AutoDock Vina, to carry out the docking simulation [30]. The crystal structures of the receptor molecules *E. coli* DNA gyrase B (PDB ID 6F86) and *S. aureus* topoisomerase IV (PDB ID : 2INR) were downloaded from protein data [28, 29]. The protein preparation was done using the reported [27] standard protocol by removing the cocrystallized ligand, deleting water molecules, and adding polar hydrogens and cofactors; then, the target protein file was prepared by leaving the associated residue with protein by using Auto Preparation of target protein file Auto Dock 4.2 (MGL tools1.5.7) [30]. The graphical user interface program was used to set the grid box for docking simulations. To surround the key amino acid residues (Ser-141, Glu-356, Glu-138, and Asn-360 for PDB ID : 2INR and Asp-73, Arg-76, and Thr-165 for PDB ID : 6F86) region in the macromolecule, grids were used. To validate the docking results, the redocking approach was used with the following grid center coordinates $63 \times 29 \times 64$ and $29 \times -24 \times 3$ for PDB ID 6f86 and PDB ID 2INR, respectively [30]. During the docking process, a maximum of nine conformers were considered for each ligand. The conformations with the most favorable (least) free binding energy were selected for analyzing the interactions between the target receptor and ligands by Discovery Studio Visualizer. The ligands are represented in different color H-bonds, and the interacting residues are represented in ball and stick model representation.

2.6. Statistics Data Analysis. The antimicrobial analysis data generated by triplicate measurements were reported as mean ± standard deviation. GraphPad Prism version 5.00 for Windows was used to perform the analysis (GraphPad Software, San Diego California USA, http://www.graphpad.com). Groups were analyzed for significant differences using a linear model of variance analysis (ANOVA) test for comparisons was performed, with significance accepted for $p < 0.05$ (supplementary information).

3. Results and Discussion

3.1. Synthesis. In the present work, a new series of [2,3′-biquinoline]-4-carboxylic acids, 2-phenylquinoline-4-carboxylic acid, and quinoline-3-carbaldehyde derivatives were synthesized by the application of Vilsmeier, Doebner, condensation, hydrolysis, and nucleophilic substitution reactions. The structures of the synthesized compounds were determined using spectroscopic methods including UV-Vis, FT-IR, and NMR.

Firstly, 2-chloroquinoline-3-carbaldehyde (1) and 2-chloro-8-methylquinoline-3-carbaldehydes (5) were, respectively, synthesized from acetanilide and 2-methylacetanilide by utilizing the Vilsmeier reaction which involves treating the corresponding acetanilide analogs with $POCl_3$ in

DMF mixture [23, 25]. These quinoline-3-carbaldehydes were treated together with pyruvic acid, aniline, and *o*-toluidine to synthesize four new (2,3′-biquinoline]-4-carboxylic acids by the application of the Doebner quinoline synthesis approach [31, 32]. Furthermore, 2-phenylquinoline-4-carboxylic acid was also prepared by the same procedure from benzaldehyde, aniline, and pyruvic acid in order to compare the antibacterial activity of biquinoline-4-carboxylic acid analogs with monoquinoline-4-carboxylic acid (Schemes 1 and 2). The Doebner method, introduced by Oscar Doebner in 1887, combines aniline with an aldehyde and pyruvic acid to give 2-substituted quinoline-4-carboxylic acid [22]. Low yield and longer reaction times, harsh reaction conditions, and the requirement of a large amount of organic solvent are the typical limitations of the Doebner method [33]. Various solvent systems including absolute ethanol [34], acetic acid [35], and solvent-free reactions [22] as well as different acid catalysts including sulfuric acid, trifluoroacetic acid, and Lewis acid [33] were employed by various researchers to overcome these limitations. A recent report showed that the replacement of trifluoroacetic acid with acetic acid and using excessive acetic acid as the solvent instead of ethanol provide better yield [35]. Based on this and other related reports, four new [2,3′-biquinoline]-4-carboxylic acids and 2-phenylquinoline-4-carboxylic acid (Schemes 1 and 2) were synthesized in glacial acetic acid. The synthesis of compounds **4**, **7**, **9**, 1**0,** and **12** (Scheme 1) was achieved by refluxing the corresponding quinoline-3-carbaldehyde analog with pyruvic acid in acetic acid for an hour followed by the treatment with aniline or *o*-toluidine. These compounds were purified using silica gel column chromatography with CH_2Cl_2 : MeOH as eluent in good yields. On the other hand, refluxing a mixture of benzaldehyde and pyruvic acid in glacial acetic acid for an hour followed by treatment with aniline gave 2-phenylquinoline-4-carboxylic acid (**12**) in 62.2% yield.

Even though glacial acetic acid was used as both solvent and catalyst, the Doebner method is very sensitive to both electronic and steric effects. An attempt made to synthesize compound **14** using the above procedure from 2-thiocyanatoquinoline-3-carbaldehyde, *o*-toluidine, and pyruvic acid failed. The major product was identified using NMR as compound **15** which is a Schiff base formed between *o*-toluidine and pyruvic acid (Scheme 2).

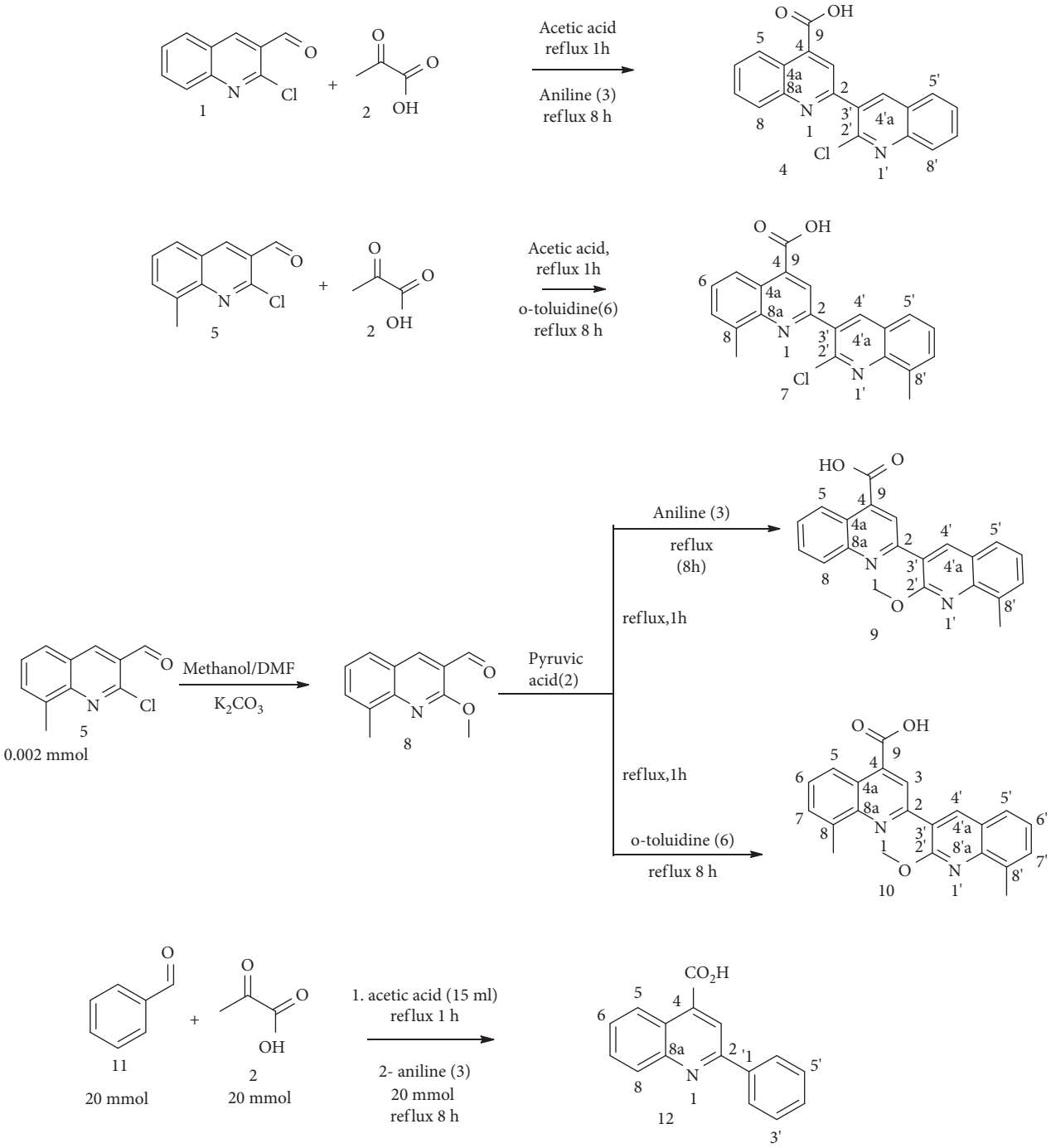
Compound **147**, which was prepared from acetanilide by utilizing Vilsmeier reaction, was added to ethanolamine and heated at 100°C for 2 hours in an oil bath. The Schiff base **169** was collected by suction filtration after adding the ethanolamine mixture into crushed ice water. Further hydrolysis of compound **169** with H_2SO_4 gave **186** in good yield.

