Research Article

Synthesis, Antibacterial, and Antioxidant Activities of Thiazolyl-Pyrazoline Schiff Base Hybrids: A Combined Experimental and Computational Study

Demis Zelelew, Milkyas Endale, Yadessa Melaku, Fedlu Kedir, Taye B. Demissie, Japheth O. Ombito, and Rajalakshman Eswaramoorthy

1 Department of Applied Chemistry, School of Applied Natural Science, Adama Science and Technology University, P.O. Box 1888, Adama, Ethiopia
2 Department of Chemistry, University of Botswana, P/Bag UB 00704, Gaborone, Botswana
3 Department of Biomaterials, Saveetha Dental College and Hospital, Saveetha University, Chennai 600 077, India

Correspondence should be addressed to Milkyas Endale; milkyasendale@yahoo.com

Received 5 July 2022; Accepted 3 September 2022; Published 27 September 2022

1. Introduction

Antimicrobial resistance (AMR) poses a major threat to human health around the world [1]. In recent years, attention has been given to the development of treatments that generate oxidative stress as part of their antibacterial mechanism of action [2]. Therefore, there is an imperative need to search for antimicrobials with antioxidant and antibacterial properties to counter the problems of bacterial resistance. A molecular hybridization is a valuable approach for the design and development of new biological molecules in which two or more active pharmacophoric units are

Thiazole-pyrazoline Schiff base hybrids have a broad range of pharmacological potential with an ability to control the activity of numerous metabolic enzymes. In this work, a greener and more efficient approach has been developed to synthesize a novel series of thiazole-pyrazoline Schiff base hybrids using ZnO nanoparticle-assisted protocol in good to excellent yields (78.3–96.9%) and examined their antibacterial activity against Gram-positive and Gram-negative bacteria, as well as their antioxidant activity.

Compound 24 (IZD equals 18.67 ± 0.58) displayed better activity against P. aeruginosa compared with amoxicillin (IZD equals 14.33 ± 2.52) at 250 µg/mL, whereas compounds 22 and 24 (IZD = 13.33 ± 0.58 mm and 17.00 ± 1.00 mm, respectively) showed better activity against E. coli compared with amoxicillin (IZD = 14.67 ± 0.58 mm) at 500 µg/mL. The remaining compounds showed moderate to weak activity against the tested bacterial strains. Compound 21 displayed significant inhibition of DPPH (IC50 equals 4.63 µg/mL) compared with ascorbic acid (IC50 equals 3.21 µg/mL). Compound 21 displayed 80.01 ± 0.07% inhibition of peroxide formation, suggesting its potential in preventing the formation of lipid peroxides. The results of the ADMET study showed that all synthesized compounds obeyed Lipinski’s rule of five. In silico pharmacokinetic study demonstrated that compound 24 had superior intestinal absorption compared with amoxicillin. In silico molecular docking analysis revealed a binding affinity of ~9.9 Kcal/mol for compound 24 against PqsA compared with amoxicillin (~7.3 Kcal/mol), whereas compounds 22 and 24 displayed higher binding affinity (~8.5 and ~7.9 Kcal/mol, respectively) with DNA gyrase B compared with amoxicillin (~7.1 Kcal/mol), in good agreement with in vitro antibacterial activity against P. aeruginosa and E. coli. In silico toxicity study showed that all synthesized compounds had LD50 (mg/kg) values ranging from 800 to 1,000 putting them in ProTox-II class 4. The in vitro antibacterial activity and molecular docking analysis showed that compound 24 is a promising antibacterial therapeutic agent against P. aeruginosa and E. coli and compound 22 is a promising antibacterial agent against E. coli, whereas compound 21 is found to be a potential natural antioxidant agent. Moreover, the green synthesis approach using ZnO nanoparticle as catalyst was found to be a very efficient method to synthesize biologically active thiazole-pyrazoline Schiff base hybrids compared with the conventional method.

1. Introduction

Antimicrobial resistance (AMR) poses a major threat to human health around the world [1]. In recent years, attention has been given to the development of treatments that generate oxidative stress as part of their antibacterial mechanism of action [2]. Therefore, there is an imperative need to search for antimicrobials with antioxidant and antibacterial properties to counter the problems of bacterial resistance. A molecular hybridization is a valuable approach for the design and development of new biological molecules in which two or more active pharmacophoric units are
combined to form a new hybrid molecule with increased potency [3, 4].

For many decades, thiazole and pyrazoline scaffolds have been studied extensively and found to possess a wide range of chemical reactivity and pharmacological activities [5, 6]. These five-membered heterocyclic compounds have been proven to possess antibacterial activity and the ability to delocalize free radicals and produce stable DPPH fragments [7]. In addition to antibacterial activity, thiazole derivatives exhibit antimicrobial [8], antiviral [9], antidiabetic [10], anticonvulsant [11], antioxidant [12], anti-HIV [13], anti-inflammatory [14], Alzheimer’s disease [15], and antitumor activities [16]. Previous studies revealed that 3,5-diaryl-4,5-dihydro-1H-pyrazole derivatives, which are members of the 2-pyrazoline class, have been extensively investigated using a variety of structural manipulations and it has been discovered that the steric and electronic properties of the various substituents at the N-1, C-3, and C-5 positions, as well as the chirality aspects of these molecules, have a critical influence on activity [17]. The N-N bond link of the pyrazoline ring is thought to be important in their biological activity [18]. Zinc oxide nanoparticle is gaining popularity as a heterogeneous catalyst for a variety of organic transformations due to their low cost, nontoxicity, waste reduction, and recycling properties [19]. The impressive catalytic ability of ZnO nanoparticles associated with morphology-tuned specific microstructures, ability of electron transportation, and their high stability when combined with the intrinsic Lewis acid of the zinc ion motivated us to consider this catalyst.

In the present work, we hereby present ZnO nanoparticle-assisted synthesis of nine novel thiazolyl-pyrazoline Schiff base hybrids, in vitro antibacterial and antioxidant activity along with molecular docking studies, in silico ADMET prediction, drug-likeness, and pharmacokinetic and toxicity properties.

2. Materials and Methods

All starting chemicals and materials used were analytical grades. Merck (Germany), Sigma-Aldrich Sigma Chemical, Co. (USA), and Loba Chemie, Pvt Ltd. (India) provided preliminary chemicals and reagents, which were purchased from commercial vendors and used immediately without additional purification unless otherwise noted. Column chromatography was performed using (Merck) silica gel (particle size 60–120 mm) with n-hexane, ethyl acetate, and dichloromethane as mobile phase. ZnO nanoparticles were obtained from the Department of Applied Chemistry, Material Chemistry Research Team of ASTU, which is characterized by UV-vis and FT-IR spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), photoluminescence (PL), and thermogravimetric analysis (TGA) techniques [20]. Amoxicillin and L-ascorbic acid (99%) were employed as standards, both obtained from Ethiopian Pharmaceutical Manufacturing Factory (EPHARM).

Melting points were determined on a Gallenkamp MFB-595 apparatus, uncorrected. UV spectra were obtained using a double-beam UV-visible spectrophotometer (Model 2201, India). The $^1$H-NMR and $^{13}$C-NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker Avance III 400 Nanospectrometer using tetramethysilane (TMS) as an internal standard. The chemical shifts are expressed in $\delta$ (ppm) and reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), d(doublet of doublets), and brs (broad singlet), respectively. Analytical TLC analysis was performed on pre-coated aluminum plate silica gel 60 F$_{254}$ (Merck, Darmstadt, Germany). Detection was carried out under UV light (254 and 365 nm). Column chromatographic separations were carried out on silica gel (60–120 mesh, Merck). SwissADME and AutoDock Vina 4.2.6 version software packages were used to undertake in silico ADMET and molecular docking simulation investigations, respectively.

2.1. Synthesis Procedures

2.1.1. Synthesis of Chalcones (1, 3-Diaryl-2-propen-1-ones, 3–5).

To an equimolar amount of p-nitro acetophenone (1, 0.06 mol) and the corresponding aldehyde derivatives (2, 0.06 mol) in 5–10 mL of ethanol, 60% (w/v) NaOH was added dropwise (3 mL) and stirred for 30 min (Scheme 1 and SI Appendix A Figure S1) [21]. The resulting solid products (5–7) were stirred in an ice bath for 30 min and then at RT until the precipitate was formed within 3 h. The reaction progress was monitored by TLC with an eluent of n-hexane: ethyl acetate (9:1). The precipitate was filtered off and washed thoroughly with cold distilled water and cold methanol (2 x 20 mL). The products (5–7) were recrystallized from absolute ethanol to yield a yellow precipitate of chalcone derivatives.

