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Synthesis, Molecular Docking and ADMET Evaluation of Thiosemicarbazide 7-chloroquinoline Hybrids as Potential Antibacterial Agents

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Abstract

Bacterial infections continue to pose a substantial global health challenge and rank among the primary causes of morbidity and mortality. The swift emergence of antimicrobial resistance has diminished the efficacy of current antibiotics, underscoring the pressing necessity for novel antibacterial agents. This study identified 7-chloroquinoline compounds for their potential biological activity. Energy minimisation of each molecule was conducted via Avogadro software to eliminate steric conflicts prior to molecular docking. Docking was subsequently performed against the Mycobacterium tuberculosis protein tyrosine phosphatase (PDB ID: 2OZ5). Ampicillin exhibited a binding energy of −8.25 kcal/mol. Compound **(5c)** had a binding energy of −8.32 kcal/mol, signifying moderate affinity, whereas **(5d)** demonstrated the most robust interaction with −10.23 kcal/mol. ADMET analysis conducted with SwissADME indicated that compounds **(5a)**, **(5b)**, and **(5d)** adhered to Lipinski's guidelines, while **(5c)** exhibited one violation but still retained drug-like properties. Intermediate **(3)** was synthesised from 4,7-dichloroquinoline and thiosemicarbazide under ultrasonic irradiation, thereafter undergoing reflux with *para*-substituted acetophenones to produce four derivatives. Structure confirmation was conducted with FTIR and ^{1}H NMR. The antibacterial assessment revealed that all compounds inhibited *E. coli*, with compound **(5d)** demonstrating the most significant inhibition zone. The data indicate that compound **(5d)** possesses significant promise for future antibacterial medication development.

Keywords: 7-chloroquinoline; Antibacterial; Molecular Docking; ADMET

Introduction

Antimicrobial resistance (AMR) has become a significant concern worldwide. The rapid development of multidrug-resistant bacteria, particularly the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), has led to a need for broad-spectrum antibacterial candidates with novel chemical scaffolds that target both Gram-positive and Gram-negative bacteria [1].

Bacteria have a complex, multilayered cell membrane that protects them from harsh environments. They can be classified into Gram-positive (G+) or Gram-negative (G-) bacteria based on their cell wall structures. The Gram stain, invented by Hans Christian Gram in 1884, is a widely used method to classify bacteria. Gram-positive bacteria have a thicker peptidoglycan cell wall, while Gram-negative bacteria have a weaker peptidoglycan cell wall. Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria, due to their modified outer membranes [2]. They also resist antimicrobials used in clinical medicine due to defense mechanisms like efflux pumps, modifications to drug binding sites, and degradation enzymes. These microorganisms increase the risk of morbidity and mortality in intensive care units.

Quinoline, an organic group compound with a double-ring structure, has been used as a scaffold for drug formulation and invention for the last decade. Quinoline derivatives have wide biological activities, including antibacterial, anticancer, antimalarial, antiviral, antifungal, and anti-inflammatory properties. 7-chloroquinoline derivatives were reported as a good starting material to create broad-spectrum antibacterial medicines [3].

Ultrasound irradiation, a green synthetic, has been used in organic synthesis for almost three decades due to its ability to modify reaction selectivity and increase reaction rate. This technology has been shown to reduce waste and energy, and can perform organic reactions with higher yield, faster reaction time, and milder conditions [4].

Molecular docking is a part of *in silico* drug discovery to predict the interaction between a small molecule ligand and a protein. The ideal drug candidate must exhibit ideal ADMET characteristics at a therapeutic dose and have adequate efficacy against the therapeutic target [5].

Quinoline, a nitrogen-containing heterocyclic aromatic ring, is a key component in drug design and development due to its potent antibacterial properties. Quinoline is found in the structures of synthetic antibiotics like Norfloxacin, Levofloxacin, and Ciprofloxacin. Researchers are now focused on discovering and developing novel drugs with quinoline and its derivatives. Thiosemicarbazide, with sulfur atoms, offers greater chemical versatility and a wide range of biological activities, making it a popular starting material for drug synthesis [6]. Quinoline dimers are interesting to explore because they have the potential to overcome the resistance of the bacteria. This is because the larger molecular mass and the bulkiness of the substituent of quinoline do not prevent their penetration into the bacterial cell walls or membranes [7]. **Figure 1** shows the chemical structures of quinoline and 7-chloroquinoline.