The synthesis of **8**, **20**, and **21** (Scheme 3) was achieved first by the application of Vilsmeier reaction and recrystallization in ethyl acetate followed by substitution of chlorine by various nucleophiles. Vilsmeier–Haack formylation uses dimethylformamide and phosphoryl chloride to furnish Vilsmeier reagent which is a mild electrophilic agent and proceeds only with activated aromatic systems [28]. Iodine is the least electronegative element of halogens, and we anticipated *p*-iodo acetanilide to provide a good yield when it is treated by the Vilsmeier reagent. However, compound **20** (Scheme 3**)** was not according to our expectations. Firstly, compound **20** was 3-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde rather than the expected 2-chloro-6-iodoquinoline-3-carbaldehyde. Secondly, its amount is only 3.7% of the total yield. The major product about 96.3% of the yield was N-(((4-iodophenyl)amino) methylene)-N-methylmethanaminium (**21**) (as confirmed by ^{1}H and ^{13}C NMR, DEPT-135). Presumably, it was formed by the attack of the Vilsmeier reagent at nitrogen giving the N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium as a major product. Schemes 4 and 5 showed the proposed mechanism for the formation of these two molecules.

Vilsmeier–Haack formylation using dimethylformamide and phosphoryl chloride involves the formation of halomethyleniminium salt **24** named as Vilsmeier reagent as an intermediate. The broad synthetic utility of this halomethyleniminium salt is not restricted to formylation but is also suitable for electrophilic substitution reactions [36]. Presumably, when an excess amount of chloromethyleniminium salt was generated and when the reaction was allowed to take place for a longer duration (22 hrs) before quenching intermediate **32** with crushed ice, further conjugation through chloromethyleniminium salt addition and elimination of protons afforded compound **20** through several sequential steps. The precise mechanism of Meth-Cohn synthesis of quinolones was not discussed explicitly [28]. Recently, Hamama et al. (2018) proposed a related mechanism using $SOCl_2$ in place of $POCl_3$ which provided a good vision on the mechanism of Vilmeier formulation [37]. Dain et al. (2004) had also proposed a related mechanism of Vilsmeier–Haack reactions of carbonyl compounds in the synthesis of substituted pyrones and pyridines [36]. Here, we suggested a related mechanism of the reaction leading to the formation of compound **20** depicted in Scheme 4.

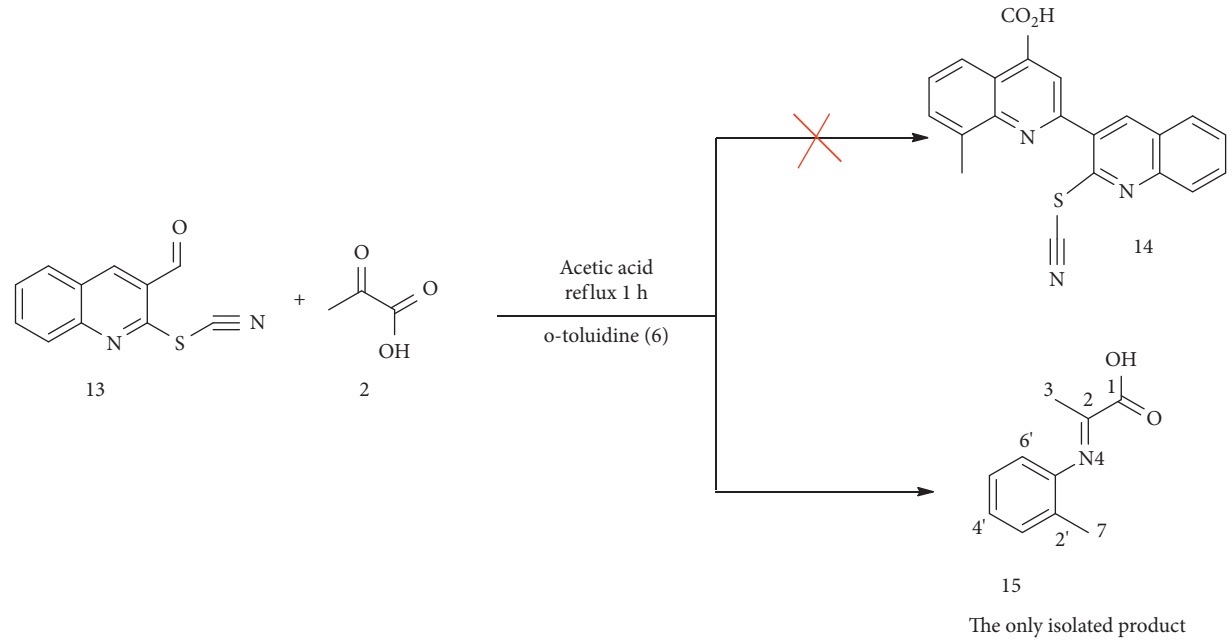
N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium salt (**21**) may be formed by direct attack of nitrogen of **19** on chloromethyleniminium salt giving an intermediate **44.** The rest of the intermediates were formed by elimination of protons from **44**, followed by electrophilic addition of $POCl_3$ affording intermediate **45**, which then underwent addition of Cl^- and elimination of H^+ to provide **46** which was further rearranged to **21** (Scheme 5).

3.2. Antibacterial Activity. All of the synthetic compounds were screened for their *in vitro* antibacterial activities by paper disc diffusion methods against two Gram-positive *Staphylococcus aureus* (ATCC25923) *and Streptococcus pyogenes* (ATCC 27853) and two Gram-negative (*Escherichia coli (ATCC 25922)* and *Pseudomonas aeruginosa* (ATCC2592) bacterial strains (Table 1). Six of the compounds, namely, **4**, **7**, **9**, **10**, **17**, and **20,** showed good activity with mean inhibition zone ranging from 9.7 ± 2.5 to 20.7 ± 1.5 mm compared with ciprofloxacin with a mean inhibition zone range of 14.3 ± 0.58 to 22.0 ± 1.0 mm, which was the positive control in the experiment. However, none of them were stronger than ciprofloxacin. Only compound **9** had an equal activity with ciprofloxacin against *Escherichia coli.* Among them, the two none quinoline Schiff base

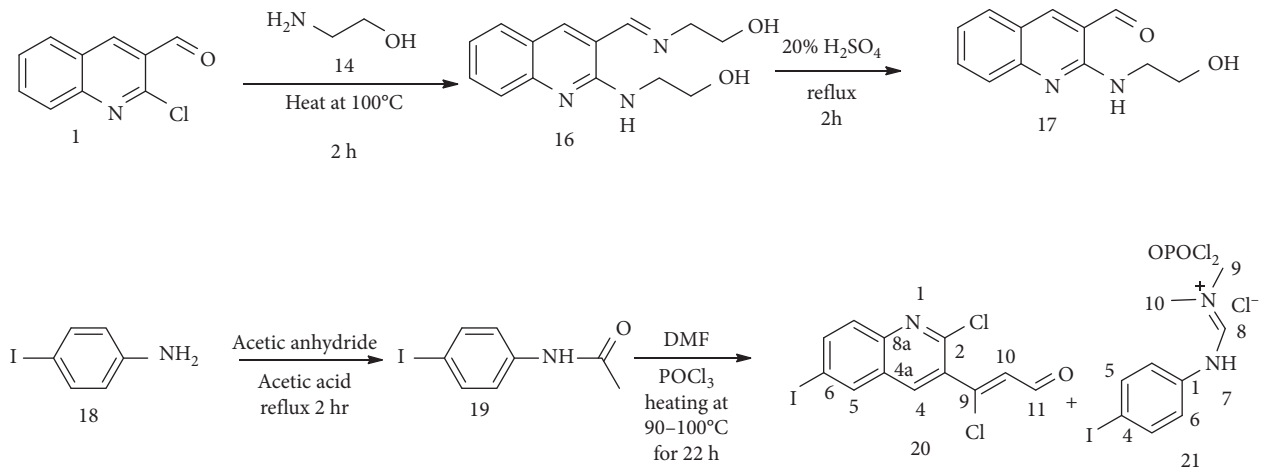


Scheme 1: Synthesis of [2,3′-biquinoline]-4-carboxylic acids and 2-phenylquinoline-4-carboxylic acid.

compounds (**15** and **21**) and 2-phenylquinoline-4-carboxylic acid (**12**) did not show any significant mean inhibition. Compounds **12, 15**, and **21** showed little activity with a mean inhibition zone of $7.0 \pm .0$ and 9.67 ± 0.58 mm against *Escherichia coli* and *Pseudomonas aeruginosa*. When the maximum mean inhibition zone was considered per bacterial strain, compound **10** exhibited 15.7 ± 0.58 mm mean inhibition against *Staphylococcus aureus* at a concentration of $0.2\,\mu g/\mu L$ while ciprofloxacin was 16.33 ± 0.58 mm at the same concentration, compound **4** showed 13.7 ± 0.58 mm against *Streptococcus pyogenes* while ciprofloxacin was16.0 ± 0.0 mm, compound **10** showed 16.0 ± 1.7 mm against *Escherichia coli* whereas ciprofloxacin was 14.3 ± 0.58 mm, and compound **9** showed 20.7 ± 1.5 mm against *Pseudomonas aeruginosa* whereas ciprofloxacin was 22.0 ± 1.0 mm at $0.2\,\mu g/\mu L$ concentration. In general, on average maximum inhibition zone (15. 6.mm) was recorded by compound **10** while (17.7 mm) by the control at the concentration of $0.2\,\mu g/\mu L$. Thus, **10** displayed the best mean inhibition zone among the synthetic compounds reported herein followed by compounds **9**, **7**, and **20**, consecutively.



