

RESEARCH PAPER

Synthesis, Structural Characterization, and Antibacterial Evaluation of Novel Rhodanine-Based Ni(II), Cu(II), and Cr(III) Complexes Bearing Substituted Thiazolidinone Moieties

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ABSTRACT

We designed, synthesized, and evaluated a novel series of substituted benzylidene rhodanine derivatives (8a–8c) and their Ni(II), Cu(II), and Cr(III) complexes for structural characteristics and antibacterial activity. The rhodanine derivatives were prepared through dithiocarbamate formation, cyclization, and subsequent Knoevenagel condensation with substituted benzaldehydes. The resulting ligands were coordinated with Ni(II), Cu(II), and Cr(III) ions under reflux conditions in ethanol to afford the corresponding metal complexes. We characterized the synthesized compounds by FT-IR, ¹H NMR, ¹³C NMR, mass spectrometry, and elemental analysis, and we further investigated the complexes using UV–visible spectroscopy, molar conductance, magnetic susceptibility, XRD, and TGA. The combined analytical data supported the proposed structures and coordination behaviour. The synthesized compounds were evaluated for antibacterial activity against selected Gram-positive and Gram-negative bacterial strains, demonstrating that benzylidene substitution and metal coordination influence their physicochemical and antibacterial properties.

Keywords: Antibacterial activity; Benzylidene rhodanine; Coordination chemistry; Cr(III) complexes; Ni(II) complexes; Rhodanine; Cu(II) complexes.

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1. Introduction

Heterocyclic compounds containing nitrogen and sulfur atoms constitute important structural frameworks in medicinal and coordination chemistry. Among these, rhodanine (2-thioxothiazolidin-4-one) is a versatile five-membered heterocyclic scaffold that has attracted considerable attention because of its synthetic accessibility, structural diversity, and broad range of reported biological activities. The rhodanine nucleus can be modified at several positions, particularly N-3 and C-5, offering opportunities to develop structurally diverse derivatives with different biological and physicochemical properties. [1–5]

Rhodanine derivatives have been extensively investigated in medicinal chemistry for antimicrobial, antiviral, anticancer, antidiabetic, antifungal, and other biological applications. Reviews of rhodanine chemistry have highlighted the

importance of structural modification in determining biological properties and have described the use of rhodanine-based compounds against diverse molecular targets. [1–7] In particular, 5-ene-rhodanines and other C-5-substituted derivatives have received considerable attention because modification at this position allows the introduction of diverse aromatic and heteroaromatic substituents while retaining the characteristic rhodanine framework. [5,6,8]

The C-5 position of rhodanine is particularly suitable for functionalization because the active methylene group readily undergoes Knoevenagel condensation with aldehydes and related carbonyl compounds. This reaction provides an efficient route to 5-arylidenes and 5-benzylidenes rhodanine derivatives containing an extended conjugated system. Various synthetic approaches to rhodanine derivatives and C-5-arylidene products have been reported, including conventional condensation

methods and more recent catalytic and microwave-assisted procedures. [8–12] Toubal et al. reported the synthesis and structural determination of novel 5-arylidene-3-N-substituted rhodanine derivatives, demonstrating the utility of Knoevenagel condensation for structural diversification of the rhodanine nucleus. [13]

The biological properties of rhodanine derivatives are strongly influenced by the nature of the substituents introduced at the N-3 and C-5 positions. Several studies have demonstrated antibacterial activity for 5-benzylidene and other substituted rhodanine derivatives against Gram-positive and Gram-negative bacteria. Zvarec et al. investigated 5-benzylidenerrhodanine derivatives and reported antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*. [14] Song et al. synthesized and evaluated 5-benzylidene rhodanine derivatives and reported activity against several Gram-positive bacterial strains, including multidrug-resistant clinical isolates. [15] AbdelKhalek et al. subsequently evaluated rhodanine derivatives against pathogenic clinical isolates and demonstrated activity against both Gram-positive and Gram-negative organisms. [16] Other studies have further investigated rhodanine derivatives as antibacterial agents through target-based approaches, including inhibition of bacterial enzymes such as enoyl-acyl carrier protein reductase. [17–20]

The antimicrobial potential of rhodanine derivatives has also been summarized in several recent reviews. These studies indicate that structural changes involving the N-3 substituent, C-5 arylidene group, and other appended pharmacophores can influence antibacterial potency and target interactions. [2,4,6,7,21] However, the biological activity of rhodanine derivatives is dependent on the individual molecular structure and experimental system. Therefore, structure–activity relationships should be interpreted based on experimental biological data rather than scaffold identity alone. [1,6]