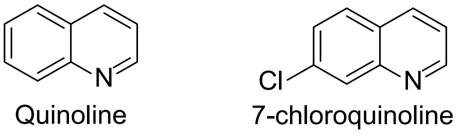


Figure 1 Chemical structures of quinoline and 7-chloroquinoline

Molecular docking is a crucial step in drug discovery, used to predict the affinity and mechanism of binding a small molecule ligand to a protein receptor. It has been the most popular computational structure-based drug design method since the 1980s. Over thirty docking programs exist, with AutoDock being the most popular. The process involves simulating the atomic-level relationship between a small molecule and a protein, predicting the ligand structure and orientation, and evaluating the binding affinity. The docking study begins with defining a binding site, which can be visualized and ranked [8].

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) is a crucial step in drug discovery and design. It forecasts the pharmacokinetic and pharmacodynamic properties of a drug candidate, aiming to identify promising candidates and reduce the probability of failure. Lipinski's 'rule of five' is used to determine oral bioavailability. Lipinski and Veber criteria are used to calculate drug-likeness descriptors using SwissADME. Other ADMET factors, such as molecular weight, topological polar surface area, rotatable bonds, and hydrogen bond acceptors, also contribute to lipophilicity [9].

Ultrasonic irradiation is a versatile technique for organic synthesis, promoting green processes and reducing energy consumption. It can speed up processes, create new reactive intermediates, and shorten reaction times. This method improves product yields, purity, and selectivity in multifunctionalized heterocycles, offering quick reactions and mild conditions [10].

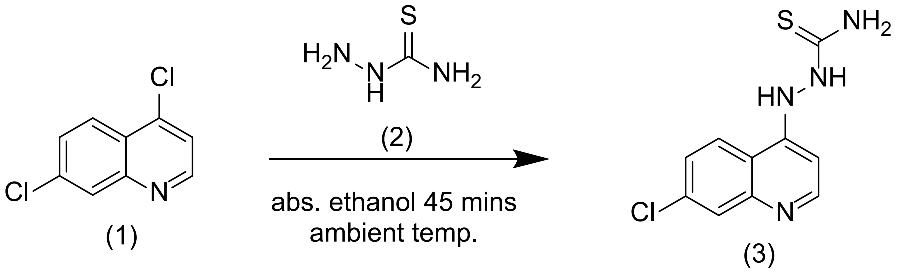
Materials and methods

Molecular Docking

The protein structure of Phosphotyrosine protein phosphatase PtpB (PDB ID:2OZ5) was retrieved from the Protein Data Bank (PDB). The structure of thiosemicarbazide 7-chloroquinoline derivatives was analyzed using a molecular visualization and structural optimization program, Avogadro, with energy minimization performed on each molecule before molecular docking. Then, the docking process was performed by using AutoDock software Auto Dock 4.2 program and dock tools 1.5.4(and the interactions of the ligand with the protein were visualized by Biovia Discovery Studio.

Preparation of 2-(7-chloroquinolin-4-yl)hydrazinecarbothioamide (3)

The study involved 4,7-dichloroquinoline (1) with thiosemicarbazide (2) in absolute ethanol, refluxing in an ultrasonic bath. TLC was conducted to determine whether the reaction had been completed. The crude was filtered and dried. The reaction was nucleophilic substitution, yielding 2-(7-chloroquinolin-4-yl)hydrazinecarbothioamide (3). The precipitate was characterized by FTIR and 1H NMR. Yielded compound (3) (light yellow powder, 50.85 %), M.p (278-280 °C); Rf value: 0.63; IR (cm-1): 3316 (primary N-H), 3130 (secondary N-H), 3088 and 3010 (sp2 C-H), 1614 (C=N), 1601 and 1441 (C=C), 1236 (C-N), 1212 (C=S), 758 (C-Cl); 1H NMR (400 MHz, DMSO-d6): δ 7.85 (1H, Ha), δ 8.50 (1H, Hb), δ 8.70 (1H, Hd), δ 6.76 (1H, He), δ 8.33 (2H, Hg), δ 10.20 (1H, Hh), δ 8.33 (1H, Hc), δ 11.39 (1H, Hf). Scheme 1 depicts the synthesis of 2-(7-chloroquinolin-4-yl)hydrazinecarbothioamide (3).