Scheme 2: Synthesis of 2-(o-tolylimino)propanoic acid.



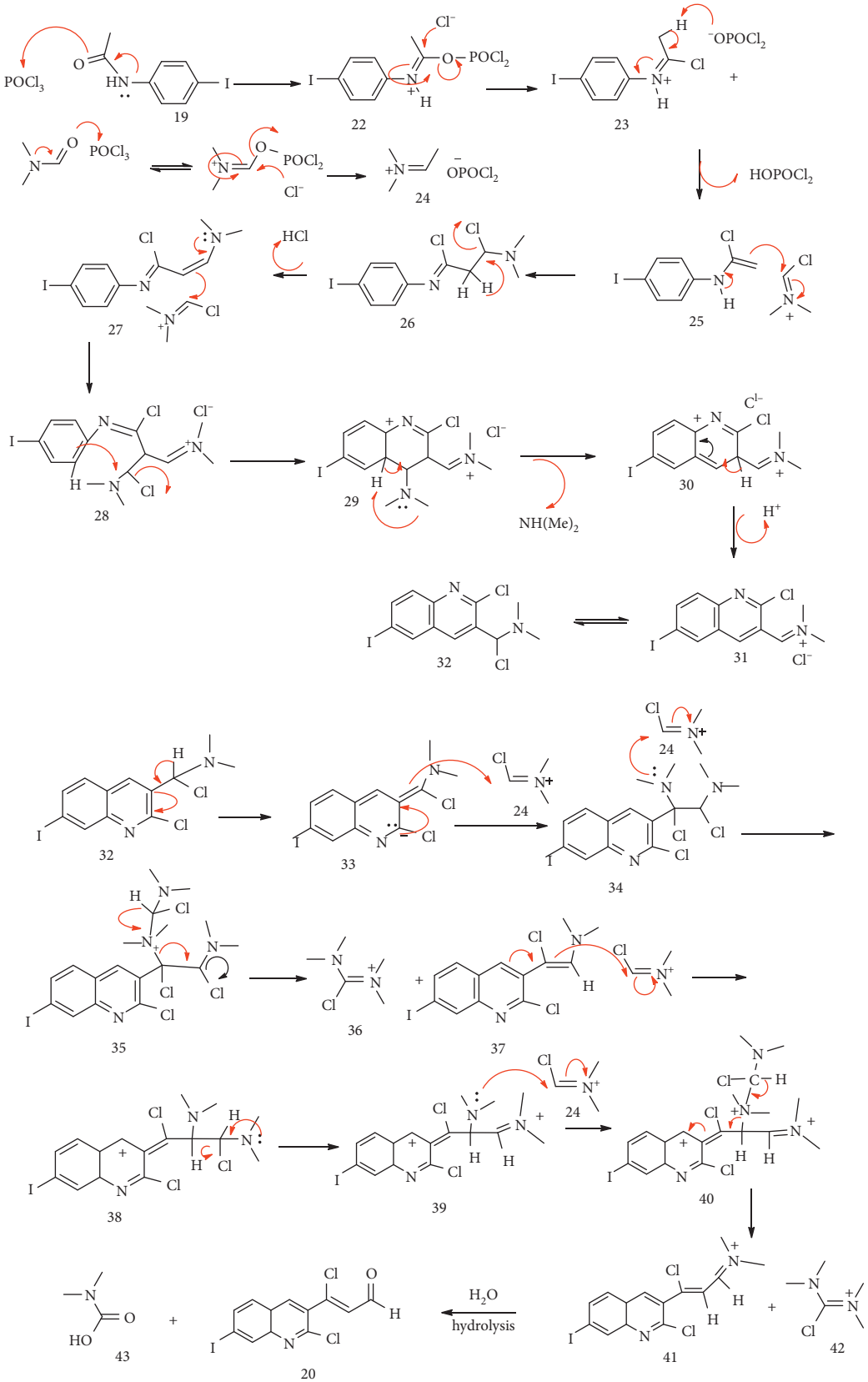
Scheme 3: Synthesis of 2-chloroquinoline-3-carbaldehyde analogs.

Literature report showed moderate activity for the antibacterial activity of 2-phenylquinoline-4-carboxylic acid (**12**) and some of its analogs; however, at the concentration used here (0.1 μg/μL and 0.2 μg/μL), **12** did not show any antibacterial activity [41,42] while the [2,3′-biquinoline]-4-carboxylic acid analogs showed much better activity compared to it.

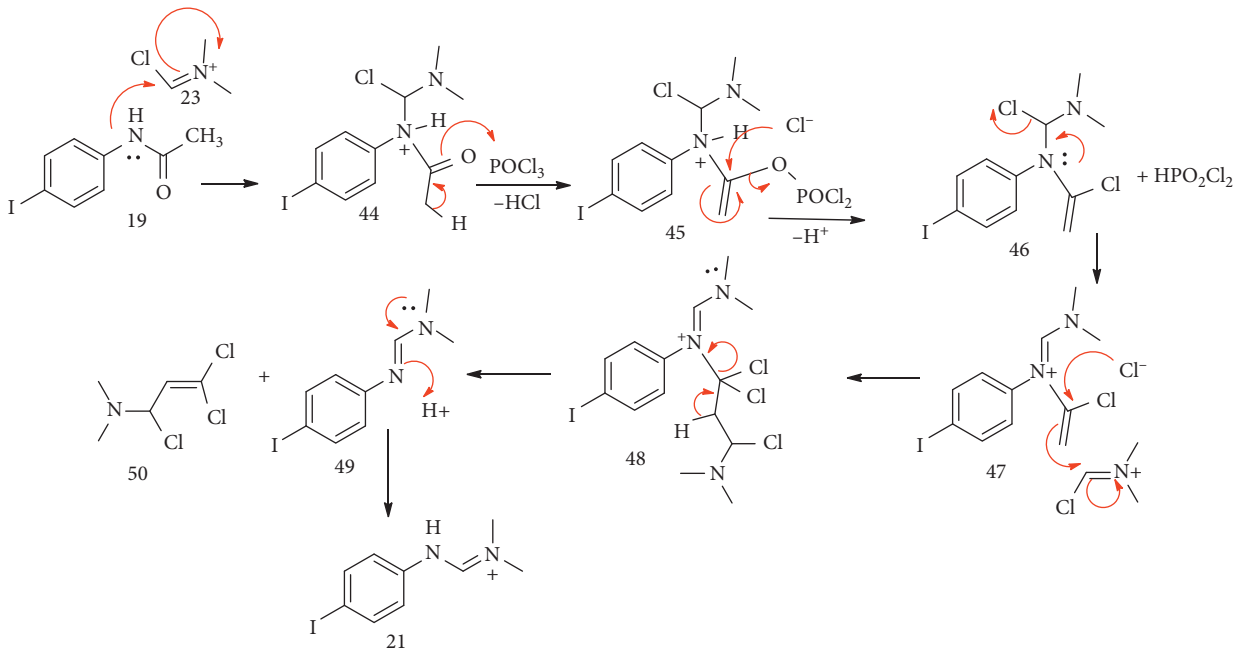
The results are expressed as mean ± SD for three experiments ($n = 3$). Means with the same letter (upper case) within the column are significantly different; means with the same letter (lower case)) in the same column are not significantly different.

The antibacterial activities of the synthetic compounds were compared with those of ciprofloxacin at 0.01 μg/mL, and the results are depicted in Figure 1. The figure clearly showed that compounds **4**, **9**, **10**, and **19** have better activities in all four bacterial strains.

3.3. The Radical Scavenging Activity of the Synthetic Compounds. This method was developed by Blois (1958) with the viewpoint to determine the antioxidant activity in a like manner by using a stable free radical α,α-diphenyl-β-picrylhydrazyl (DPPH) [38]. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants [38]. DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. Qualitatively, antioxidants decolorize the purple color of DPPH, and the intensity of the



Scheme 4: Proposed mechanism of formation of 2-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde.



Scheme 5: Proposed mechanism of N-(((4-iodophenyl)amino)methylene)-N-methylmethanaminium.