2.1.2. Synthesis of 2-Amino-4-(substituted-phenyl)-1, 3-thiazole Derivatives (10–12).

The thiazole moiety was synthesized by utilizing Hantzsch’s modified approach (Scheme 2), which involved a solvent-free reaction of substituted acetophenone (6–8) derivatives with thiourea (9) and iodine under thermal conditions resulting in 2-amino-4-aryl thiazole derivatives (10–12, SI Appendix A, Figure S2) [22]. Thiourea (40 mmol, 2 molar equiv.) was mixed with iodine beads (20 mmol, 1 molar equiv.) in a mortar, and the mixture was homogenized with a pestle. The solids were scratched off into a 250 mL round-bottom flask with a magnetic stirring bar and charged with the corresponding acetophenone (20 mmol, 1 molar equiv.). The flask was heated in an oil bath under an air-cooled condenser at 100°C with stirring. The reaction progress was monitored using TLC in n-hexane and ethyl acetate (4:1). After 3 hours, the mixture was cooled and it solidified again. A saturated aqueous solution of sodium thiosulfate (Na$_2$S$_2$O$_3$.5H$_2$O, 5%) was added with concomitant mechanical disruption of the solid matrix in a sufficient amount to reduce the remaining iodine. The product as a suspension was filtered off and carefully washed with 20 mL of diethyl ether to remove the residues of unreacted acetophenone. The crude product was dissolved in hot water, filtered, and the hot filtrate was adjusted to pH = 9 by aqueous NaHCO$_3$. After cooling, the precipitated product was collected and recrystallized using ethanol.

| Chemical | Used as | Source |
|----------|---------|--------|
| Amoxicillin | Standard | EPHARM |
| L-Ascorbic Acid | Standard | EPHARM |
| Silica Gel | Silica gel | Merck |
| UV-Visible Spectrophotometer | Equipment | Model 2201 |
| TLC Analysis | Analysis | Unpublished |
| Hantzsch Approach | Synthesis | Scheme 2 |
| Thiourea | Reagent | 40 mmol |
| Iodine | Reagent | 40 mmol |
| Sodium Thiosulfate | Reagent | 5% |
| Hot Water | Solvent | Solution |
| Hot Filtrate | Solvent | Adjustment |

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2-(2-Amino-thiazol-4-yl) phenol (12). Pale yellow solid powder; yield: 43.2%; m.p.: 138–142°C; Rf (20% EtOAc in n-hexane) = 0.73; 1H-NMR (400 MHz, DMSO-d6): δ 11.56 (s, 1H, NH), 7.29 (d, J = 7.7 Hz, 1H, ArH), 7.00 (s, 1H, CH of thiazole), 6.70 (t, J = 7.7 Hz, 1H, ArH), 6.61 (d, J = 8.2 Hz, 1H, ArH), 6.38 (t, J = 8.1 Hz, 1H, ArH), 2.09 (d, J = 2.7 Hz, 2H, -NH2); 13C-NMR (100 MHz, DMSO-d6): δ 168.6, 155.5, 147.3, 129.3, 126.4, 119.3, 118.4, 117.1, 100.8.

2.1.3. Synthesis of 3, 5-Diphenyl-4,5-dihydro-1H-pyrazol-1-carbaldehydes (13–15). Chalcones 13–15 (0.04 mol), 9.72 mL hydrazine hydrate (80%, 0.2 mol), and ethanol (15 mL) mixture were heated under reflux for 10 minutes until the chalcone was entirely consumed [23]. The progress of the reaction was monitored using TLC in n-hexane and ethyl acetate (9:1) as the mobile phase. When the reaction was completed, the warm reaction mixture was poured into ice-cold water (50 mL), and the precipitate was filtered and rinsed with water and ethanol using suction filtration. The solid produced was recrystallized with ethanol to yield N-formyl pyrazoline derivatives in high purity with good yields (SI Appendix A Figure S3).

4,5-Dihydro-3-(4-nitrophenyl)-5-phenylpyrazole-1-carbaldehyde (13). Reddish solid powder; yield: 87.3%; m.p.: 130–132°C; Rf (30% EtOAc in n-hexane) = 0.54; 1H-NMR (400 MHz, DMSO-d6): δ 8.48 (s, 1H, CHO), 7.82 (d, J = 8.5 Hz, 2H), 7.50 (s, 2H), 7.26 (dd, J = 16.9, 9.1 Hz, 2H), 6.85 (d, J = 8.2 Hz, 2H), 6.16 (d, J = 8.4 Hz, 1H), 5.11 (dd, J = 11.72 Hz, 3J = 4.92 Hz, 1H, C4-H2), 3.54 (dd, J = 17.47 Hz, 3J = 11.60 Hz, 1H, C1-HM), 3.01 (dd, J = 17.74 Hz, 3J = 4.91 Hz, 1H, C4-HM); 13C-NMR (100 MHz, DMSO-d6): δ 165.9, 161.1, 155.4, 148.9, 141.7, 137.5, 128.6, 126.5, 124.8, 114.3, 59.9, 42.9.

5-(4-Bromophenyl)-4,5-dihydro-3-(4-nitrophenyl)pyrazole-1-carbaldehyde (14). Yellow solid powder; yield: 78.9%; m.p.: 202–204°C; Rf (30% EtOAc in n-hexane) = 0.61; 1H-NMR (400 MHz, DMSO-d6): δ 8.50 (s, 1H, CHO), 7.87 (dd, J = 8.8, 3.5 Hz, 4H), 7.38 (d, J = 8.6 Hz, 3H), 7.11 (d, J = 8.0 Hz, 5H), 6.89 (d, J = 8.1 Hz, 3H), 4.55 (dd, J = 11.81 Hz, 3J = 5.29 Hz, 1H, C4-H2), 3.38 (dd, J = 17.27 Hz, 3J = 11.71 Hz, 1H, C1-HM), 3.10 (dd, J = 17.24 Hz, 3J = 4.81 Hz, 1H, C4-HM); 13C-NMR (100 MHz, DMSO-d6): δ 166.5, 160.3, 159.9, 146.2, 141.9, 131.3, 128.8, 125.9, 123.9, 120.3, 63.3, 48.6.

4,5-Dihydro-5-(2-hydroxyphenyl)-3-(4-nitrophenyl) pyrazole-1-carbaldehyde (15). Pale yellow solid powder; yield: 92.5%; m.p.: 175–177°C; Rf (30% EtOAc in n-hexane) = 0.79; 1H-NMR (400 MHz, DMSO-d6): δ 10.65 (s, 1H), 8.55 (s, 1H, CHO), 7.58 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 7.7 Hz, 2H), 7.00 (d, J = 7.6 Hz, 2H), 5.81 (dd, J = 11.71 Hz, 3J = 4.94 Hz, 1H, C4-H2), 2.76 (dd, J = 17.27 Hz, 3J = 11.71 Hz, 1H, C1-HM), 2.10 (dd, J = 17.14 Hz, 3J = 5.29 Hz, 1H, C4-HM); 13C-NMR (100 MHz, DMSO-d6): δ 167.3, 163.7, 159.4, 148.5, 134.2, 131.8, 128.0, 124.5, 124.4, 124.3, 120.5, 118.8, 79.78, 49.4.

2.1.4. Synthesis of Thiazolyl-Pyrazoline Schiff Base Hybrids in the Presence of ZnO Nanoparticles as a Catalyst Derived from Chalcones (16–24). The target Schiff base hybrids were synthesized by condensation of an equimolar ethanolic solution (20 mL) of N-formyl pyrazoline derivatives, 13–15 (3 mmol), with substituted 2-amino-4-(substituted-phenyl)
thiazole derivatives, 10–12 (3 mmol equiv.), in ZnO nanoparticles (43 mg, 0.2 mmol, 20 mol %) as a catalyst in methanol (20 mL) under reflux for 3 h. The progress of the reaction was monitored using TLC at an appropriate time interval using n-hexane: ethyl acetate (7:3). At the end of the reaction, the solution was slowly evaporated, and the precipitate was filtered, washed with ethanol, and dried under a vacuum. Finally, the resulting solid was recrystallized from ethanol to provide a microcrystalline solid product (Scheme 3 and SI Appendix Figure S4).

(18E)-N-((4,5-dihydro-3-(4-nitrophenyl)-5-phenylpyrazol-1-yl)methylene)-4-phenylthiazole-2-amine (16). Reddish yellow solid powder; yield: 94.7%; m.p.: 258–260°C; Mol. wt. = 453.52; chemical formula: C_{25}H_{14}N_{3}O_{3}S; R_{f} (20% EtOAc in n-hexane) = 0.17; UV-visible (MeOH) \( \lambda_{\text{max}} = 285 \text{ and } 385 \text{ nm}; \)

1H NMR (400 MHz, DMSO-\( d_{6} \)): \( \delta \) 8.44 (s, 1H, CH =N), 7.78 (d, \( J = 8.4 \) Hz, 2H), 7.71 (d, \( J = 11.8 \) Hz, 1H), 7.50 (d, \( J = 8.4 \) Hz, 1H), 7.29 (d, \( J = 8.2 \) Hz, 1H), 7.25 (d, \( J = 8.7 \) Hz, 1H), 6.97 (d, \( J = 8.4 \) Hz, 1H), 6.96–6.54 (m, 5H), 6.51 (s, 1H, Ar-H of thiazole-H), 6.42 (d, \( J = 6.5 \) Hz, 1H), 6.14 (d, \( J = 8.2 \) Hz, 1H), 5.10 (dd, \( J = 12.82, 3 \) Hz, 1H, C_{5}-H_{X}), 4.54 (dd, \( J = 18.32, 3 \) Hz, 1H, C_{4}-H_{M}), 3.50 (dd, \( J = 18.43, 3 \) Hz, 1H, C_{4}-H_{M}), 

13C-NMR (100 MHz, DMSO-\( d_{6} \)): \( \delta \) 169.1, 160.6, 155.0, 148.5, 141.6, 137.1, 134.1, 129.5, 128.4, 125.9, 124.3, 118.2, 114.0, 102.2, 59.2, 42.5.

