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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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-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 IC50 values of 1.25 and 1.75 μg/mL, respectively. At the same concentration, the IC50 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.
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 is typically 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], antitussive [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-carboxaldehyde 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 Janson 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-carboxaldehyde 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 CH₂Cl₂: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 (υ C–H str.), 2838 (υ C–H str.), 1675 (υ acidic C–N), 1562 (υ aromatic C–N), 1304 (υ aromatic C–H) 2904 (υ C–H str.), 2848 (υ C–H str.).

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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); 13C NMR (100 MHz, DMSO-d6): δC 17.8 (C-10), 18.6 (C-11), 53.9 (C-12), 123.0 (C-3'), 123.7 (C-3), 123.9 (C-5), 124.1 (C-4a'), 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), 153.0(C-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; 1H NMR (400 MHz, DMSO-d6): δH 7.56 (3H, m, H-3, H-4, H-5), 7.72 (1H, t, J = 7.02 Hz, H-6), 7.89 (1H, d, 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); 13C NMR (100 MHz, DMSO-d6): δC 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-((2-Hydroxyethyl)amino)quinoline-3-Carbaldehyde (13). To the mixture of methanol (15 mL), potassium bicarbonate (1.10 g, 0.020 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 (1.70 g, 0.008 mol) was refluxed for 4 hours in a mixture of methanol (15 mL), potassium bicarbonate (1.10 g, 0.020 mol), and DMF (10 mL). The mixture was refluxed for 12 hours while the progress of the reaction was monitored occasionally with TLC. The yield was 1.4 g (82.4%). 2-Methoxy-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, 0.020 mol), and DMF (10 mL). The mixture was refluxed for 12 hours while the progress of the reaction was monitored occasionally with TLC. The yield was 1.4 g (82.4%). 2-Methoxy-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, 0.020 mol), and DMF (10 mL). The mixture was refluxed for 12 hours while the progress of the reaction was monitored occasionally with TLC. The yield was 1.4 g (82.4%). 2-Methoxy-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, 0.020 mol), and DMF (10 mL). The mixture was refluxed for 12 hours while the progress of the reaction was monitored occasionally with TLC. The yield was 1.4 g (82.4%). 2-Methoxy-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, 0.020 mol), and DMF (10 mL). The mixture was refluxed for 12 hours while the progress of the reaction was monitored occasionally with TLC. The yield was 1.4 g (82.4%). 2-Methoxy-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, 0.020 mol), and DMF (10 mL). The mixture was refluxed for 12 hours while the progress of the reaction was monitored occasionally with TLC. The yield was 1.4 g (82.4%).
by suction filtration and allowed to dry in the air (0.62 g, 2.4 mmol). The dried product was refluxed in 20% H₂SO₄ (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): 1H NMR (400 MHz, DMSO-d₆): δ₁0 3.65 (4H, H-10, H-11), 4.96 (1H, s, H-9), 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); 13C NMR (100 MHz, DMSO-d₆): δC 37.0 (CH₃), 43.6 (CH₃), 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 dimethyl sulfoxide (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 inculcated 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:

\[
\%\text{inhibition} = \frac{A_0 - A_1}{A_0} \times 100
\]

where \(A_0\) is the absorbance of the control and \(A_1\) is the absorbance of the sample. The results are averages of three measurements. The IC₅₀ 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

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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 crystallized 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 × 29 × 64 and 29 × −24 × 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-carboxaldehyde 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 acetaldehyde and 2-methylacetanilide by utilizing the Vilsmeier reaction which involves treating the corresponding acetaldehyde analogs with POCl3 in

| Compounds  | Concentration (µg/mL) | Staphylococcus aureus | Streptococcus pyogenes | Escherichia coli | Pseudomonas aeruginosa |
|------------|-----------------------|-----------------------|-----------------------|-----------------|------------------------|
| 4          | 0.10                  | 11.0 ± 1.0 ^ab        | 11.0 ± 1.0 ^ab        | 12.7 ± 2.5 ^ab  | 15.7 ± 1.5 ^D          |
|            | 0.20                  | 12.3 ± 0.58 ^b        | 13.7 ± 0.58 ^b        | 13.0 ± 2.0 ^C   | 19.3 ± 3.4 ^D          |
| 7          | 0.10                  | 10.3 ± 0.58 ^ab       | 10.0 ± 0.0 ^c         | 9.7 ± 2.5 ^C    | 11.3 ± 2.3 ^D          |
|            | 0.20                  | 13.0 ± 1.7 ^ab        | 13.3 ± 2.1 ^B         | 12.7 ± 1.2 ^C   | 19.7 ± 1.5 ^D          |
| 9          | 0.10                  | 11.0 ± 3.0 ^ab        | 10.3 ± 1.2 ^ab        | 14.3 ± 1.5 ^C   | 12.0 ± 2.0 ^D          |
|            | 0.20                  | 14.3 ± 1.5 ^ab        | 12.3 ± 1.2 ^b         | 14.0 ± 1.5 ^C   | 20.7 ± 1.5 ^D          |
| 10         | 0.10                  | 11.0 ± 1.0 ^c         | 11.0 ± 1.0 ^b         | 13.7 ± 2.5 ^C   | 14.0 ± 1.0 ^D          |
|            | 0.20                  | 15.7 ± 0.58 ^c        | 11.0 ± 1.7 ^b         | 16.0 ± 1.7 ^C   | 19.7 ± 1.5 ^D          |
| 12         | 0.10                  | 0.0 ± 0.0^c           | 7.0 ± 0.0 ^c          | 7.0 ± 0.0 ^ab   | 0.0 ^ab                |
|            | 0.20                  | 7.0 ± 0.0^c           | 7.0 ± 0.0^c           | 7.0 ± 0.0 ^ab   | 0.0 ^ab                |
| 15         | 0.10                  | 8.33 ± 0.58 ^c        | 7.0 ± 0.0^D           | 7.0 ± 0.58 ^c   | 7.33 ± 0.58 ^ab        |
|            | 0.20                  | 9.67 ± 0.58 ^c        | 8.33 ± 0.58 ^D        | 8.67 ± 0.58 ^c  | 9.67 ± 0.58 ^ab        |
| 17         | 0.10                  | 9.7 ± 2.3 ^c          | 0.0 ± 0^c             | 9.0 ± 1.0 ^c    | 0 ± ^ab                |
|            | 0.20                  | 10.7 ± 2.1 ^c         | 0.0 ± 0^c             | 9.7 ± 2.9 ^c    | 9.3 ± 2.1 ^D          |
| 20         | 0.10                  | 12.7 ± 0.58 ^c        | 8.7 ± 2.1 ^B          | 13.7 ± 2.5 ^c   | 13.3 ± 1.5 ^D          |
|            | 0.20                  | 13.3 ± 1.5 ^c         | 11.0 ± 0.0 ^B         | 14.7 ± 2.1 ^C   | 19.7 ± 1.5 ^D          |
| 21         | 0.10                  | 0^c                   | 0±^b                  | 7.7 ± 0.58 ^c   | 0 ± ^ab                |
|            | 0.20                  | 0^c                   | 0±^b                  | 7.7 ± 0.58 ^c   | 0 ± ^ab                |
| Ciprofloxacin | 0.10               | 14.7 ± 0.58 ^c        | 14.7 ± 0.58 ^bc       | 14.3 ± 0.58 ^bc | 19.0 ± 0.6 ^Dx        |
|            | 0.20                  | 16.33 ± 0.58 ^c       | 16.0 ± 0.0^bc         | 14.3 ± 2.1 ^Ec  | 22.0 ± 1.4 ^Db         |
| DMSO       | 0                     | 0                     | 0                     | 0               | 0                      |