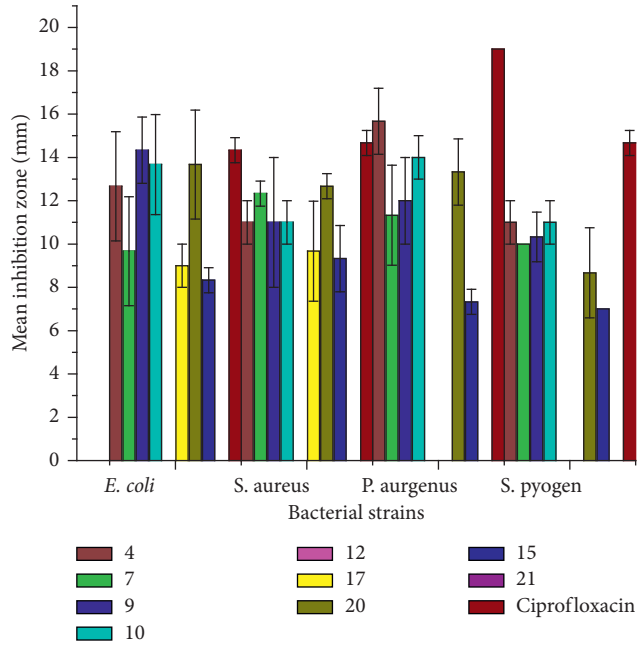


Figure 1: The inhibition zone (mm) of synthetic compounds at 0.1 μg/μL. The error bar indicates the standard deviation.

color notifies the extent of the reaction. Quantitatively, the change in absorbance at 517 nm was used to quantify the radical scavenging capacity of substances. The DPPH assay is based on both electron transfer (SET) and hydrogen atom transfer (HAT) reactions [39]. DPPH assay is relatively an easy, economic, and rapid method to evaluate the radical scavenging activity of nonenzymatic antioxidants [39].

Because of these merits, DPPH was used to determine the radical scavenging capacity of the synthetic compounds. The measurements were made after DPPH-sample mixtures were kept at 37°C in a dark oven for 30 minutes to attain steady-state equilibrium. As shown in Table 2, most of the synthetic compounds showed very good radical scavenging activity. Two of the synthetic compounds, namely, **9** and **20**, have IC_{50} values less than 8 μg/mL. And the IC_{50} values of **9** and **20** are 1.25 and 1.75 μg/mL, respectively, which are lower than the IC_{50} of ascorbic acid (4.5 μg/mL). Thus, these two compounds were the strongest antioxidant agents. Structurally, **9** has an acidic proton and electron-rich aromatic nucleus and **20** possesses labile iodine and

Table 2: The % inhibition of the synthetic compounds.

Compounds	Concentrations in μg/mL					IC_{50} (μg/mL)
	25	20	15	10	5	
4	29.22 ± 1.37	27.38 ± 0.49	26.67 ± 1.07	24.96 ± 0.49	21.98 ± 2.45	85.4
7	58.78 ± 2.53	52.20 ± 0.65	50.64 ± 186	40.36 ± 1.59	34.75 ± 2.46	17.2
9	83.26 ± 1.72	80.85 ± 0.43	79.86 ± 0.99	77.72 ± 1.05	52.27 ± 2.98	1.25
10	17.64 ± 1.31	14.61 ± 0.25	13.48 ± 0.25	10.35 ± 0.25	2.55 ± 0.0	75.1
12	52.48 ± 1.23	51.34 ± 0.49	27.52 ± 1.72	25.81 ± 0.49	7.81 ± 0.25	22.40
15	25.25 ± 0.88	27.23 ± 0.0	24.82 ± 0.25	11.20 ± 0.25	4.40 ± 0.24	42.75
17	33.48 ± 1.48	24.54 ± 0.88	24.68 ± 0.43	22.13 ± 1.86	9.36 ± 1.27	41.8
20	89.50 ± 0.65	84.78 ± 0.37	80.30 ± 0.97	64.52 ± 1.12	53.41 ± 0.87	1.75
21	45.53 ± 1.28	44.40 ± 0.24	31.34 ± 0.98	29.32 ± 1.08	6.52 ± 0.25	25.0
Ascorbic acid	95.83 ± 1.19	86.46 ± 0.54	76.60 ± 0.53	64.40 ± 0.62	48.91 ± 0.49	4.5

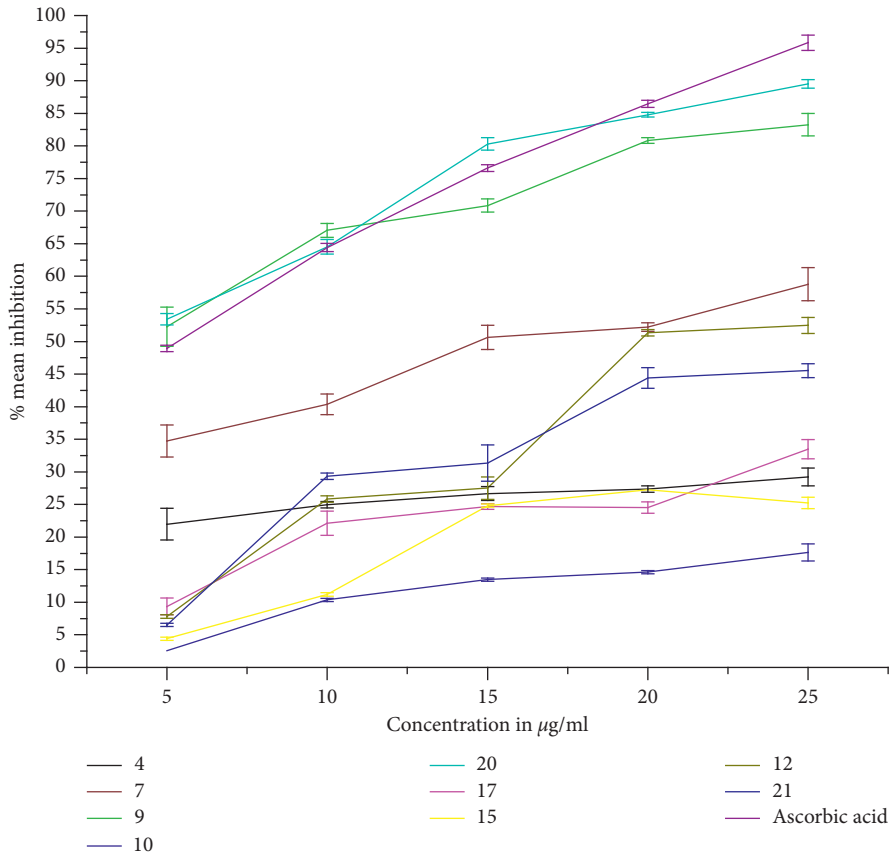


Figure 2: The percent inhibition of the compounds and the standard.

α,β-conjugated bond which may be involved in an electron transfer process with DPPH. For the other three compounds, **7**, **12**, and **21**, the IC_{50} values are between 15 and 25 μg/mL showing that these have also significant antioxidants even though they are weaker than ascorbic acid. Structurally, these compounds contain C=C, carboxylic acid, and iodo as labile functional groups.

The remaining four compounds **4, 10**, **15**, and **17** are moderate in their radical scavenging activities. Their IC_{50} values vary from 41 to 85 μg/mL. The antioxidant properties of these compounds have been compared with ascorbic acid (Figure 2).

In Silico Molecular Docking Evaluation. Bacterial DNA gyrase is vital for the survival of bacteria. Recently, researchers have explored a range of synthetic inhibitors as antibacterial drugs to target DNA gyrase [38, 39]. Therefore, we have carried out the molecular docking analysis for the synthesized compounds to elucidate their binding interactions with DNA gyrase and compared them with the clinical drug inhibitor (ciprofloxacin). The synthesized compounds (**4–21**) were found to have maximum binding energy ranging from −5.4 to −7.9 kcal/mol (Table 3), with the best result achieved using compound **10** (−7.9 kcal/mol). The binding affinity, H-bond, and residual interaction of nine

Table 3: Molecular docking value of synthetic compounds (**4–21**) against *E. coli* DNA gyrase B (PDB ID : 6F86).

Compounds	Affinity (kcal/mol)	H-bond	Residual amino acid interactions	
			Hydrophobic/Pi-cation/Pi-anion/Pi-alkyl interactions	Van der walls interactions
4	−6.9	Glu-50, Pro-79, Asn-46	Ile-94, Ile-78	Asp-73, Asp-49, Gly-77
7	−6.8	Pro-79, Asn-46	Ile-78, Ile-94, Glu-50	Asp-73, Asp-49, Gly-77, Thr-165
9	−6.9	Thr-165	Asn-46, Ile-78, Pro-79, Ile-94, Glu-50	Asp-73, Ala-47, Arg-76, Gly-77
10	−7.9	Asp-73, Thr-165, Pro-79, Glu-50	Ala-47, Val-167, Ile-78, Ile-94	Val-43
12	−7.2	Gly-77, Thr-165, Asn-46	Ala-47, Val-43, Val-167, Ile-78	Gly-75
15	−6.8	Asp-73, Thr-165, Gly-77	Asn-46, Ile-78, Ile-94	Val-43, Ala-47, Arg-76, Pro-79, Gly-75, Glu-50
17	−6.1	--	Pro-79, Ile-78, Arg-76, Glu-50	Asp-73, Asn-46, Ala-47, Gly-77, Thr-165
20	−5.4	Asp-73, Val-43	Glu-50, Ile-78, Pro-79	Gly-77, Asn-46, Ala-47, Val-167
21	−5.4	Ala-47, Asp-73	Asn-46, Ile-78	Val-43, Glu-50, Thr-165
Ciprofloxacin	−7.2	Asp-73, Arg-76, Thr-165	Glu-50, Gly-77, Ile-78, Asn-46	Ala-47

Table 4: Molecular docking results of synthesized compounds against *S. aureus* topoisomerase IV (PDB ID 2INR).