(18E)-N-((5-(4-bromophenyl)-4,5-dihydro-3-(4-nitrophenyl)pyrazol-1-yl)methylene)-4-(4-nitrophenyl)thiazole-2(amine) (20). Yellow solid powder; yield: 89.8%; m.p.: 247–249°C; R_{f} (20% EtOAc in n-hexane) = 0.71; UV-visible (MeOH) \( \lambda_{\text{max}} = 285 \text{ and } 385 \text{ nm}; \)

1H NMR (400 MHz, DMSO-\( d_{6} \)): \( \delta \) 8.44 (s, 1H, CH =N), 7.78 (d, \( J = 8.4 \) Hz, 2H), 7.71 (d, \( J = 11.8 \) Hz, 1H), 7.50 (d, \( J = 8.4 \) Hz, 1H), 7.29 (d, \( J = 8.2 \) Hz, 1H), 7.25 (d, \( J = 8.7 \) Hz, 1H), 6.97 (d, \( J = 8.4 \) Hz, 1H), 6.96–6.54 (m, 5H), 6.51 (s, 1H, Ar-H of thiazole-H), 6.42 (d, \( J = 6.5 \) Hz, 1H), 6.14 (d, \( J = 8.2 \) Hz, 1H), 5.10 (dd, \( J = 12.82, 3 \) Hz, 1H, C_{5}-H_{X}), 4.47 (dd, \( J = 18.22, 3 \) Hz, 1H, C_{4}-H_{M}), 3.50 (dd, \( J = 18.43, 3 \) Hz, 1H, C_{4}-H_{M}).
OH), 8.54 (s, 1H, CH =N), 7.88 (d, J = 8.7 Hz, 1H), 7.80 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.33–7.22 (m, 1H), 6.99 (d, J = 7.0 Hz, 1H), 6.75 (s, 1H, Ar-H of thiazole-H), 6.56 (d, J = 4.7 Hz, 1H), 5.17 (dd, J = 11.35, J = 4.59 Hz, 1H, C_{5}-H_{X}), 3.94 (dd, J = 17.49, J = 4.59 Hz, 1H, C_{4}–H_{3}), 3.25 (dd, J = 17.92, J = 4.92 Hz, 1H, C_{4}'–H_{A}), 3.88 (dd, J = 17.0Hz, 2H), 6.75 (s, 1H, Ar-H of theiazole-H), 6.56 (d, J = 5.22 Hz, 1H, C_{5}–H_{X}), 3.94 (dd, J = 17.51, J = 5.25 Hz, 1H, C_{4}–H_{3}), 3.88 (dd, J = 17.52, J = 5.22 Hz, 1H, C_{4}'–H_{A}); 13C-NMR (100 MHz, DMSO-d$_6$): δ 169.1, 163.3, 161.8, 158.9, 156.4, 148.7, 146.3, 143.7, 131.3, 133.8, 131.5, 128.6, 126.7, 124.4, 124.0, 120.1, 120.1, 118.4, 116.9, 107.1, 66.3, 49.0.

2-((E)-(2-thiazol-4-yl)phenol)methyl)-4,5-dihydro-3-(4-nitrophenyl)-1H-pyrazol-5-yl)phenol (24). Bright yellow solid powder; yield: 92.9%; m.p.: 220–222°C; Rf (20% EtOAc in n-hexane) = 0.72; UV-visible (MeOH) λ max = 285 and 395 nm; $^1$H-NMR (400 MHz, DMSO-d$_6$): δ 10.98 (s, 1H, OH), 10.77 (s, 1H, CH =N), 8.83 (s, 1H, Ar-H of thiazole-H), 7.85 (d, J = 9.0 Hz, 4H), 7.83–7.76 (m, 1H), 7.80–7.63 (m, 5H), 7.33–7.18 (m, 5H), 7.02–6.93 (m, 5H), 6.84–6.61 (m, 1H), 6.59 (d, J = 17.0 Hz, 2H), 6.55–6.45 (m, 2H), 6.38 (d, J = 7.9 Hz, 2H), 6.20 (s, 1H, Ar-H of thiazole-H), 5.57 (dd, J = 11.95, J = 5.22 Hz, 1H, C_{5}–H_{X}), 3.94 (dd, J = 17.51, J = 5.29 Hz, 1H, C_{4}–H_{3}), 3.88 (dd, J = 17.52, J = 5.22 Hz, 1H, C_{4}'–H_{A}); 13C-NMR (100 MHz, DMSO-d$_6$): δ 168.8, 163.9, 163.3, 159.1, 156.4, 155.7, 154.7, 148.4, 147.5, 143.6, 133.7, 131.4, 128.2, 127.7, 126.6, 124.0, 120.1, 120.1, 118.4, 116.8, 101.1, 49.0, 40.1.

2.2. Biological Activity Evaluations

2.2.1. In Vitro Antibacterial Activity. The antibacterial susceptibility of each bacterial strain to all synthesized compounds was assessed using the Clinical and Laboratory Standards Institute (CLSI) procedures using agar disc diffusion assay. The inhibition zone technique shows the ability of a compound or drug to prevent microbial growth [24]. The Adama Public Health Research and Referral Laboratory Center provided four strains of pathogenic microorganisms of two Gram-positive bacteria, including the MRSA strain of Staphylococcus aureus (ATCC 25923) and Streptococcus pyogenes (ATCC 27853), and two Gram-negative bacteria, Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853). Colony morphology and Gram staining, as well as routine biochemical assays, were used to identify and authenticate the bacterial strains, according to Bergey’s Manual of Determinative Bacteriology [25]. The antibacterial activity was assessed at 250 and 500 μg/mL concentrations by measuring the zone of inhibition against the test organisms and comparing it with amoxicillin as the reference antibiotic. Amoxicillin (25 and 50 mg/mL) was the standard drug used as a positive control, and DMSO was used as a negative control. The experiments were repeated three times, and the average clear zone (inhibition zone) around each compound was measured in millimeters (mm) [26].

2.2.2. DPPH Free Radical Scavenging Assay. By bleaching the purple color of a methanolic solution of DPPH, the ability of the related compounds to transfer hydrogen atoms or electrons was measured. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) approach was used to examine the in vitro radical scavenging activity of the synthesized compounds with minor adjustments [27]. To a solution of 1 ml of 0.004% methanolic solution of diphenyl picrylhydrazyl (DPPH), 4 ml of various sample concentrations was added. After 60 minutes in the dark, the absorbance was measured against a blank at 517 nm using a double-beam UV-vis spectrophotometer. The blank reading solution was methanol, while the standard solution was ascorbic acid, which was calculated by the following equation:

$$% \text{DPPH free radical scavenging} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100,$$

(1)
where $A_{\text{control}}$ = the absorbance of the control (DPPH without compound) and $A_{\text{sample}}$ = the absorbance of the test sample. To determine the IC$_{50}$, different concentrations of the test compounds in methanol (12.5 to 200 g/mL) were added to a methanolic solution of DPPH (the concentration that makes 50% inhibition of the DPPH color). All IC$_{50}$ values were computed from the corresponding sigmoidal dose-response curve according to their best-fit shapes based on at least five reaction points using the Office Excel 2007 software (Microsoft, Redmond, WA, USA). All experiments were carried out in triplicates to calculate the mean and standard deviation statistically.

2.2.3. In Vitro Lipid Peroxidation Inhibition Assay. Lipid peroxidation proceeds in the form of a self-sustaining chain reaction and is very harmful. The formation of lipid-free radicals and lipid peroxide radicals in the cell membranes is considered an important feature of the cell damage caused by reactive oxygen species [28]. Ascorbic acid was used as a positive control. The percentage of lipid peroxidation inhibition was calculated from the following equation:

\[
\% \text{ lipid peroxidation inhibition} = \left( \frac{A_{\text{control}} - A_{\text{compound}}}{A_{\text{compound}}} \right) \times 100, \tag{2}
\]

where $A_{\text{sample}}$ = absorbance of the sample and $A_{\text{control}}$ = absorbance of control. The percentage of lipid peroxidation inhibition was plotted against the synthetic compound concentration to determine the concentration required to achieve 50% inhibition of phospholipid oxidation (IC$_{50}$) in linoleic acid; ascorbic acid was used as a positive control.

2.2.4. In Silico Computational Studies

1) ADMET, Drug-Likeness, and Toxicity Prediction. The prediction of the in silico ADMET parameters before the experimental studies is one of the most important aspects of drug discovery and development processes. ADMET studies play a critical role in helping to optimize the pharmacokinetic and pharmacodynamic properties of new drugs [29]. This prediction is based on an already established concept by the Lipinski rule of five [30] and the Veber rule [31]. Properties of the compounds were estimated using ADME descriptors by using SwissADME (https://www.swissadme.ch/), Molinspiration (https://www.molinspiration.com/), and PreADMET (https://preadmet.webservice.bmdrc.org/) online servers. Structures of compounds were submitted to SwissADME tools and converted to their canonical simplified molecular-input line-entry system (SMILES), to estimate in silico pharmacokinetic parameters and other molecular properties based on the reported method [32]. The organ toxicity profiles and toxicological endpoints of the ligands and toxicity class were predicted using ProTox-II Web server explorer (https://tox-new.charite.de/).