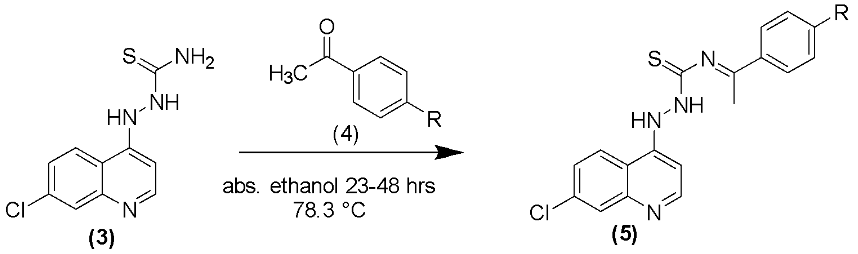
Scheme 1 Synthesis of 2-(7-chloroquinolin-4-yl)hydrazinecarbothioamide (3)

Synthesis of (E)-2-(7-chloroquinolin-4-yl)-N-(1-phenylethylidene)hydrazine-1-carbothioamide **5(a)**
The reaction of acetophenone **(4a)** with 2-(7-chloroquinolin-4-yl)hydrazinecarbothioamide **(3)** in absolute ethanol was carried out under reflux for 23 hours at 78.3 °C. The product was filtered and dried, and TLC analysis confirmed the reaction was complete. Yielded compound **5(a)** (light yellow powder, 49.22 %), M.p (265-270 °C); R_f value: 0.55; IR (cm^{-1}): 3131 (secondary N-H), 3088 and 3010 (sp^2 C-H), 1615 (C=N), 1601 and 1441 (C=C), 1236 (C-N), 1212 (C=S), 778 (C-Cl).

Synthesis of (*E*)-2-(7-chloroquinolin-4-yl)-N-(1-(p-toyl)ethylidene) hydrazine-1-carbothioamide **5(b)**
The reaction involved refluxing 4-methylacetophenone (4b) with 2-(7-chloroquinolin-4-yl)hydrazinecarbothioamide (3) in absolute ethanol for 48 hours at 78.3 °C. The TLC was conducted using ethyl acetate and n-hexane. The product was filtered, dried, and analyzed for completion, confirming the previous results. Yielded compound 5(b) (light yellow powder, 18.18 %), M.p (265-270 °C); Rf value: 0.54; IR (cm-1): 3129 (secondary N-H), 3089 and 3010 (sp2 C-H), 2960 and 2879 (sp3 C-H), 1615 (C=N), 1602 and 1442 (C=C), 1236 (C-N), 1213 (C=S), 778 (C-Cl).

*Synthesis of (E)-N-(1-(4-bromophenyl)ethylidene)-2-(7-chloroquinolin-4-yl) hydrazine carbothioamide **5(c)***
The reaction involved refluxing 4-bromoacetophenone (4c) with 2-(7-chloroquinolin-4-yl)hydrazinecarbothioamide (3) in absolute ethanol for 48 hours at 78.3 °C. The TLC was conducted using ethyl acetate and chloroform. The product was filtered, dried, and analyzed for completion, confirming the previous results. Yielded compound 5(c) (light yellow powder, 39.91 %), M.p (265-270 °C); Rf value: 0.53; IR (cm-1): 3129 (secondary N-H), 3087 and 3010 (sp2 C-H), 1615 (C=N), 1601 and 1440 (C=C), 1236 (C-N), 1211 (C=S), 811 (C-Br), 778 (C-Cl).

*Synthesis of (E)-2-(7-chloroquinolin-4-yl)-N-(1-(4-nitrophenyl)ethylidene) hydrazine-1-carbothioamide **5(d)***
The reaction involved refluxing 4-nitroacetophenone **(4d)** with 2-(7-chloroquinolin-4-yl)hydrazinecarbothioamide **(3)** in absolute ethanol for 48 hours at 78.3 °C. The TLC was conducted using ethyl acetate and *n*-hexane. The product was filtered, dried, and TLC analysis confirmed the reaction was complete. Yielded compound **5(d)** (light yellow powder, 39.99 %), M.p (265-270 °C); R_f value: 0.53; IR (cm^{-1}): 3131 (secondary N-H), 3088 and 3011 (sp^2 C-H), 1615 (C=N), 1602 and 1442 (C=C), 1551 and 1381(N-O), 1236 (C-N), 1213 (C=S), 778 (C-Cl). Scheme 2 represents the synthesis of 7-chloroquinoline derivatives **5(a-d)**.