Beyond their medicinal chemistry applications, rhodanine derivatives are of interest in coordination chemistry because their heteroatom-containing functional groups can interact with metal ions. Early investigations established the formation of transition-metal complexes of rhodanine, including Ni(II), Co(II), Zn(II), Cd(II), and Hg(I/II) complexes. [22–24] Spectroscopic and physicochemical studies have subsequently been used to investigate the coordination behaviour and structural characteristics of rhodanine-based metal complexes. [22–25] More recent work has demonstrated the preparation of Ni(II) and Cu(II) complexes of rhodanine and evaluated their physicochemical and antibacterial properties, with the complexes showing enhanced antibacterial

activity compared with the corresponding parent ligand under the reported experimental conditions. [26]

Combining rhodanine-based ligands with transition-metal ions therefore provides an opportunity to investigate how ligand substitution and metal coordination affect molecular structure, solid-state properties, thermal behaviour, and antibacterial activity. Previous studies on rhodanine and related thiazolidinone systems have demonstrated that both ligand structure and metal identity can influence the resulting physicochemical and biological characteristics. [22–26]

In the present study, we synthesized and characterized a series of 3-phenethyl-5-(substituted benzylidene)-2-thioxothiazolidin-4-one derivatives (8a–8c) and their Ni(II), Cu(II), and Cr(III) complexes. The rhodanine derivatives were modified at the N-3 and C-5 positions, followed by coordination with selected transition-metal ions. We characterized the synthesized compounds using spectroscopic and analytical techniques. We also investigated the metal complexes by UV–visible spectroscopy, molar conductivity, magnetic susceptibility, powder X-ray diffraction, and TG/DTG analysis. We evaluated the antibacterial activities of the ligands and complexes against selected Gram-positive and Gram-negative bacterial strains. This study examined how structural modification and metal coordination affect the physicochemical and antibacterial properties of this rhodanine-based series.

2. MATERIALS AND METHODS

2.1 Experimental

2.1.1 Materials and instrumentation

All chemicals and solvents used in the present study were of analytical reagent (AR) grade and were used as received without further purification unless otherwise stated. 2-Phenylethan-1-amine (phenethylamine), carbon disulfide (CS₂), bromoacetic acid, substituted aromatic halides, piperidine, toluene, and the metal salts CuCl₂·2H₂O, NiCl₂·6H₂O, and CrCl₃·6H₂O were procured from Sigma-Aldrich, Merck, or Spectrochem. Ethanol, methanol, dimethyl sulfoxide (DMSO), glacial acetic acid, acetone, diethyl ether, and other solvents used for synthesis and purification were of analytical grade. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel plates. Melting points were determined using a suitable melting-point apparatus and are reported without correction.

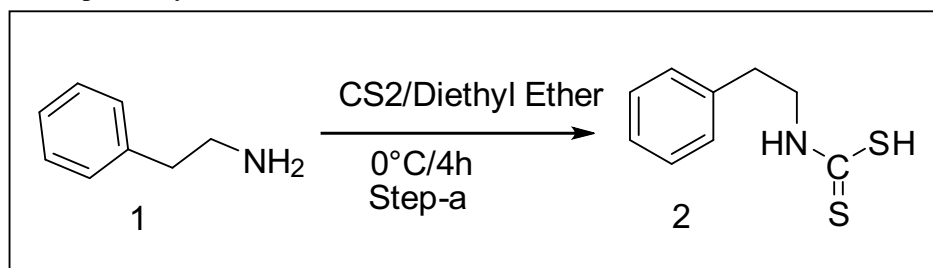
FT-IR spectra were recorded in the range of 4000–400 cm^{–1} using KBr pellets on a Shimadzu FT-IR spectrophotometer. ¹H and ¹³C NMR spectra were recorded in DMSO-d₆ on a Bruker Avance 400 MHz spectrometer, using tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ) are reported in parts per million (ppm). We recorded mass spectra

using an appropriate mass spectrometric technique. The synthesized metal complexes were further characterized by UV-visible spectroscopy, molar conductance, magnetic susceptibility measurements,

powder X-ray diffraction (PXRD), and thermogravimetric analysis (TGA), as described in the respective sections.