2.2.5. Molecular Docking Study. A molecular docking study was performed to investigate the binding mode between the synthesized compounds against target enzymes. ChemDraw program (Chem Create 16.0) was used to draw chemical structures of compounds with suitable 2D orientation, and ChemDraw Ultra 8.0 was employed to minimize the energy of ligands and standard drugs. The protein preparation and docking simulation were then carried out using the energy-minimized ligand molecules as input to AutoDock Vina 4.2.6 [33]. The DNA gyrase B (PDB ID: 6f86), the N-terminal domain of PqsA in complex with 6-fluoroanthraniloyl-AMP (PDB ID:5oe3), Pyruvate Kinase in complex with bis-indole alkaloid (PDB ID:3t07), LuxS of S. pyogenes (Uniprot id: P0C0C7), and Human peroxiredoxin 5 (PDB ID: 1HD2) crystal structures were downloaded from the Protein Data Bank (https://www.rcsb.org) to investigate the binding interaction of the synthesized compounds with the active site of protein targets using the following grid box parameters (PDB ID: 6f86) [62] [64], [15 × 14 × 19], (PDB ID:5oe3) [38] [17], (PDB ID:3t07) [19 × 21 × 14], (PDB ID:5oe3) [18 × 14 × 16], (Uniprot id: P0C0C7) [46 × 45 × 39], (PDB ID:1HD2) [6 × 44 × 33], respectively [34]. The best-docked conformation between ligand and protein was searched using the docking algorithm with AutoDock Vina. During the docking process, a maximum of nine conformers were considered for each ligand. Meanwhile, the Discovery Studio Visualizer 2021 and PyMOL (version 2.4) were used to investigate the possible interactions and ligand orientations between the target receptor and ligands by identifying conformations to optimize the docking function [35].

3. Results and Discussion

A series of novel thiazolyl-pyrazoline Schiff base hybrids were synthesized and characterized using spectroscopic methods with good to excellent yields (78.3–96.9%). Shorter reaction times were achieved for the ZnO nanoparticle-catalyzed approach compared with the conventional glacial acetic acid-catalyzed reactions under reflux conditions. Physical properties and NMR spectral data of the synthesized compounds are presented in supporting information (SI Appendix B Figures 19–45).

3.1. Recyclability Study of the Catalyst. A good catalyst for scale-up applications should have high activity and stability. After the reaction was completed, the catalyst was filtered, washed with dichloromethane and methanol, and dried at 100°C for 5 hr before being used in the next cycle with no apparent loss of activity, making this technique cost-effective and environmentally benign (SI Appendix A, Table S3) [36]. The catalyst ZnO NPs were found to have good catalytic activity for six consecutive runs with no appreciable catalytic activity loss and delivered a modest decrease in reaction yield (Figure 1). ZnO nanoparticle catalysis provides a shorter time and a higher product yield compared with typical acid catalysts [37, 38].
3.2. General Synthesis

3.2.1. General Synthesis of Chalcone Derivatives (3–5). Chalcone scaffolds were synthesized at room temperature by combining p-nitro acetoephone with various substituted aryl aldehydes via a basic catalyzed Claisen–Schmidt condensation reaction (Scheme 1 and SI Appendix A Figure S1). The aryl ring substitution pattern was carefully chosen to provide a variety of electronic environments that would affect the target molecules’ antibacterial and antioxidant activity patterns. In the 1H-NMR spectrum of compounds (3–5), H-β and H-α protons of α, β-conjugated system of chalcone appeared as a broad doublet in between δ 7.75–7.73 and δ 7.32–7.39, respectively, with a coupling constant of J = 15.6 Hz, which agrees with a trans-configuration. For compound 3, AAXX′ spin system aromatic protons for ring A and monosubstituted ring B are evident at δ 7.43–7.95. For compound 4, two sets of AAXX′ spin system aromatic protons are observed between δ 7.23–7.46 and δ 7.52–7.96. For compound 5, AAXX′ spin system aromatic protons for ring A and disubstituted ring B are evident at δ 7.70–7.88 and δ8.00–8.04, respectively. In the 13C-NMR spectrum of compounds (3–5), the C-β and C-α signals appeared at δ138.4 and 120.1, respectively, while the carbonyl group appeared between δ 167 and 189.9.

3.2.2. General Synthesis of 2-Amino-4-(substituted-phenyl) thiazole Derivatives (10–12). Thiazole amine derivatives 10–12 were synthesized (Scheme 2, SI Appendix A Figure S2) by reacting substituted acetophenone and thiourea in the presence of a catalyst, and the intermediate 4-((substituted-phenyl) thiazol-2-aminos (10–12) were obtained from good to excellent yields in a single Step (48.2–96.6%). The singlet at δ 2.9 in the 1H-NMR spectrum of 10–12 confirms the existence of the –NH2 group. The singlet at δ 6.97–7.80 suggests the presence of sp2 methine in the thiazole ring. Aromatic methine peaks were observed between δ 6.93 and 7.52. The proposed thiazole derivative molecular structures are in good agreement with the 13C-NMR spectrum, i.e., phenyl ring peaks at δ 119.2, 127.6, 128.4, 131.2, and 143.3, and peaks that belong to thiazole ring appeared at δ 107.4, 148.2, and 165.1.

3.2.3. General Synthesis of N-Formyl Pyrazoline Derivatives (13–15). Chalcones (3–5) were used as the starting material for a nucleophilic cycloaddition reaction with hydrazine to form intermediates of N-formyl pyrazolines (13–15). The N-formyl pyrazoline ring is produced by cyclization and dehydration of chalcone derivatives after a 1,4-Michael addition of hydrazine. The synthesis of N-formyl pyrazoline derivatives (13–15) was found to be in good to excellent yields (80–96%) in two steps (Scheme 4 and SI Appendix A Figure S3). In the 1H-NMR spectrum, the diastereotopic protons Hα, Hβ, and Hγ that correspond to the pyrazoline nucleus display an AMX spin pattern where Hα and Hβ each appear as a doublet of a doublet at Hα appeared at δ 2.91–3.06 (dd, 3JAM = 17.41–17.87, 3JAX = 4.91–6.87 Hz, C4–H4 of pyrazoline) and Hβδ = 3.42–3.90 (dd, 3JAM = 17.27–17.47 Hz, 3JAX = 11.60–11.71 Hz, C4–H4 of pyrazoline). The Hγ methine (C–H) proton of pyrazoline moiety appeared in the range of δ 4.60–5.65 with coupling constants of (dd, 3JAX = 11.71–11.81, 3JMX = 4.92–5.29 Hz, CH–H of pyrazoline) suggesting vicinal coupling of methine with magnetically nonequivalent methylene protons. The aldehyde proton signal appeared as a singlet at δ 8.93–9.23. The 13C-NMR spectrum shows that the methylene carbon, sp2 methine, and sp2 quaternary carbons of the pyrazoline ring appeared at C-4 at δ 42.2–44.09, C-5 at δ 62.7–65.02, and C-3 at δ 155.8, respectively, whereas the aldehyde carbonyl carbon appeared in between at δ 160.1 and 167.5 range confirming the formation of N-formyl pyrazoline derivatives (13–15). All the other aromatic were observed in the expected regions.

3.2.4. General Synthesis of Thiazolyl-Pyrazoline Schiff Base Hybrids (16–24). A series of novel thiazolyl-pyrazoline Schiff base hybrids were synthesized via a Schiff base reaction between 2-amino-4-((substituted-phenyl)thiazole (10–12) and N-formyl-3,5-diphenyl-4,5-dihydro-(1H) pyrazole derivatives (13–15) in the presence of ZnO nanoparticles as a heterogeneous catalyst in ethanol reflux (Scheme 3). The scope of the optimized method was checked with electron-donating and electron-withdrawing substituents at phenyl rings. 1H-NMR spectra of target products (16–24) revealed three doublets of doublets as an AMX spin pattern with varying coupling constant (J) values, two diastereotopic methylene protons of Hα and Hβ and sp3 methine proton Hγ of pyrazoline nucleus. Protons Hα and Hβ appeared as two doublets of a doublet in the range of δ 3.09–3.35 (dd, JAM = 17.2–17.85 Hz, JAX = 4.78–6.88 Hz, C4–H4) and δ 3.42–3.90 (dd, JAM = 17.60–17.97 Hz, JMX = 11.20–11.88 Hz, C4–H4 of pyrazoline) due to vicinal and geminal coupling, respectively. Proton Hγ exhibited a signal having doublet of doublet in the region δ 4.60–5.64 with JAX = 11.38–11.87 Hz, JMX = 4.92–6.80 Hz, Cα–H of due to vicinal coupling with two
nonequivalent methylene protons of pyrazoline nucleus. A signal having singlet seen at δ 7.00-8.68 attributed to sp² methine proton of thiazole ring. The azomethine sp² methine proton (CH=N) was also detected as a singlet at δ 9.19. In addition, all the other aromatic protons were found in the expected regions. The ¹³C-NMR spectrum of the compounds (16–24) showed peaks at δ 151.1 attributed to sp³ methine of azomethine group (-CH=N) ring, respectively. The signals at δ 164.8-165.1, 148.6-152.3, and 102.8–109.5 attributed to sp² quaternary carbons of thiazole ring rings. Moreover, sp² quaternary carbon, sp³ methine, and methylene carbons of the pyrazoline moiety were observed around δ 152.4–153.8, δ 63.2-64.7, and δ 43.4-43.9, respectively. Furthermore, aromatic ring sp² methines and sp² quaternary carbons appeared within expected ranges of δ 117.4–164.1 (16–24, SI Appendix A Figure S4). A plausible mechanism for the synthesis of thiazolyl-pyrazoline Schiff base hybrids via ZnO nanoparticles as a catalyst was proposed as shown in Scheme 5.