(3) **(5)**

4(a) : R = H
4(b) : R = 4-Me
4(c) : R = 4-Br
4(d) : R = 4-NO_2

5(a) : R = H
5(b) : R = 4-Me
5(c) : R = 4-Br
5(d) : R = 4-NO_2

Scheme 2: Synthesis of 7-chloroquinoline derivatives **5(a-d)**

Antibacterial Assay

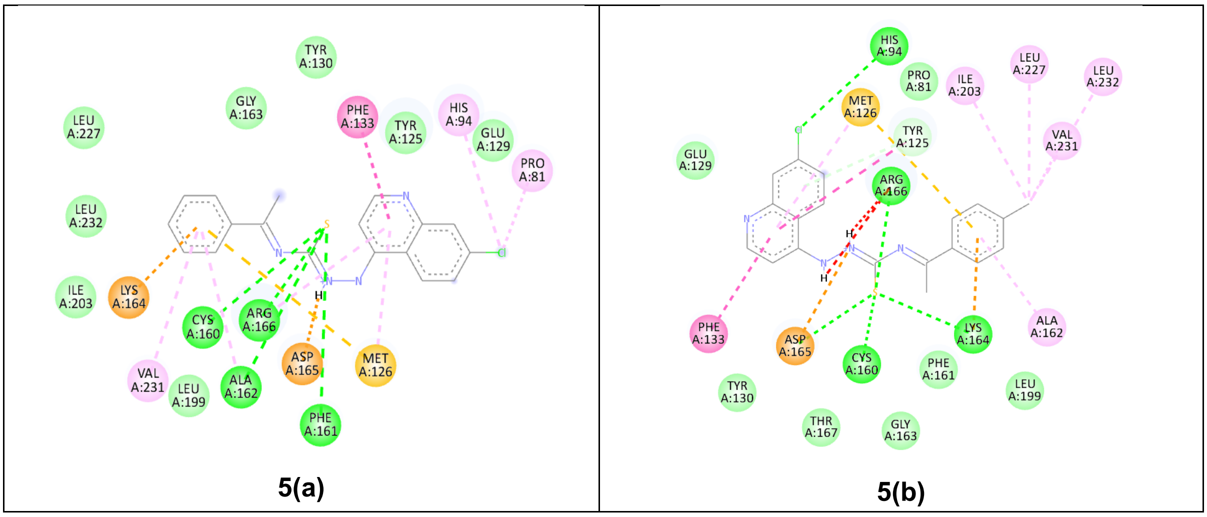
Nutrient agar was dissolved in distilled water, and Ringer's solution was prepared and sterilized by autoclaving. The test samples were dissolved in methanol and DMSO. A laminar flow cabinet was sterilized, and agar was poured into petri dishes. Bacterial inoculum was prepared with an *E. coli* concentration. Inoculating loops were heated until red, and *E. coli* colonies were mixed with the Ringer's solution. The agar was punched into two holes and incubated overnight. The inhibition zones were measured to determine bacterial growth.