2.2 Synthesis of the Ligands

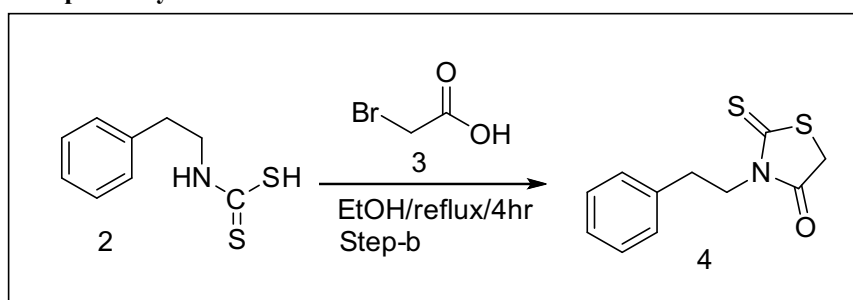
2.2.1. Synthesis of phenethylcarbamodithioic acid



To a stirred solution of 2-(4-fluorophenyl)ethan-1-amine (1.0 equiv) in diethyl ether, the reaction mixture was cooled to 0 °C. Carbon disulfide (CS₂, 1.2 equiv) was then added slowly, and the mixture was stirred at 0 °C for 30

min. A white precipitate formed, which was collected by filtration, washed with diethyl ether, and dried. The resulting dithiocarbamic acid intermediate was used directly in the subsequent step without further purification.

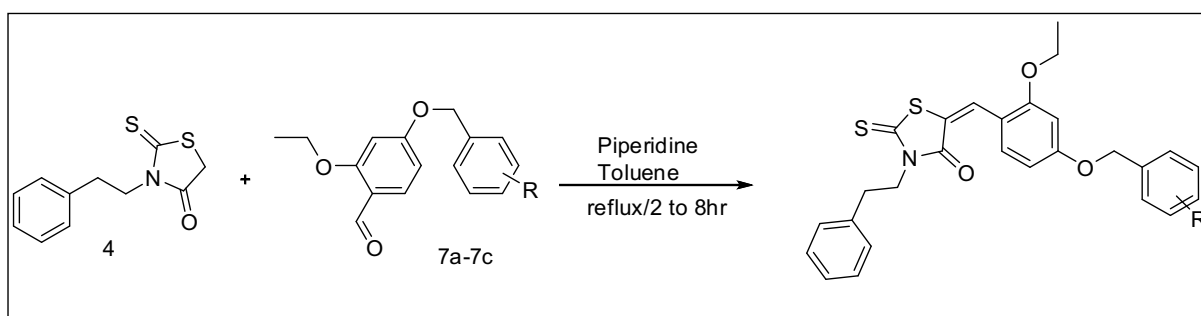
2.2.2. Synthesis of 3-phenethyl-2-thioxothiazolidin-4-one



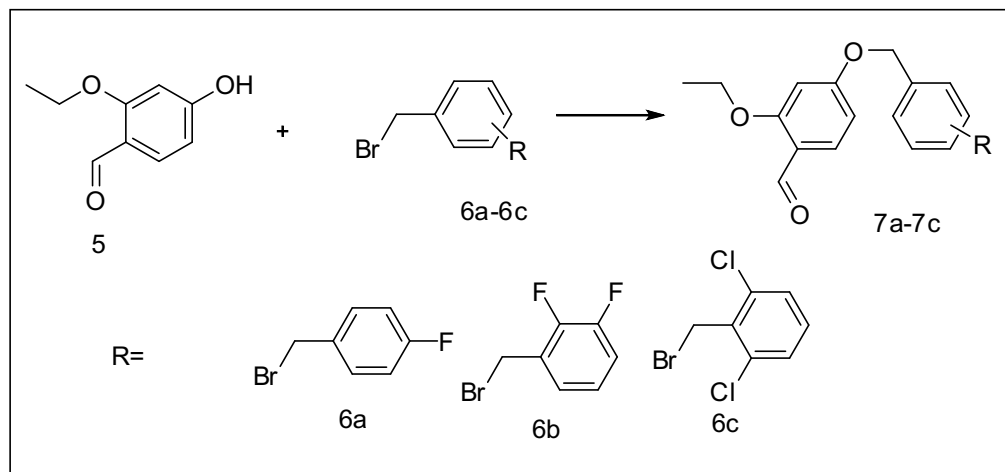
The step-01 product was treated with 2-bromoacetic acid (1.3 equiv) in ethanol, and the resulting reaction mixture was refluxed at 80 °C for 4 h. Upon completion of the reaction, the solvent was removed by distillation. The residue was treated with water (50 mL) and ethyl acetate (50 mL) and stirred thoroughly; then the organic layer was separated. The organic phase was concentrated under reduced pressure to afford the crude product. The crude material was purified by column chromatography over silica gel (100–200 mesh), using ethyl acetate/hexane (20:80 to 30:70, v/v) as the eluent to afford the desired product.

A stirred solution of 3-phenethyl-2-thioxothiazolidin-4-one (1.0 equiv) in toluene was treated with the appropriate substituted aldehyde (1.1 equiv) and piperidine (0.2 mL) at room temperature. The reaction mixture was refluxed for 6 h and monitored by TLC until completion. After completion, the reaction mixture was concentrated under reduced pressure. The resulting crude material was diluted with ethyl acetate and washed with water. The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to afford the crude product. The crude product was purified by column chromatography over silica gel (100–200 mesh), using ethyl acetate/hexane (30:70 to 40:60, v/v) as the eluent to afford the corresponding benzylidene rhodanine derivatives.