Compounds	Affinity (kcal/mol)	H-bond	Residual amino acid interactions	
			Hydrophobic/Pi-cation/Pi-anion/Pi-alkyl interactions	Van-der walls interactions
4	−5.9	Glu-356	--	Arg-35, Ser-349, His-353, Asn-352, Asp-335, Ile-355, Ala-359
7	−5.8	Ser-141	Glu-138, Asn-360	Phe-142, Thr-139, Val-140
9	−5.9	Ser-141, Asn-360	Glu-138, Thr-139	Glu-356, Val-140, Phe-142
10	−5.9	Ser-141, Asn-360, Thr-139	Glu-138	Glu-356, Val-140
12	−5.7	Ser-141, Asn-360, Thr-139	Phe-142	Val-140
15	−4.7	Val-140	Phe-142, Asn-360	Ser-141, Thr-139
17	−4.5	Glu-138	--	Ser-141, Phe-142, Val-140, Asn-360, Thr-139
20	−4.8	Asn-352	Arg-35, Glu-356, Ile-355, Ser-349, Asp-335	His-353
21	−5.4	Val-140	Glu-138	Thr-139, Phe-142
Ciprofloxacin	−4.9	Glu-138, Ser-141, Asn-360	Thr-139, Glu-356, Arg-35	Met-154

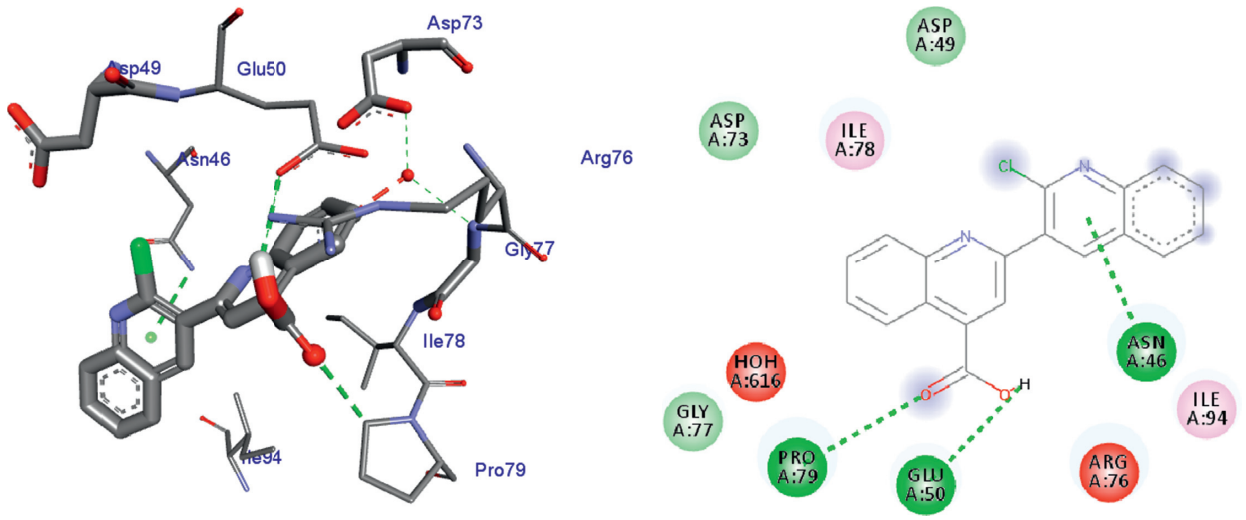


Figure 3: The binding interactions of **4** against *E. coli* DNA gyrase A (PDB ID : 6F86).

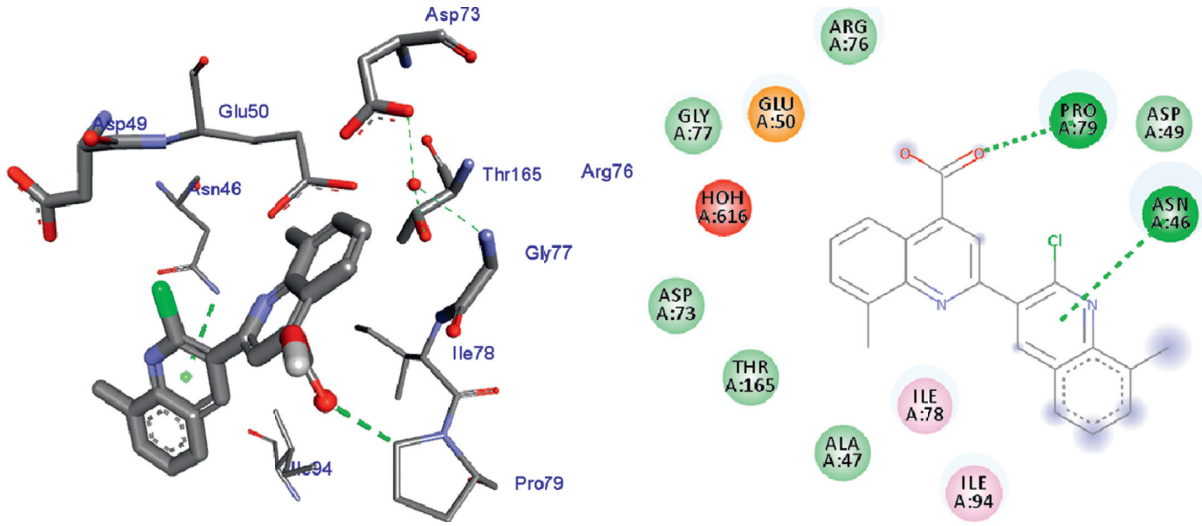


Figure 4: The binding interactions of **7** against *E. coli* DNA gyrase A (PDB ID : 6F86).

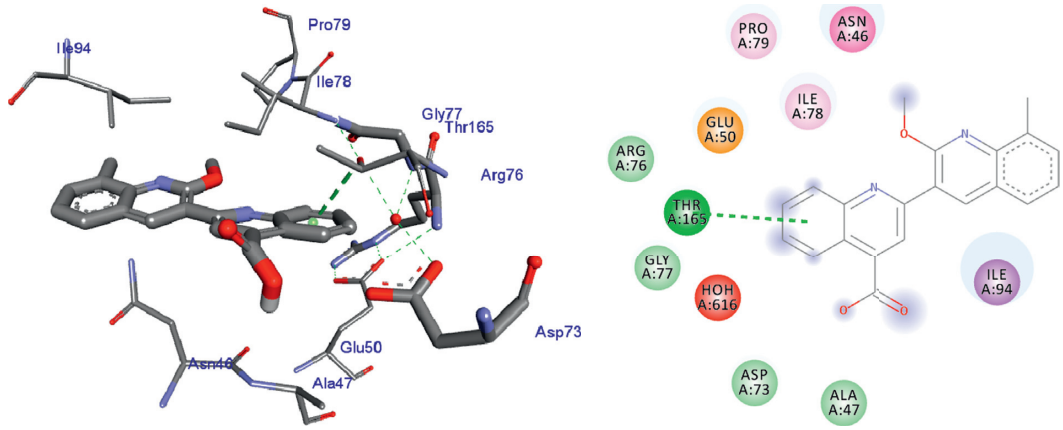


Figure 5: The binding interactions of **9** against *E. coli* DNA gyrase A (PDB ID : 6F86).