Results and discussion

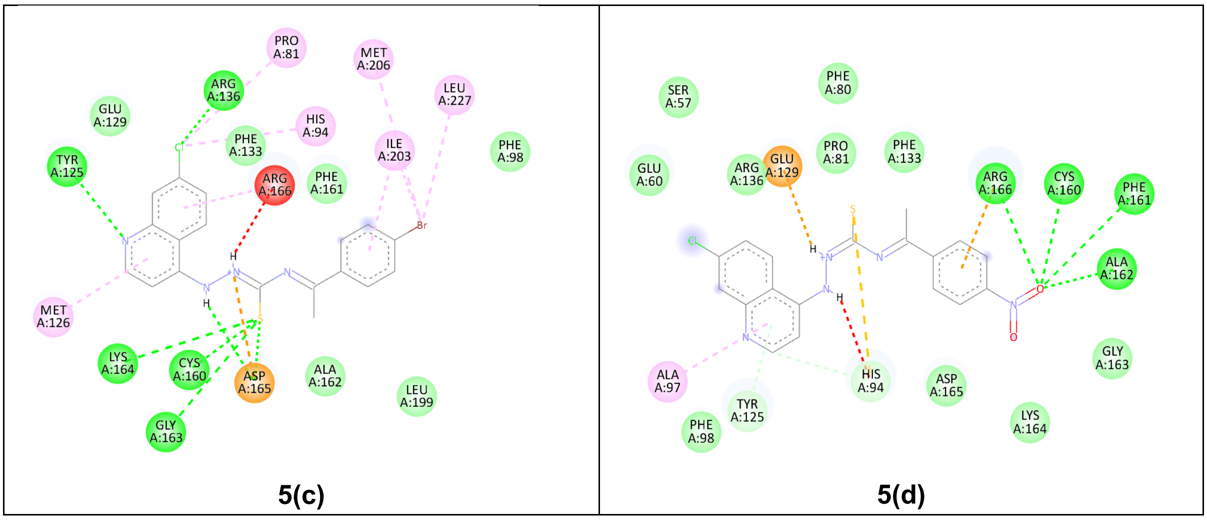
Molecular Docking and ADMET Analysis

The grid map displays interaction energy based on coordinates (x = 5.361 Å, y = 60.884 Å, z = 6.140 Å). Protein-ligand binding energies were evaluated after molecular docking. The lowest binding energy indicates the best conformational position of the compound within the target protein's active region. Compound **5(d)** has the lowest binding energy of -10.23 kcal/mol, indicating good binding affinity with the protein. Meanwhile, compound **5(c)** has the highest binding energy of -8.32 kcal/mol, indicating a weaker binding affinity.

The Discovery Studio Visualizer was used to analyze hydrophobic and hydrogen bond interactions between thiosemicarbazide 7-chloroquinoline compounds and 2OZ5 protein. **Figure 2** shows conventional hydrogen bonds, π-donor hydrogen bonds, carbon-hydrogen bonds, van der Waals interactions, π-sulfur and π-cation interactions, salt bridge interactions, and unfavorable donor-donor interactions found in the interaction of compounds **5(a)**, **5(b)**, **5(c)** and **5(d)** with 2OZ5 residues [11].



5(a) **5(b)**



5(c) **5(d)**

Figure 2 Interactions of compounds **5(a)**, **5(b)**, **5(c)** and **5(d)** with 2OZ5 residues

The synthesized compounds **5(a)-5(d)** were evaluated for drug likeness using Lipinski's rule of five. All compounds met the rule without violation, except for compound **5(c)**, which had a violation. All compounds were found to be potential candidates for antibacterial studies. The bioavailability radar representations showed that all compounds had Lipophilicity (LIPO) values between +2.73 and +4.93, Molecular Weight (MW) values between 223 and 390, Polarity (POLAR) TPSA values between 45 and 70, Solubility (INSOLU) values below 6, Saturation (INSATU) values below 0.15, and Flexibility (FLEX) values below nine rotatable bonds [12].. The compounds were classified as drug-like based on their physicochemical properties.

Characterization of Compounds by using ATR-FTIR and ^{1}H NMR Spectroscopy

FTIR Spectrum for 7-chloroquinoline Derivatives

The ATR-FTIR spectrum identified secondary amine (N-H) at 3131 cm^{-1}, aromatic C=C at 1601 cm^{-1} and 1441 cm^{-1}, sp^2 C-H stretching at 3088 cm^{-1} and 3010 cm^{-1}, and absorption bands of C=N and C=S at 1615 cm^{-1} and 1213 cm^{-1}, respectively, for all synthesized compounds **5(a)**, **5(b)**, **5(c)**, and **5(d)**.

*^{1}H NMR Spectrum for 2-(7-chloroquinolin-4-yl)hydrazinecarbothioamide **(3)***

The ^{1}H NMR spectrum of intermediate **(3)** revealed the presence of all nine hydrogens, with four doublet signals at δ (ppm) 7.85, 8.50, 8.70, and 6.76, three singlet signals at δ (ppm) 8.33, 10.20, 8.13, and 11.39, and one broad singlet signal at δ (ppm) 8.13.

Antibacterial Assay

The newly synthesized compounds **5(a)-5(d)** were tested against Gram-negative bacteria (*E. coli*) using the agar well diffusion method. *E. coli* was chosen due to its standardized, easy-to-handle nature and is commonly used as a reference strain for evaluating antimicrobial susceptibility. The compounds were dissolved in methanol and DMSO, with a 1:1 ratio. Results showed positive results against *E. coli*, with ampicillin as a positive control showing a positive result at higher concentrations. Compound **5(d)** showed the largest inhibition zone, with an average diameter of 28.7 mm, indicating good antibacterial activity.

Conclusion

The study successfully docked compounds **5(a), 5(b), 5(c),** and **5(d)** using AutoDock Tools and Avogadro software. Compound **5(d)** showed the strongest binding affinity with the protein (2OZ5) compared to ampicillin. The compounds obeyed Lipinski's rule of five and were synthesized using reflux heating methods. The compounds were characterized by ATR-FTIR and ^{1}H NMR. The agar well diffusion method was used to determine the antibacterial activity of the compounds against Gram-negative bacteria (*E. coli*). Compound **5(d)** with a nitro group substituent at the *para* position was identified as the best potential antibacterial agent.

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