3.3. In Vitro Antibacterial Activity. The antibacterial activity of the synthesized thiazolyl-pyrazoline Schiff base hybrids (16–24) was evaluated by in vitro disk diffusion bioassay and measuring inhibition zone diameters (IZDs, expressed as mean ± SD mm) at 500 and 250 µg/mL against the following strains of bacteria: Staphylococcus aureus (ATCC 29213), Streptococcus pyogenes (ATCC 27853), Escherichia coli (ATCC 25922), and Pseudomonas aeruginosa (ATCC 27853) [39, 40]. Compounds 22 and 24 displayed potent inhibitory activity against P. aeruginosa (13.33 ± 0.58 mm and 17.33 ± 0.58 mm, respectively) at 500 µg/mL (Table 1) compared with amoxicillin (15.00 ± 1.00 mm). Compounds 19 and 24 displayed promising activity against E. coli (12.00 ± 1.00 mm and 17.00 ± 1.00 mm, respectively) at 500 µg/mL compared with amoxicillin (14.67 ± 0.58 mm). Compounds 18, 19, 21, and 24 displayed promising activity against S. pyogenes (19.33 ± 0.58 mm, 19.33 ± 0.58 mm, 18.67 ± 2.52 mm, and 15.67 ± 0.58 mm, respectively) at 500 µg/mL, whereas compounds 18 and 21 displayed promising activity against S. aureus (10.00 ± 1.00 mm and 11.00 ± 1.00 mm, respectively) at 500 µg/mL compared with amoxicillin (33.33 ± 1.53 mm and 15.00 ± 1.00 mm, respectively). The rest of the compounds showed moderate to weak activity against the tested bacterial strains. Thus, these potent antibacterial compounds could be considered promising lead compounds to develop new antibacterial drugs (Figure 2, SI Appendix C Figure S6).

3.3.1. Structure-Activity Relationships (SARs). Structure-activity-based mechanism of antimicrobial action is critical for the design and improvement of novel antimicrobial and antioxidant agents. The results demonstrate that compound 17, with a p-NO₂ substituent on the aromatic ring of thiazole moiety, resulted in a decreasing pattern in antibacterial activity against all strains compared with compound 16, which has a hydrogen atom at para position of the aromatic ring of thiazole moiety. Compound 18, with a p-OH substituent on the aromatic ring of thiazole moiety, resulted in an increase in antibacterial activity towards S. aureus at 250 µg/mL and S. pyogenes at 250 and 500 µg/mL compared with compound 16. Compound 20, with a p-NO₂ substituent on the aromatic ring of thiazole moiety, resulted in an increase in antibacterial activity towards S. aureus at 500 µg/mL and S. pyogenes at 250 µg/mL compared with compound 19, which has hydrogen at the para position of the aromatic ring of thiazole moiety. Compound 21, with a p-OH substituent on the aromatic ring of thiazole moiety, resulted in an increase in antibacterial activity towards S. aureus and S. pyogenes at 500 µg/mL compared with compound 19, which has hydrogen at the para position of the aromatic ring of thiazole moiety, whereas compound 23, with a p-NO₂ substituent on the aromatic ring of thiazole moiety, resulted in an increasing antibacterial activity trend towards S. aureus and S. pyogenes at 500 µg/mL compared with compound 22. On the other hand, compound 21, with a p-OH substituent on the aromatic ring of thiazole moiety, resulted in an increase in antibacterial activity towards S. pyogenes, E. coli, and P. aeruginosa at 500 µg/mL compared with compound 19, which has hydrogen at the para position of the aromatic ring of thiazole moiety. In addition, the presence of p-Br and p-OH group pyrazoline moiety bearing with substituted-phenyl rings might be the most relevant cause for enhancing the antibacterial activities. The results suggest that the antibacterial activity of the investigated compounds was influenced by the physicochemical properties of the type and position of substituent on the aromatic rings of thiazole and pyrazoline moieties.

3.4. Antioxidant Activities

3.4.1. DPPH Free Radical Scavenging and Lipid Peroxidation Inhibition. The antioxidant activity of compounds 16–24 was determined by the DPPH assay. From the results obtained (Table 2), only compound 21 displayed significant
antioxidant activity (IC\textsubscript{50} = 4.63 μg/mL) compared with the positive control, ascorbic acid (IC\textsubscript{50} = 3.21 μg/mL) (Figure 3). The rest of the compounds showed moderate to weak activity. Most of the synthesized compounds (except 17 and 22) showed greater than 80% of DPPH radical scavenging activity at a concentration of 200 μg/mL (Figure 4). The presence of electron-donating hydroxyl functionality at an ortho position of thiazole phenyl groups could be the most possible reason, which might stabilize the radicals for their strong electron-donating nature [41]. Compounds 21 and 24 inhibited peroxide formation by 80.01 ± 0.07% and 79.44 ± 0.05%, respectively, demonstrating their potential in preventing lipid peroxide formation as presented (Table 2). Thus, the results showed that the studied thiazole-pyrazoline-based Schiff base hybrids remove the free radicals, especially the lipid peroxide radicals, by giving them their hydrogen atom and thus inhibiting the lipid peroxidation reaction.

3.5. \textit{In Silico} ADMET Prediction. SwissADME is a Web-based tool that provides important information about drug-likeness and pharmacokinetic properties of bioactive molecules [42, 43]. In this perspective, the theoretical agreement of synthesized compounds to Lipinski’s rule of five and Veber’s rule [30, 31, 44], as well as the pharmacokinetic and toxicity parameters, was estimated using ADMET descriptors by a SwissADME [31] and PreADMET [45] online servers. The percent absorption (%Abs) of the synthesized compounds was computed using the formula
\[ \%\text{Abs} = 109 - 0.345TPSA \] [46]. The filter that combines Lipinski’s “rule of five” refers physicochemical properties of a molecule to become a drug (i.e., MW ≤ 500 Daltons, iLog P ≤ 5, H-bond donors ≤ 5, and H-bond acceptors ≤ 10) [44] and Veber’s parameters (TPSA ≤ 140 Å\textsuperscript{2} and rotatable bonds ≤ 10) [30]. ProTox-II Web server exploration was used to forecast the ligands’ organ toxicity profiles and toxicological endpoints, as well as their LD\textsubscript{50} [46, 47]. The results showed that all of the compounds obeyed Lipinski’s rule of five (Table 3), with only minor deviations for 19–21 and 23, and thus, they have the potentials to be developed as orally bioavailable drugs. All synthesized compounds have a higher bioavailability score of 0.55 and were suitable for oral administration, indicating drug-like qualities [48]. Their HBD values are < 3, indicating that they are highly soluble in cellular membranes, with HBA values ranging from 5 to 8.
Table 1: Antibacterial activity of compounds 16–24 and amoxicillin against selected Gram-positive and Gram-negative strains.

| Synthesized compounds | Concentration (µg/mL) | Mean inhibition zone diameter (IZD) in mm | Gram-positive bacteria | Gram-negative bacteria |
|-----------------------|-----------------------|------------------------------------------|------------------------|------------------------|
|                       |                       |                                          | *Staphylococcus aureus* (ATCC 29213) | *Streptococcus pyogenes* (ATCC 27853) | *Escherichia coli* (ATCC 25922) | *Pseudomonas aeruginosa* (ATCC 27853) |
| 16                    | 500                   | 8.67 ± 0.58                             | 9.00 ± 0.00            | 7.33 ± 0.58            | 8.67 ± 0.58            | 6.00 ± 0.00            |
|                       | 250                   | 8.33 ± 0.58                             | 7.33 ± 0.58            | 6.67 ± 0.58            | 8.00 ± 1.00            |
| 17                    | 500                   | 8.33 ± 0.58                             | 6.33 ± 0.58            | 6.00 ± 0.00            | 7.33 ± 0.58            | 8.00 ± 0.00            |
|                       | 250                   | 8.33 ± 0.58                             | 6.00 ± 0.00            | 6.67 ± 0.58            | 8.00 ± 0.00            |
| 18                    | 500                   | 8.00 ± 1.00                             | 19.33 ± 0.58           | 6.67 ± 0.58            | 6.67 ± 0.58            | 6.67 ± 0.58            |
|                       | 250                   | 10.00 ± 1.00                            | 18.33 ± 0.58           | 6.00 ± 0.00            | 6.00 ± 0.00            |
| 19                    | 500                   | 7.33 ± 0.58                             | 19.33 ± 0.58           | 12.00 ± 1.00           | 9.67 ± 0.58            | 9.67 ± 0.58            |
|                       | 250                   | 6.33 ± 0.58                             | 17.00 ± 1.00           | 9.33 ± 0.58            | 8.00 ± 0.00            | 8.00 ± 0.00            |
| 20                    | 500                   | 9.00 ± 0.00                             | 8.00 ± 1.00            | 7.67 ± 0.58            | 7.33 ± 0.58            | 7.33 ± 0.58            |
|                       | 250                   | 8.33 ± 0.58                             | 14.00 ± 2.00           | 7.00 ± 0.00            | 7.33 ± 0.58            | 7.33 ± 0.58            |
| 21                    | 500                   | 11.00 ± 1.00                            | 18.67 ± 2.52           | 8.00 ± 0.00            | 7.33 ± 0.58            | 7.33 ± 0.58            |
|                       | 250                   | 11.67 ± 0.58                            | 22.33 ± 1.53           | 6.33 ± 0.58            | 7.00 ± 0.00            | 7.00 ± 0.00            |
| 22                    | 500                   | 6.67 ± 0.58                             | 6.00 ± 0.00            | 13.33 ± 0.58           | 13.33 ± 0.58           | 13.33 ± 0.58           |
|                       | 250                   | 7.00 ± 0.00                             | 6.00 ± 0.00            | 11.33 ± 0.58           | 7.00 ± 0.00            | 7.00 ± 0.00            |
| 23                    | 500                   | 9.67 ± 0.58                             | 9.67 ± 0.58            | 6.00 ± 0.00            | 8.00 ± 0.00            | 8.00 ± 0.00            |
|                       | 250                   | 8.67 ± 0.58                             | 7.00 ± 0.00            | 8.00 ± 0.00            | 8.00 ± 0.00            | 8.00 ± 0.00            |
| 24                    | 500                   | 8.33 ± 0.58                             | 15.67 ± 0.58           | 17.00 ± 1.00           | 17.33 ± 0.58           | 17.33 ± 0.58           |
|                       | 250                   | 7.67 ± 0.58                             | 10.67 ± 0.58           | 8.00 ± 0.00            | 18.67 ± 0.58           | 18.67 ± 0.58           |
| Amoxicillin           | 500                   | 15.00 ± 1.00                            | 33.33 ± 1.53           | 14.67 ± 0.58           | 15.00 ± 1.00           | 15.00 ± 1.00           |
|                       | 250                   | 14.00 ± 1.00                            | 29.67 ± 2.52           | 10.33 ± 0.58           | 14.33 ± 2.52           | 14.33 ± 2.52           |
| DMSO                  | 6 ± 0.00              | 6 ± 0.00                                | 6 ± 0.00               | 6 ± 0.00               | 6 ± 0.00               | 6 ± 0.00               |