Table 1: The antibacterial activity of the synthetic compounds.

0 = inhibition zone was not observed. Mean zone of inhibition in mm (mean ± S.D) n = 3.
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 excess 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, 10, 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 CH2Cl2: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-thio-cyanatoquinoline-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 H2SO4 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 1H and 13C 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 SOCl2 in place of POCl3 which provided a good vision on the mechanism of Vilsmeier 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.

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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 POCl3 affording intermediate 45, which then underwent addition of Cl− and elimination of H+ to provide 46 which was further rearranged to 21 (Scheme 5).
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### 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...
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 ± 0.0 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 μg/μL while ciprofloxacin was 16.3 ± 0.58 mm. Compound 4 showed 13.7 ± 0.58 mm against *Streptococcus pyogenes* while ciprofloxacin was 16.0 ± 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 μg/μL 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 μg/μL. Thus, 10 displayed the best mean inhibition zone among the synthetic compounds reported herein followed by compounds 9, 7, and 20, consecutively.

**Scheme 1:** Synthesis of [2,3′-biquinoline]-4-carboxylic acids and 2-phenylquinoline-4-carboxylic acid.
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 a,a-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 2: Synthesis of 2-(o-tolylimino)propanoic acid.

Scheme 3: Synthesis of 2-chloroquinoline-3-carbaldehyde analogs.
Scheme 4: Proposed mechanism of formation of 2-chloro-3-(2-chloro-6-iodoquinolin-3-yl)acrylaldehyde.
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 IC₅₀ values less than 8 µg/mL. And the IC₅₀ values of 9 and 20 are 1.25 and 1.75 µg/mL, respectively, which are lower than the IC₅₀ 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

**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.**
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 IC₅₀ 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 IC₅₀ values vary from 41 to 85 μg/mL. The antioxidant properties of these compounds have been compared with ascorbic acid (Figure 2).

### Table 2: The % inhibition of the synthetic compounds.

| Compounds | Concentrations in μg/mL | IC₅₀ (μg/mL) |
|-----------|-------------------------|-------------|
| 4         | 29.22 ± 1.37            | 85.4        |
| 7         | 58.78 ± 2.53            | 17.2        |
| 9         | 83.26 ± 1.72            | 12.5        |
| 10        | 17.64 ± 1.31            | 75.1        |
| 12        | 52.48 ± 1.23            | 22.40       |
| 15        | 25.25 ± 0.88            | 42.75       |
| 17        | 33.48 ± 1.48            | 41.8        |
| 20        | 89.50 ± 0.65            | 1.25        |
| 21        | 45.53 ± 1.28            | 25.0        |
| Ascorbic acid | 95.83 ± 1.19 | 4.5        |

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

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 | Van der walls interactions |
|-----------|---------------------|--------|----------------------------------|---------------------------|
|           |                     |        | Hydrophobic/Pi-cation/Pi-anion/Pi-alkyl 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, Gly-77 | Asp-73, Ala-47, Arg-76, Gly-77 |
| 10        | −7.9                | Asp-73, Thr-165, Asn-46, 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, Gly-50 |
| 17        | −6.1                | -- | Pro-79, Ile-78, Arg-76, Gly-77 | 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, Gly-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 | Van-der walls interactions |
|-----------|---------------------|--------|----------------------------------|---------------------------|
|           |                     |        | Hydrophobic/Pi-cation/Pi-anion/Pi-alkyl 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 | Gly-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).
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
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 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).
exhibited good antibacterial activities against both Gram-negative and Gram-positive bacteria in both \textit{in vitro} and molecular docking evaluations.

The 3-dimensional binding interaction of nine compounds and ciprofloxacin against \textit{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/mL 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 IC50 values of two of the compounds, namely, 9 and 20, are less than 2 μg/mL. Particularly, 9 and 20 with IC50 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)

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