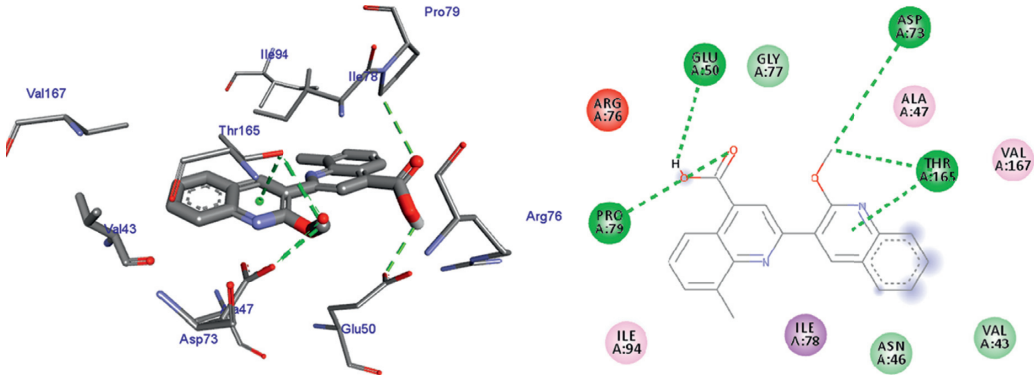


Figure 6: The binding interactions of **10** against *E. coli* DNA gyrase A (PDB ID : 6F86).

compounds and ciprofloxacin were summarized in Table 3. Compared to ciprofloxacin, compounds **4–21** show similar residual interactions with amino acid residues Glu-50, Gly-77, Ile-78, Asn-46, Ile-94, and Ala-47 and H-bond with Asp-73, Arg-76, and Thr-165. Compounds **4, 7,** and **10** have additional hydrogen bonding interaction with amino acid residue Pro-79. Compounds **4** (Asn-46, Glu-50), **5** (Asn-46), **12** (Asn-46, Gly-77), and **17** (Gly-77) have shown additional hydrogen bonding interaction with amino acid residues. The synthetic compound **10** recapitulates the residual amino acid interactions of ciprofloxacin against DNA gyrase (6f86). In this study, the residual interaction of compounds (**4–21**) was

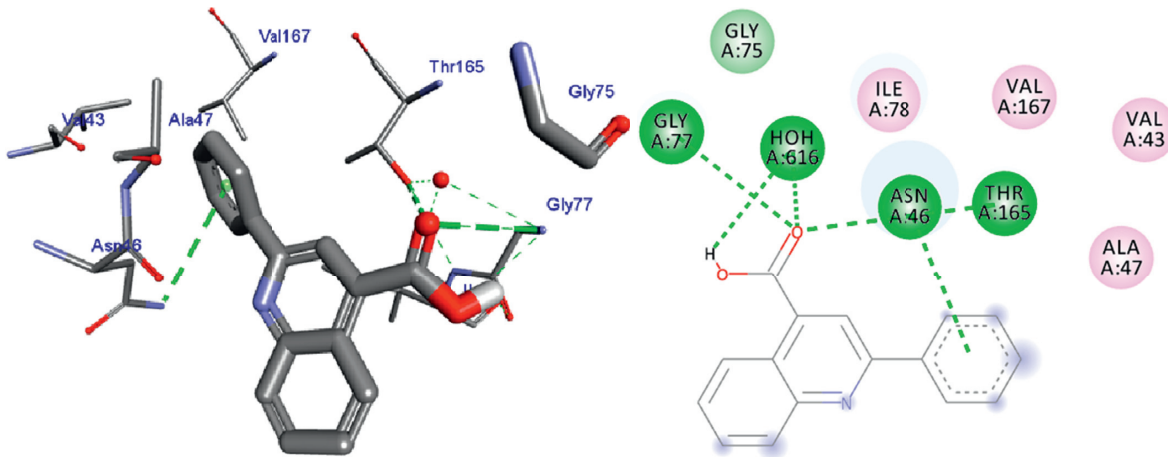


Figure 7: The binding interactions of **12** against *E. coli* DNA gyrase A (PDB ID : 6F86).

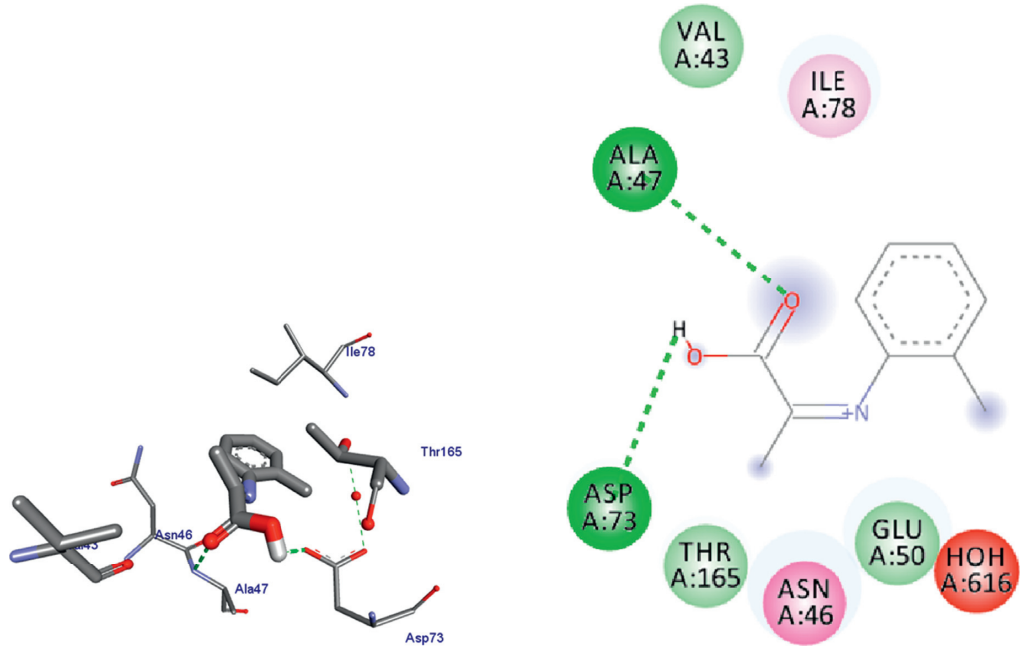


Figure 8: The binding interactions of **15** against *E. coli* DNA gyrase A (PDB ID: 6F86).

in good agreement with the previously reported binding modes that include the essential interactions between the ligand, Asp-73, and the water molecule. The molecular docking analysis results are in good agreement with *in vitro* analysis of the synthesized compounds, **4** (−6.9 kcal/mol), **7** (−6.8 kcal/mol), **9** (−6.9 kcal/mol), **10** (−7.9 kcal/mol), **12** (−7.2 kcal/mol), and **17** (−6.8 kcal/mol) activities against *E. coli*. The *in silico* analysis shows that compounds **10** (−7.9 kcal/mol) and **12** (−7.2 kcal/mol) revealed better activity. Compounds **15** (−5.4 kcal/mol, **20** (−6.1 kcal/mol), and **21** (−5.4 kcal/mol)) docking results were partially matching the ciprofloxacin interactions with amino acid residues. Based on the *in silico* molecular docking analysis results, compounds **10** and **12** show comparable residual interactions and docking scores of ciprofloxacin. Therefore, compound **10** might have better antibacterial agents than the other compounds reported herein. The binding affinity, H-bond, and residual interaction of ten compounds were summarized in Table 3.

The molecular docking analysis for the synthesized compounds was also carried out against *S. aureus* topoisomerase IV (PDB ID 2INR) to elucidate their binding interactions with topoisomerase IV in the case of Gram-positive bacteria and compared them with the clinical drug inhibitor (ciprofloxacin). The synthesized compounds (**4**–**21**) here were also found to have maximum binding energy ranging from −4.5 to −5.9 kcal/mol (Table 4), with the best result achieved using compound **10** (−5.9 kcal/mol) and ciprofloxacin (−4.9 kcal/mol). The same three synthetic compounds, namely, **4**, **9**, and **10**, displayed the maximum binding affinity with topoisomerase IV of *S. aureus*. Thus, compounds **4**, **9**, and **10**

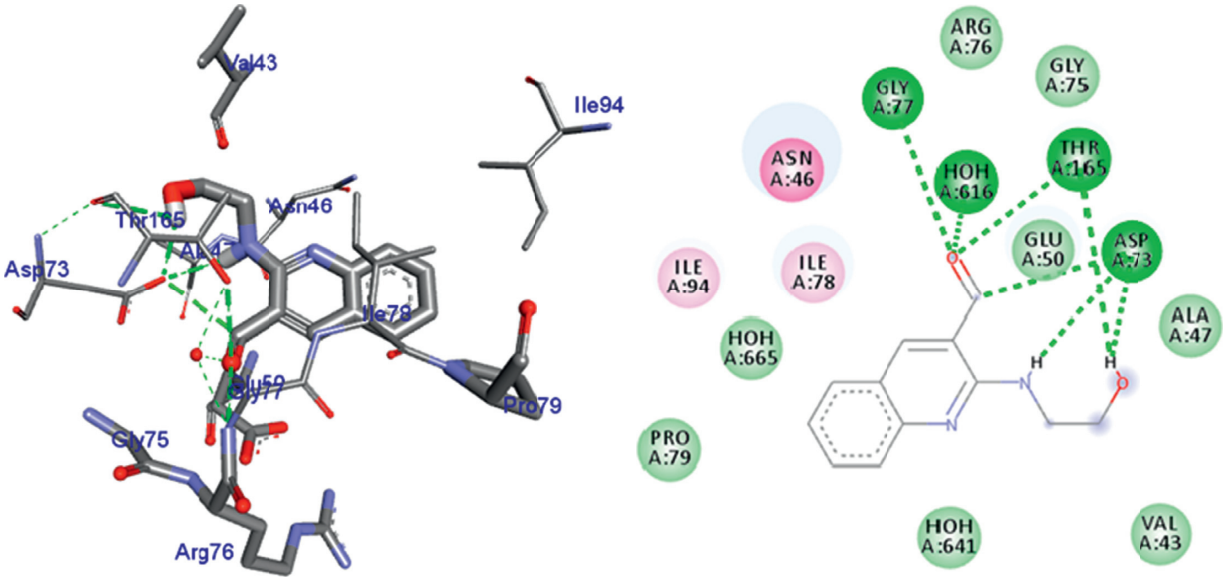


Figure 9: The binding interactions of **17** against *E. coli* DNA gyrase A (PDB ID: 6F86).