aThe experiment was carried out in triplicate and the average zone of inhibition was calculated. bATCC: American Tissue Culture Collection. cIZD, inhibition zone diameter.

Figure 2: Minimum diameter inhibition zone of the synthetic compounds (16-24) in mm (mean ± SD) at 250 and 500 µg/mL concentrations.
Table 2: *In vitro* antioxidant and anti-lipid peroxidation inhibition test with various concentrations of 16–24.

| Conc. (µg/mL) | 16     | 17     | 18     | 19     | 20     | 21     | 22     | 23     | 24     | Ascorbic acid* |
|--------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------------|
| 12.5         | 32.32 ± 0.50 | 22.75 ± 0.31 | 29.67 ± 0.24 | 22.69 ± 0.54 | 19.00 ± 0.01 | 35.32 ± 0.03 | 18.13 ± 0.55 | 25.96 ± 0.08 | 34.02 ± 0.02 | 38.94 ± 0.04 |
| 25.0         | 47.25 ± 0.36 | 33.59 ± 0.41 | 40.95 ± 0.12 | 44.90 ± 0.02 | 32.73 ± 0.11 | 55.73 ± 0.04 | 33.82 ± 0.36 | 38.99 ± 0.07 | 49.63 ± 0.25 | 57.16 ± 0.13 |
| 50.0         | 63.9 ± 0.30 | 56.0 ± 0.36 | 67.9 ± 0.16 | 60.2 ± 0.20 | 55.4 ± 0.39 | 76.3 ± 0.11 | 56.9 ± 0.41 | 58.1 ± 0.28 | 62.9 ± 0.16 | 77.6 ± 0.18 |
| 100.0        | 74.33 ± 0.29 | 68.21 ± 0.74 | 76.92 ± 0.03 | 77.61 ± 0.06 | 78.92 ± 0.04 | 80.44 ± 0.11 | 67.83 ± 0.11 | 73.19 ± 0.21 | 77.53 ± 0.34 | 83.73 ± 0.06 |
| 200.0        | 80.25 ± 0.35 | 78.44 ± 0.38 | 83.74 ± 0.17 | 81.22 ± 0.14 | 85.74 ± 0.23 | 91.49 ± 0.05 | 78.45 ± 0.30 | 80.53 ± 0.13 | 87.41 ± 0.02 | 95.71 ± 0.13 |
| IC<sub>50</sub> (µg/mL)* | 34.25 | 70.93 | 39.79 | 27.34 | 64.62 | 4.63 | 27.32 | 57.35 | 29.08 | 3.21 |
| % Anti-lipid peroxidation inhibition | 55.74 ± 0.09 | 68.05 ± 0.54 | 65.00 ± 0.14 | 70.66 ± 0.52 | 69.07 ± 0.52 | 80.01 ± 0.07 | 58.88 ± 0.25 | 69.66 ± 0.11 | 79.44 ± 0.05 |

*IC<sub>50</sub> is the 50% inhibitory concentration of the samples, and the results were represented as mean ± SD. * Ascorbic acid was used as a positive control.
The 

% inhibition of the synthesized compounds (16–24) and the standard drug at different concentration ranges.

The iLogP values for all compounds are <5, notably for 20 and 22–24 indicating the compounds have good lipophilicity characteristics, which may protect them against ROS degradation. TPSA ranged from 86.68 to 152.73 Å², while the consensus log $\text{p}_\text{ow}$ (indicator of lipophilicity) was observed in an optimal range from 2.06 to 3.64. Compounds that satisfy the parameters of TPSA ≤ 140 Å² are expected to have adequate solubility, oral bioavailability, and cell permeability [49]. All compounds (16–24) exhibited better percent absorption (% Abs) than amoxicillin suggesting good oral bioavailability.

In silico pharmacokinetic characteristic results (Table 4) revealed that compounds 16–24 had superior intestinal absorption to amoxicillin, ranging from 90.80 to 99.82% absorption with HIA values near 100%. In vitro, Caco2 and MDCK cells have moderate to high permeability, notably 18, 22, and 24, and especially 19 and 24, which are better than amoxicillin (0.39), respectively. Skin permeability (SP, LogKp) was found to be somewhat below the permitted range of −5.42 to −6.59 cm/sec, and a substance with a low negative LogKp value has higher absorption into human skin. The results indicate that the synthesized compounds are showing good absorption properties to cross the gut wall by passive diffusion mechanism to reach the target. All of the compounds were poorly absorbed into the CNS in vivo, showing that they have a decreased ability to cross the CNS, with a total clearance value of 24 (0.07), which is comparable to amoxicillin (0.06). All of the substances have a high PPB%, with values of more than 90%, suggesting that they are strongly bound. The results revealed that compounds 18 (99.75) and 24 (97.31) had high PPB values, indicating longer half-lives than amoxicillin (23.35) [50]. Furthermore, glycoprotein (P-gp) inhibition measurements are utilized to forecast the target drugs’ excretion properties. P-gp and cytochrome P450 (CYP) are known to aid in the protective efflux (biotransformation) of biological membranes (GI tract or brain) from xenobiotics to protect tissues [51]. The compounds’ metabolic properties were predicted by the behavior of cytochrome P450 and their isomorph enzymes. In this investigation, compounds 16–24 showed no inhibitory activity against CYP2C19, CYP2C9, CYP2C6, or CYP3A4, except 3b and 3c, which showed inhibitory activity against CYP2D9. In our study, none of the synthesized compounds inhibited P-gp substrates, except 16 and 19.

Toxicity data must be connected with in vitro safety investigations before being examined in relevant laboratory animal models [52]. In addition, all compounds were predicted for their computational toxicity risks, such as the AMES test (for mutagenicity prediction), tumorigenicity (mouse/rat) (for carcinogenicity prediction), and skin irritation (for checking cardiac toxicity), and cytotoxicity effect (Table 5). Based on toxicity profiles, all compounds (16–24) and amoxicillin showed mutagenic behavior in the AMES test. All compounds, except for 17, 20, 23, 24, and amoxicillin, exhibited no possible carcinogenic effect in mice and no potential carcinogenic effect in rats. All of the compounds exhibited a low risk of being cardiototoxic or cytotoxic, as well as being safer than standard drugs. All of the synthesized compounds tested had predicted LD₅₀ (mg/kg) values ranging from 800 to 1,000 putting them in ProTox-II class 4, indicating that they could be harmful if swallowed (300LD₅₀ ≤ 2000) [53]. The bioavailability radar plots of the tested compounds (16–24) indicated that all of the compounds were completely within the pink area, indicating that they have drug-like qualities with an improved bioavailability profile (SI Appendix C Figure S7) [54]. Even though the chemical violates type Fsp3 0.25, unsaturation score (INSATU), it is verifiable that it does not violate any of Lipinski and Veber’s standards, satisfying the qualifications within the ideal spectrum that combines drug-likeness criteria. Using wLogP and TPSA, the BOILED-Egg server predicts this pharmacokinetics [55]. As a result, pharmacokinetics were evaluated using the BOILED-Egg model, which appeared in the white ellipse (HIA) justifying their high probability of being passively absorbed by the gastrointestinal tract, with red points for 18, 21, and 24 (non-
### Table 3: Parameters of in silico bioavailability prediction and physicochemical properties of compounds (16–24) and amoxicillin.

| Compound | MW (g/mol) | iLogP | HBD (n<sub>H2O/HNH</sub>) | HBA (n<sub>O/N</sub>) | n rotb | MR | TPSA (Å<sup>2</sup>) | % ABS | Lipinski violations | Bioavailability score |
|----------|------------|-------|---------------------------|----------------------|--------|----|---------------------|-------|-------------------|----------------------|
| Lipinski* | ≤500       | 5     | ≤5                        | ≤10                  | ≤10    | –  | –                   | ≤140  | –                 | 100%                 |
| Veber**  | 16         | 453.52| 3.64                      | 0                    | 5      | 6  | 140.68              | 86.68 | 79.09             | 0.55                 |
| 17       | 498.51     | 3.31  | 0                         | 7                    | 7      | 149.51 | 325.12             | 0.55 |
| 18       | 469.52     | 3.09  | 1                         | 6                    | 6      | 142.71 | 106.91             | 0.55 |
| 19       | 532.41     | 3.23  | 0                         | 5                    | 6      | 148.38 | 86.68              | 79.09 | 1                 | 0.55                 |
| 20       | 577.41     | 3.81  | 1                         | 7                    | 7      | 157.21 | 325.12             | 0.55 |
| 21       | 548.41     | 3.54  | 1                         | 6                    | 6      | 150.41 | 106.91             | 72.11 | 1                 | 0.55                 |
| 22       | 469.52     | 2.66  | 1                         | 6                    | 6      | 142.71 | 325.12             | 0.55 |
| 23       | 514.51     | 2.06  | 1                         | 8                    | 7      | 151.53 | 152.73             | 56.30 | 0.17              |                      |
| 24       | 485.51     | 2.57  | 2                         | 7                    | 6      | 144.73 | 106.91             | 65.13 | 0.55              |                      |
| Amoxicillin | 365.4 | 1.46  | 4                         | 6                    | 6      | 94.59  | 132.96             | 63.12 | 0.55              |                      |

*Reference values of Lipinski; **Reference values of Veber; (–): not tested. MW, molecular weight; iLogP, lipophilicity (O/W, octanol-water partitioning coefficient); HBD, number of hydrogen bond donors (OH and NH groups); HBA, number of hydrogen bond acceptors (O and N atoms); nVs, number of Lipinski rule violations; n rotb, number of rotatable bonds; MR, molar refractivity; TPSA, topological polar surface area (Å<sup>2</sup>); % ABS, percentage of absorption (%Abs = 109–(0.345 × TPSA)).

### Table 4: Pharmacokinetic (ADME) property prediction of compounds (16–24) and amoxicillin using PreADMET online server.

| Compound | HIA (%)<sup>a</sup> | Caco2 (nm/sec)<sup>b</sup> | SP, LogK<sub>p</sub> (cm/hour)<sup>c</sup> | MDCK (nm/sec)<sup>d</sup> | P<sub>B</sub> (%)<sup>e</sup> | BBB (c.brain/c.blood)<sup>f</sup> | CYP2C19<sup>g</sup> | CYP2C9<sup>h</sup> | CYP2D6<sup>i</sup> | P<sub>gp</sub>-inhibition<sup>k</sup> |
|----------|----------------------|-----------------------------|------------------------------------------|-------------------------|-----------------|--------------------------|-----------------|-----------------|-----------------|-----------------|
| 16       | 99.82                | 15.60                       | –5.80                                    | 0.047                   | 96.53           | 0.80                     | Non             | Non             | Non             | Non             |
| 17       | 96.14                | 9.39                        | –5.81                                    | 0.047                   | 95.90           | 0.21                     | Non             | Non             | Non             | Non             |
| 18       | 98.56                | 4.35                        | –5.61                                    | 0.044                   | 99.75           | 0.34                     | Non             | Non             | Non             | Non             |
| 19       | 98.43                | 27.34                       | –6.59                                    | 0.021<sup>*</sup>       | 93.54           | 1.59                     | Non             | Non             | Non             | Non             |
| 20       | 99.40                | 17.52                       | –6.60                                    | 0.018<sup>*</sup>       | 93.82           | 0.41                     | Non             | Non             | Non             | Non             |
| 21       | 98.02                | 18.88                       | –6.40                                    | 0.030<sup>*</sup>       | 93.30           | 0.63                     | Non             | Non             | Non             | Non             |
| 22       | 98.56                | 7.78                        | –5.61                                    | 0.044                   | 94.41           | 0.24                     | Non             | Non             | Non             | Non             |
| 23       | 90.80                | 11.84                       | –5.62                                    | 0.044                   | 94.60           | 0.12                     | Non             | Non             | Non             | Non             |
| 24       | 95.95                | 5.52                        | –5.42                                    | 0.044                   | 97.31           | 0.07                     | Non             | Non             | Non             | Non             |
| Amoxicillin | 62.93 | 0.34  | –0.21                      | 0.398                   | 23.35           | 0.06                     | Non             | Non             | Non             | Non             |

<sup>a</sup> HIA: human intestinal absorption; <sup>b</sup>Caco2: Caco2 cell permeability; <sup>c</sup>SP, Log<sub>Kp</sub>: skin permeability; <sup>d</sup>MDCK: Madin–Darby canine kidney; <sup>e</sup>P<sub>B</sub>: plasma protein binding; <sup>f</sup>BBB level: blood-brain barrier level; <sup>g</sup>-<sup>h</sup>CYP2D6: cytochrome P<sub>450</sub>; <sup>i</sup>P<sub>gp</sub>: glycoprotein inhibition.

### Table 5: In silico toxicity prediction of compounds (16–24) and amoxicillin using PreADMET and ProTox-II protocols.

| Compound | AMES (mutagenicity prediction) | Carcino<sub>mouse</sub> (for carcinogenicity prediction of the tested compounds) | Carcino<sub>rat</sub> (for carcinogenicity prediction of the tested compounds) | hERG<sub>inhibition</sub> (for checking the cardiac toxicity) | Cytotoxicity | <sup>a</sup>L<sub>D50</sub> (mg/kg) | Class |
|----------|--------------------------------|------------------------------------------------------------------|-----------------------------------------------------------------|-------------------------|-------------|-------------------------------|-------|
| 16       | Mutagen                       | Negative                                                         | Positive                                                        | low_risk               | Negative   | 1000                          | 4     |
| 17       | Mutagen                       | Positive                                                         | Positive                                                        | low_risk               | Negative   | 1000                          | 4     |
| 18       | Mutagen                       | Negative                                                         | Positive                                                        | low_risk               | Negative   | 1000                          | 4     |
| 19       | Mutagen                       | Negative                                                         | Positive                                                        | low_risk               | Negative   | 1000                          | 4     |
| 20       | Mutagen                       | Negative                                                         | Positive                                                        | low_risk               | Negative   | 1000                          | 4     |
| 21       | Mutagen                       | Negative                                                         | Positive                                                        | low_risk               | Negative   | 1000                          | 4     |
| 22       | Mutagen                       | Negative                                                         | Positive                                                        | low_risk               | Negative   | 1000                          | 4     |
| 23       | Mutagen                       | Negative                                                         | Positive                                                        | low_risk               | Negative   | 1000                          | 4     |
| 24       | Mutagen                       | Negative                                                         | Positive                                                        | low_risk               | Negative   | 1000                          | 4     |
| Amoxicillin | Mutagen | Negative                                                         | Negative                                                        | low_risk               | Negative   | 15000                         | 4     |

<sup>a</sup>L<sub>D50</sub>: lethal dose parameter.
substrate of P-gp) and blue points for 16 and 19 (substrate of P-gp), respectively (SI Appendix C Figure S8). They were predicted to be no P-gp substrate, despite their promising intestinal absorption and bioavailability, and they cannot pass through the blood-brain barrier (BBB), demonstrating they are not able to cross the blood-brain barrier to the brain when it attaches to certain receptors.

3.6. In Silico Molecular Docking Simulation. Studies of docking simulations were carried out to identify the binding pattern of the synthesized compounds (16–24) to selected protein target active sites (i.e., DNA gyrase B, PqsA, pyruvate kinase, LuxS, and human peroxiredoxin 5) [56]. The binding affinity, hydrogen bond, and amino acid residual interactions (hydrophobic/π-cation/π-anion/π-alkyl and van der Waals interactions) of ligands (16–24) and standards are presented (SI Tables 7–11). The majority of the hybrids investigated had the best docking scores and good interactions (2D and 3D) with different amino acid residues (SI Appendix D Figure S5(a)–S5(e), 1–10).

3.6.1. Molecular Docking Studies of Synthesized Compounds (16–24) against E. coli DNA Gyrase B (PDB ID: 6f86). The synthesized compounds (16–24) were docked towards the bacterial DNA gyrase B receptor to determine their binding affinity, and the results were compared to amoxicillin (SI Appendix D Figure S5(a), 1–10, and Table S7) [57]. The minimum binding energies of the synthesized compounds (16–24) ranged from −7.4 to −8.5 kcal/mol, showing that thiazolyl-pyrazoline Schiff base hybrids have a thermodynamically favorable inhibitory action on E. coli DNA gyrase B. Compounds 19 (−8.0 Kcal/mol), 20 (−7.8 Kcal/mol), 21 (−7.9 Kcal/mol), 22 (−8.5 Kcal/mol), and 24 (−7.9 Kcal/mol) have high binding interactions with DNA gyrase B, respectively (SI Figure 5 and SI Appendix D Figure 5(a), 10) compared with amoxicillin (−7.1 Kcal/mol). The synthesized compounds displayed similar residual interactions with amino acid residues of Ile-78 and Ile-94, as well as hydrogen bonding interaction patterns with Val-120, Asn-46, and Ser-121 when compared to standard amoxicillin. The docking results (high negative binding affinity) are in good agreement with the in vitro antibacterial results of compounds 19, 22, and 24 against Gram-negative E. coli bacteria.