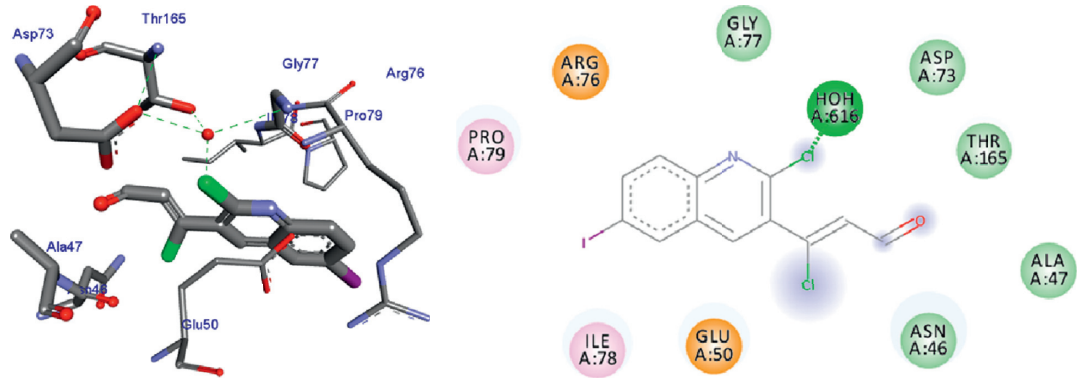


Figure 10: The binding interactions of **20** against *E .coli* DNA gyrase A (PDB ID: 6F86).

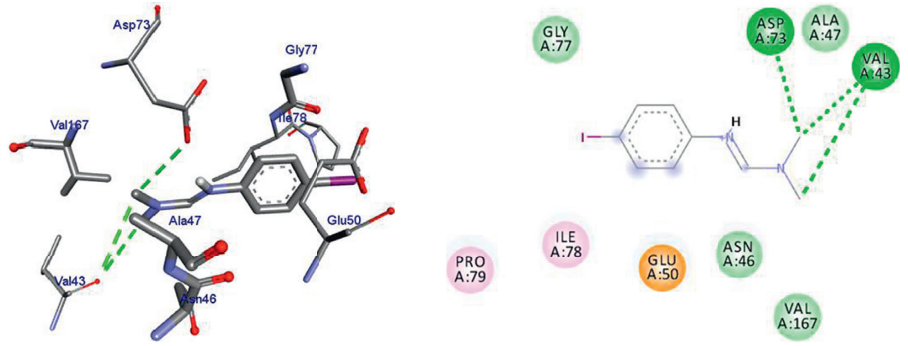


Figure 11: The binding interactions of **21** against *E. coli* DNA gyrase A (PDB ID: 6F86).

exhibited good antibacterial activities against both Gram-negative and Gram-positive bacteria in both *in vitro* and molecular docking evaluations.

The 3-dimensional binding interaction of nine compounds and ciprofloxacin against *E. coli* gyrase B complex is illustrated in Figures 3–11.

4. Conclusion

In conclusion, new [2,3′-biquinoline]-4-carboxylic acid and quinoline-3-carbaldehyde analogs were synthesized by the application of Vilsmeier–Haack, Doebner, nucleophilic substitution, and acid hydrolysis reactions. The antibacterial activities of the novel compounds were screened against *S. aureus* (ATCC25923, *S. pyogenes* (ATCC 27853), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC25923) with paper disc diffusion method. Seven of the synthetic compounds showed good antibacterial activities in all four bacterial strains relative to ciprofloxacin, the positive control in the experiment. Among them, compounds **4**, **9**, and **10** displayed the best mean inhibition zone (13.7 ± 0.58 to 20.7 ± 1.5 mm) at a concentration of 0.2 μg/μL against some of the bacterial strains studied. The radical scavenging activities of the compounds were evaluated with DPPH radical assay. Most of them showed very good radical scavenging activities. The IC_{50} values of two of the compounds, namely, **9** and **20,** are less than 2 μg/mL. Particularly, **9** and **20** with IC_{50} values of 1.25 and 1.75 μg/mL showed a stronger radical scavenging activity than ascorbic acid (4.5 μg/mL). The molecules were also docked against *E. coli DNA gyrase B* and *topoisomerase IV* of *S. aureus* to study the maximum affinity of the compounds for the enzyme within the active site of the enzymes. The results of *in silico* molecular docking evaluation of the compounds against *E. coli* DNA gyrase A were also in good agreement with *in vitro* antibacterial analysis. Three of the synthetic compounds, **4** (−6.9 kcal/mol), **9** (−6.9 kcal/mol), and **10** (−7.9 kcal/mol), exhibited the highest binding affinity comparable to ciprofloxacin against *E. coli*. These compounds also displayed the maximum mean inhibition zone *in vitro* analysis showing a nice agreement between the two experiments. [40–48]

Data Availability

The data supporting these results are available from the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper**.**

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Supplementary Materials

The NMR spectra of the synthesized compounds and molecular docking figures. (*Supplementary Materials*)

References

[1] M. I. Hutchings, A. W. Truman, and B. Wilkinson, "Antibiotics: past, present and future," *Current Opinion in Microbiology*, vol. 51, pp. 72–80, 2019.

[2] F. Moges, M. Endris, A. Mulu et al., "The growing challenges of antibacterial drug resistance in Ethiopia," *Journal of Global Antimicrobial Resistance*, vol. 2, no. 3, pp. 148–154, 2014.

[3] I. Roca, "The global threat of antimicrobial resistance: Science for intervention," *New Microbes and New Infections*, vol. 6, pp. 22–29, 2015.

[4] M. T. Moghadam, "How phages overcome the challenges of drug resistant bacteria in clinical infections," *Infection and Drug Resistance*, vol. 13, pp. 45–61, 2020.

[5] W. C Reygaert, "An overview of the antimicrobial resistance mechanisms of bacteria," *AIMS Microbiology*, vol. 4, no. 3, pp. 482–501, 2018.

[6] J. L. Martínez, "Mechanisms of action and of resistance to quinolones," *Antibiotic Drug Resistance*, vol. 20195, no. 2, pp. 39–55, 2019.

[7] G. R. Dwivedi, "Synergy of clavine alkaloid 'chanoclavine' with tetracycline against multi-drug-resistant E. coli," *Antibiotic Drug Resistance*, vol. 37, no. 5, 2019.

[8] L. Freire-Moran, B. Aronsson, C. Manz et al., "Critical shortage of new antibiotics in development against multidrug-resistant bacteria-Time to react is now," *Drug Resistance Updates*, vol. 14, no. 2, pp. 118–124, 2011.

[9] D. Hooper, "Emerging mechanisms of fluoroquinolone resistance," *Emerging Infectious Diseases*, vol. 7, no. 2, pp. 337–341, 2001.

[10] A. Talebi Bezmin Abadi, A. A. Rizvanov, T. Haertlé, and N. L. Blatt, "World health organization report: current crisis of antibiotic resistance," *BioNanoScience*, vol. 9, no. 4, pp. 778–788, 2019.

[11] M. A. S. Abdelwahid, T. Elsaman, M. S. Mohamed, S. A. Latif, M. M. Mukhtar, and M. A. Mohamed, "Synthesis, characterization, and antileishmanial activity of certain quinoline-4-carboxylic acids," *Journal of Chemistry*, vol. 2019, Article ID 2859637, 6 pages, 2019.

[12] M. A. Abd-El-Maksoud, H. A. Tawfik, S. S. Maigali, F. M. Soliman, M. E. Moharam, and M. F. Dondeti, "Synthesis, antimicrobial and molecular docking evaluation of some heterocycles containing quinoline moiety," *Der Pharma Chemica*, vol. 8, no. 13, pp. 291–301, 2016.

[13] X. Wen, S.-B. Wang, D.-C. Liu, G.-H. Gong, and Z.-S. Quan, "Synthesis and evaluation of the anti-inflammatory activity of quinoline derivatives," *Medicinal Chemistry Research*, vol. 24, no. 6, pp. 2591–2603, 2015.

[14] S. Eswaran, A. V. Adhikari, I. H. Chowdhury, N. K. Pal, and K. D. Thomas, "New quinoline derivatives: synthesis and investigation of antibacterial and antituberculosis properties," *European Journal of Medicinal Chemistry*, vol. 45, no. 8, pp. 3374–3383, 2010.

[15] J. Zhang, S. Wang, Y. Ba, and Z. Xu, "1,2,4-Triazole-quinoline/quinolone hybrids as potential anti-bacterial agents," *European Journal of Medicinal Chemistry*, vol. 174, pp. 1–8, 2019.

[16] X.-M. Chu, C. Wang, W. Liu et al., "Quinoline and quinolone dimers and their biological activities: an overview," *European Journal of Medicinal Chemistry*, vol. 161, pp. 101–117, 2019.

[17] R. Musiol, J. Jampilek, V. Buchta et al., "Antifungal properties of new series of quinoline derivatives," *Bioorganic & Medicinal Chemistry*, vol. 14, no. 10, pp. 3592–3598, 2006.

[18] S. Mukherjee and M. Pal, "Quinolines: a new hope against inflammation," *Drug Discovery Today*, vol. 18, no. 7-8, pp. 389–398, 2013.

[19] Y.-Q. Hu, C. Gao, S. Zhang et al., "Quinoline hybrids and their antiplasmodial and antimalarial activities," *European Journal of Medicinal Chemistry*, vol. 139, pp. 22–47, 2017.

[20] K. Kaur, M. Jain, R. P. Reddy, and R. Jain, "Quinolines and structurally related heterocycles as antimalarials," *European Journal of Medicinal Chemistry*, vol. 45, no. 8, pp. 3245–3264, 2010.

[21] L. W. Deady, A. J. Kaye, G. J. Finlay, B. C. Baguley, and W. A. Denny, "Synthesis and antitumor properties of N-[2-(dimethylamino)ethyl]carboxamide derivatives of fused tetracyclic quinolines and quinoxalines: a new class of putative topoisomerase inhibitors," *Journal of Medicinal Chemistry*, vol. 40, no. 13, pp. 2040–2046, 1997.

[22] G. Ramann and B. Cowen, "Recent advances in metal-free quinoline synthesis," *Molecules*, vol. 21, no. 8, pp. 986–8, 2016.

[23] Y. Erugu, B. Sangepu, B. Gandu, G. Anupoju, and V. R. Jetti, "Design, synthesis of novel quinoline-4-carboxylic acid derivatives and their antibacterial activity supported by molecular docking," *World Journal Of Pharmacy & Pharmaceutical Sciences*, vol. 3, no. 12, pp. 1612–1634, 2014.

[24] S. Narwal, S. Kumar, and P. K. Verma, "Synthesis and therapeutic potential of quinoline derivatives," *Research on Chemical Intermediates*, vol. 43, no. 5, pp. 2765–2798, 2017.

[25] V. D. Boyarshinov, A. I. Mikhalev, T. A. Yushkova, S. V. Ukhov, and T. M. Kon'shina, "Synthesis and biological activity of quinoline-2-carboxylic acid aryl esters and amides," *Pharmaceutical Chemistry Journal*, vol. 51, no. 5, pp. 351–354, 2017.

[26] C. Mugnaini, S. Pasquini, and F. Corelli, "The 4-quinolone-3-carboxylic acid motif as a multivalent scaffold in medicinal chemistry," *Current Medicinal Chemistry*, vol. 16, no. 14, pp. 1746–1767, 2009.

[27] C. Zehiroglu and S. B. Ozturk Sarikaya, "The importance of antioxidants and place in today's scientific and technological studies," *Journal of Food Science and Technology*, vol. 56, no. 11, pp. 4757–4774, 2019.

[28] O. Meth-Cohn, B. Narine, B. Tarnowski et al., "A versatile new synthesis of quinolines and related fused pyridines. Part 9. Synthetic application of the 2-chloroquinoline-3-carbaldehydes," *Journal of the Chemical Society, Perkin Transactions*, vol. 1, no. 1520, pp. 2509–2517, 1981.

[29] E. Bergogne-Bérézin and K. J. Towner, "Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features," *Clinical Microbiology Reviews*, vol. 9, no. 2, pp. 148–165, 1996.

[30] A. Allouche, "Software news and updates gabedit—a graphical user interface for computational chemistry softwares," *Journal of Computational Chemistry*, vol. 32, pp. 174–182, 2012.

[31] S. B. Carr, G. Makris, S. E. V. Phillips, and C. D. Thomas, "Crystallization and preliminary X-ray diffraction analysis of two N-terminal fragments of the DNA-cleavage domain of topoisomerase IV fromStaphylococcus aureus," *Acta Crystallographica Section F Structural Biology and Crystallization Communications*, vol. 62, no. 11, pp. 1164–1167, 2006.

[32] S. Narramore, C. E. M. Stevenson, A. Maxwell, D. M. Lawson, and C. W. G. Fishwick, "New insights into the binding mode of pyridine-3-carboxamide inhibitors of *E. coli* DNA gyrase," *Bioorganic & Medicinal Chemistry*, vol. 27, no. 16, pp. 3546–3550, 2019.

[33] L.-M. Wang, L. Hu, H.-J. Chen, Y.-Y. Sui, and W. Shen, "One-pot synthesis of quinoline-4-carboxylic acid derivatives in water: ytterbium perfluorooctanoate catalyzed Doebner reaction," *Journal of Fluorine Chemistry*, vol. 130, no. 4, pp. 406–409, 2009.

[34] D. B. Patel, R. H. Vekariya, K. D. Patel, N. P. Prajapati, and S. Mahesh, "Recent advances in synthesis of quinoline-4-carboxylic acid and their biological evaluation: a review," *Journal of Chemical and Pharmaceutical Research*, vol. 9, no. 2, pp. 216–230, 2017.

[35] X. Wang, X. Xie, Y. Cai et al., "Design, synthesis and antibacterial evaluation of some new 2-phenyl-quinoline-4-carboxylic acid derivatives," *Molecules*, vol. 21, no. 3, p. 340, 2016.

[36] A. D. Thomas, C. Josemin, and C. V. Asokan, "Vilsmeier-Haack reactions of carbonyl compounds: synthesis of substituted pyrones and pyridines," *Tetrahedron*, vol. 60, no. 23, pp. 5069–5076, 2004.

[37] W. S. Hamama, M. E. Ibrahim, A. A. Gooda, and H. H. Zoorob, "Recent advances in the chemistry of 2-chloroquinoline-3-carbaldehyde and related analogs," *RSC Advances*, vol. 8, no. 16, pp. 8484–8515, 2018.

[38] M. Saha, S. Hasan, R. Akter et al., "*In vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* Linn," *Bangladesh Journal of Veterinary Medicine*, vol. 6, no. 2, pp. 197–202, 1970.

[39] N. Liang and D. Kitts, "Antioxidant property of coffee components: assessment of methods that define mechanisms of action," *Molecules*, vol. 19, no. 11, pp. 19180–19208, 2014.

[40] N. A. Liberto, J. B. Simões, S. de Paiva Silva et al., "Quinolines: microwave-assisted synthesis and their antifungal, anticancer and radical scavenger properties," *Bioorganic & Medicinal Chemistry*, vol. 25, no. 3, pp. 1153–1162, 2017.

[41] M. Yamato, Y. Takeuchi, K. Hashigaki et al., "Synthesis and antitumor activity of fused tetracyclic quinoline derivatives. 1," *Journal of Medicinal Chemistry*, vol. 32, no. 6, pp. 1295–1300, 1989.

[42] N. Muruganantham, R. Sivakumar, N. Anbalagan, V. Gunasekaran, J. T. Leonard, and "Synthesis, anticonvulsant and antihypertensive activities of 8-substituted quinoline derivatives," *Biological and Pharmaceutical Bulletin*, vol. 27, no. 10, pp. 1683–1687, 2004.

[43] D. J. Brenner, J. T. Staley, and N. R. Krieg, "Bergey ' s manual ® of systematic Bacteriology classification of procaryotic organisms and the concept of bacterial speciation," *Clinical Microbiology Reviews*, vol. 3, 2006.

[44] T. P. Forrest, G. A. Dauphinee, and W. F. Miles, "On the mechanism of the Doebner-Miller reaction," *Canadian Journal of Chemistry*, vol. 47, no. 11, pp. 2121-2122, 1969.

[45] S. E. Denmark and S. Venkatraman, "On the mechanism of the Skraup–Doebner–Von miller quinoline synthesis," *The Journal of Organic Chemistry*, vol. 71, no. 4, pp. 1668–1676, 2006.

[46] S. J. Wadher, N. A. Karande, D. S. Borkar, and P. G. Yeole, "Synthesis and biological evaluation OF SCHIFF bases OF cinchophen as antimicrobial agents," *International Journal of ChemTech Research*, vol. 1, no. 4, pp. 1297–1302, 2009.

[47] H. Hailekiros, F. Kedir, and M. Endale, "In silico molecular docking, dft analysis and ADMET studies of carbazole alkaloid and coumarins from roots of clausena anisata: a potent inhibitor for quorum sensing molecular docking analysis," *AutoDock*, vol. 14, pp. 13–24, 2021.

[48] M. M. Ghorab, A. S. Alqahtani, A. M. Soliman, and A. A. Askar, "Novel N - (substituted) thioacetamide quinazolinone benzenesulfonamides as antimicrobial agents," *International Journal of Nanomedicine*, vol. 15, pp. 3161–3180, 2020.