3.6.2. Molecular Docking Studies of Synthesized Compounds (16–24) against the N-Terminal Domain of PqsA in a Complex with 6-Fluoroanthraniloyl-AMP (PDB ID:5oe3). The binding affinity of synthesized compounds (16–24) was evaluated towards the N-terminal domain of PqsA in the 6-fluoroanthranilamidoyl-AMP complex [58] and compared with amoxicillin (SI Appendix D Figure S5(b), 1–10, and Table S8). The synthesized compounds (16–24) have minimum binding energy in the binding pocket of the N-terminal domain of PqsA ranging from −7.8 to −10.7 kcal/mol. Compounds 17 (−9.5 Kcal/mol), 19 (−9.6 Kcal/mol), 20 (−9.5 Kcal/mol), 22 (−10.7 Kcal/mol), 23 (−9.6 Kcal/mol), and 24 (−9.9 Kcal/mol) have large binding interactions. Higher binding affinity was observed for compounds 22 and 24, with the binding affinity of −10.7 and −9.9 Kcal/mol, respectively, against PqsA compared with amoxicillin (−7.3 Kcal/mol), suggesting that the compounds are promising antibacterial agents against P. aeruginosa (Figure 6 and SI Appendix D Figure 5(b), 10), in good agreement with the in vitro antibacterial activity against Gram-negative P. aeruginosa observed. The synthesized compounds revealed similar amino acid residue interactions with Thr-232, Arg-128, Asp-231, Asp-132, Ala-124, Ala-126, Phe-209, Gly-302, and H-bonding interaction profiles with Asn-61, Ser-65, and Pro-129 as compared to the amoxicillin drug.

3.6.3. Molecular Docking Studies of Synthesized Compounds (16–24) against S. aureus Pyruvate Kinase (PDB ID:3t07). The synthesized compounds (16–24) were subjected to a molecular docking analysis to determine their binding affinity towards pyruvate kinase in a complex containing the natural bisindole alkaloid, and the results were compared to amoxicillin (SI Appendix D Figure S5(c), 1–10, and Table S9) [59,60]. It was found that the synthesized compounds have minimum binding energy in the range of −4.8 to −6.4 kcal/mol. Compounds 18 (−5.5 Kcal/mol), 19 (−5.0 Kcal/mol), 20 (−5.5 Kcal/mol), 21 (−5.2 Kcal/mol), 22 (−5.9 Kcal/mol), 23 (−6.4 Kcal/mol), and 24 (−5.0 Kcal/mol) have better binding interactions, with the best docking score obtained for compounds 22, 21, and 23 with binding affinity of −5.2, −5.9, and −6.4 Kcal/mol, respectively, against pyruvate kinase compared with amoxicillin (−4.7 Kcal/mol) (Figure 7 and SI Appendix D Figure 5(c), 10). This suggests that these compounds are effective antibacterial agents against S. aureus. Amino acid residue interactions with Pro-402, Lys-425, Leu-355, Ile359, and H-bonding interaction patterns with Ala-358, Thr-464, Gly-462, and Ser-383 were similar in the synthesized compounds. The in vitro biological results of 21 against Gram-positive bacteria of S. aureus are in good agreement with molecular docking results to some extent.

3.6.4. Molecular Docking Studies of Synthesized Compounds (16–24) against LuxS of S. pyogenes (UniProt Id: P0C0C7). The binding affinity of synthesized compounds (16–24) was examined against LuxS and compared with amoxicillin (SI Appendix D Figure S5d, 1–10, and Table S10) [61]. The synthesized compounds (16–24) had minimal binding energies ranging from −3.7 to −5.1 kcal/mol (SI Table S10). The binding affinity of 21 against LuxS was nearly identical (−5.1 Kcal/mol) to that of amoxicillin (−5.5 Kcal/mol), indicating that these compounds are promising antibacterial agents against S. pyogenes (Figure 8 and SI Appendix D Figure 5d, 10), in good agreement with the in vitro biological results of observed by 21 against Gram-positive bacteria of S. pyogenes. The synthesized compounds have similar amino
acid residue interactions with Ala-64, His-61, Cys-127, Pro-120, Pro-79, Leu-56, and H-bonding interaction profiles with His-57, Gly-128, and His-61 when compared to a standard antibiotic drug (amoxicillin).

3.6.5. Molecular Docking Studies of Synthesized Compounds (16–24) against Human Peroxiredoxin 5 (PDB ID: 1hd2). Target synthetic compounds (16–24) were docked to the binding site of human protein target peroxidase 5 receptor and compared to ascorbic acid (SI Appendix D Figure S5(e), 1–10, and Table S11) [62]. The synthesized compounds had minimum binding energies ranging from −6.7 to −7.8 kcal/mol of which compounds 17 (−7.4 Kcal/mol), 18 (−7.4 Kcal/mol), 19 (−7.2 Kcal/mol), 21 (−7.8 Kcal/mol), 22 (−7.5 Kcal/mol), and 24 (−7.3 Kcal/mol) have high binding interactions. A higher docking score was observed for compound 21 (−7.8 Kcal/mol) higher than ascorbic acid (−5.2 Kcal/mol), suggesting that the compound is a promising antioxidant (Figure 9 and SI Appendix D Figure 5(e), 10), in good agreement with the in vitro antioxidant result displayed by compound 21.
Figure 7: 3D (a) and 2D (b) representations of the binding interactions of 23 against S. aureus pyruvate kinase.

Figure 8: 3D (a) and 2D (b) representations of the binding interactions of 21 against LuxS of S. pyogenes.

Figure 9: 3D (a) and 2D (b) representations of the binding interactions of 21 against human peroxiredoxin 5.
4. Conclusion and Future Outlooks

In this study, we reported the conventional and green synthesis of nine thiazolyl-pyrazoline Schiff base hybrids together with their ADMET profile predictions and molecular docking study as antibacterial and antioxidant agents. The structures of the synthesized compounds were determined using combined spectroscopic techniques (UV−Vis, 1H-NMR, and 13C−NMR). The results of antibacterial screening indicate that compound 24 (I\text{ZD} = 18.67 \pm 0.58 \text{mm}) was more potent than amoxicillin (I\text{ZD} = 14.33 \pm 2.52 \text{mm}) against P. aeruginosa at 250 \mu g/mL, whereas compounds 22 and 24 (I\text{ZD} = 13.33 \pm 0.58 \text{mm} and 17.00 \pm 1.00 \text{mm}, respectively) showed better activity compared with amoxicillin (I\text{ZD} = 14.67 \pm 0.58 \text{mm}) at 500 \mu g/mL against E. coli. The remaining compounds showed moderate to weak activity against the tested bacterial strains. Compound 21 displayed significant inhibition of DPPH (I\text{C}_{50} = 4.63 \mu g/mL) compared with aspirin acid (I\text{C}_{50} = 3.21 \mu g/mL). Compound 21 displayed 80.01 \pm 0.07% inhibition of peroxide formation, suggesting its potential in preventing the formation of lipid peroxides. The results of the ADMET study showed that all synthesized compounds obeyed Lipinski’s rule of five. In silico pharmacokinetic study demonstrated that compound 24 had superior intestinal absorption compared with amoxicillin.

Compounds 22 and 24 displayed binding affinities of -10.7 and -9.9 Kcal/mol, respectively, against PqsA compared with amoxicillin (−7.3 Kcal/mol) in agreement with the observed in vitro antibacterial activity against P. aeruginosa. Compound 21 displayed a binding affinity of −5.1 Kcal/mol and −7.8 Kcal/mol with LuxS and human peroxiredoxin 5, respectively, compared with amoxicillin and ascorbic acid (−5.5 and −5.2 Kcal/mol, respectively). Compounds 22 and 24 displayed higher binding affinity (−8.5 and −7.9 Kcal/mol, respectively) with DNA gyrase B compared with amoxicillin (−7.1 Kcal/mol). In silico toxicity study showed that all synthesized compounds had LD\text{_{50}} (mg/kg) values ranging from 800 to 1,000 putting them in ProTox-II class 4. The in vitro antibacterial activity and molecular docking analysis showed that compound 24 is a promising antibacterial therapeutic agent against P. aeruginosa and E. coli, whereas compound 22 is a promising antibacterial agent against E. coli. Compound 21 is found to be a promising antioxidant agent. Moreover, the green synthesis approach using ZnO nanoparticles as catalysts was found to be a very efficient method to synthesize biologically active thiazole-pyrazoline Schiff base hybrids compared with the conventional method. Future attempts should be carried out to synthesize a series of related thiazole-based Schiff base hybrids for comprehensive structure-activity relationship and in vivo studies to develop lead antioxidant and antibacterial drugs.

Data Availability

The data used to support the findings of this study are included within the manuscript and supplementary information.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this study.

Acknowledgments

Demis Zelelew acknowledges the Department of Applied Chemistry, School of Applied Natural Science, Adama Science and Technology University, and Wachemo University for PhD opportunity study and leave of absence, respectively. The support rendered by Adama Public Health Research and Referral Laboratory Center, Adama, Ethiopia, during the antibacterial study is gratefully acknowledged.

Supplementary Materials

The UV-visible, 1H, and 13C-NMR spectra of all synthesized compounds, molecular docking scores, and 3D and 2D representations of the binding interactions of synthesized compounds (16–24) and amoxicillin/ascorbic acid against target proteins (i.e., DNA gyrase B, PqsA, pyruvate kinase, LuxS, and human peroxiredoxin 5), bioavailability radar, and BOILED-Egg model of the compounds (16–24) are all presented in the supplementary information. (Supplementary Materials)

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