2.2.3. Synthesis of (E)-5-(4-(benzyloxy)-2-ethoxybenzylidene)-3-phenethyl-2-thioxothiazolidin-4-one:



2.2.4. Synthesis of Common Intermediate-7a to 7c:



Potassium carbonate (1.0 g) was added to a stirred solution of 2-ethoxy-4-hydroxybenzaldehyde (1.0 g) in DMF (10 mL), and the mixture was stirred at room temperature for 30 min. Subsequently, 1-(chloromethyl)-substituted benzene (2.0 g) was added, and the reaction mixture was stirred at room temperature for 6 h. TLC monitored the reaction progress. After complete consumption of the starting material, the reaction mixture was poured into cold water to precipitate the product. The solid was collected by filtration, washed with cold water and pentane, and dried to afford the corresponding 2-ethoxy-4-[(substituted benzyl)oxy]benzaldehyde as an off-white solid (2.0 g, 84%).

2.3. General Procedure for the Synthesis of Metal Complexes

The ligands 8a–8c (1.0 mmol) were individually dissolved in methanol (20 mL) and heated under reflux. A solution of the corresponding metal salt, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, or $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ (1.0 mmol), in methanol (10 mL) was added dropwise to the respective ligand solution with constant stirring. The reaction mixture was refluxed at 70 °C for 4 h. After completion, the mixture was cooled to room temperature and concentrated under reduced pressure. The resulting precipitate was collected by filtration, washed successively with methanol and diethyl ether, and dried under vacuum. The synthesized Ni(II), Cu(II), and Cr(III) complexes were characterized by appropriate spectroscopic and analytical techniques.

2.4. Characterisation of Ligands 8a-c

Synthesis of 8a (E)-5-(4-(4-fluorobenzoyloxy)-2-ethoxybenzylidene)-3-phenethyl-2-thioxo-4-one.

¹H NMR: 7.73 (s, 1H, Ar-H), 7.55 – 7.48 (m, 2H, Ar-H), 7.37 – 7.18 (m, 10H), 5.19 (s, 2H, PhCH_2O), 4.26 (t, J = 7.6 Hz, 2H), 4.21 (q, J = 6.8 Hz, 2H), 2.96 (t, J = 7.6 Hz, 2H), 1.37 (t, J = 6.4 Hz, 3H). ¹³C NMR: 193.43, 167.15, 163.33, 161.11, 150.02,

138.09, 133.49, 130.40, 129.07, 127.00, 125.21, 119.70, 115.60, 114.58, 69.70, 64.51, 45.73, 32.59, 15.07. Chemical Formula: $\text{C}_{27}\text{H}_{24}\text{FNO}_3\text{S}_2$ Molecular Weight: 493.61 Elemental Analysis: C, 65.70; H, 4.90; F, 3.85; N, 2.84; O, 9.72; S, 12.99

Synthesis of 8b (E)-5-(4-(3,4-difluorobenzoyloxy)-2-ethoxybenzylidene)-3-phenethyl-2-thioxo-4-one.

¹H NMR: 7.72 (s, 1H, Ar-H), 7.58 – 6.93 (m, 2H, Ar-H), 6.88 – 6.38 (m, 9H, Ar-H), 5.21 (s, 2H, PhCH_2O), 4.27 (t, J = 7.6 Hz, 2H), 4.23 (q, J = 6.8 Hz, 2H), 2.96 (t, J = 7.6 Hz, 2H), 1.37 (t, J = 6.4 Hz, 3H). ¹³C NMR: 193.47, 167.16, 150.70, 149.13, 138.10, 134.93, 133.92, 129.00, 127.11, 126.78, 125.12, 125.00, 119.83, 118.22, 117.20, 115.01, 69.16, 64.56, 45.74, 32.59, 15.06. Chemical Formula: $\text{C}_{27}\text{H}_{23}\text{F}_2\text{NO}_3\text{S}_2$ Molecular Weight: 511.60 Elemental Analysis: C, 63.39; H, 4.53; F, 7.43; N, 2.74; O, 9.38; S, 12.54

Synthesis of 8c (E)-5-(4-(2,6-dichlorobenzoyloxy)-2-ethoxybenzylidene)-3-phenethyl-2-thioxo-4-one.

¹H NMR (400 MHz, DMSO-d_6 , δ ppm): 7.72 (s, 1H, Ar-H), 7.58–6.93 (m, 2H, Ar-H), 6.88–6.38 (m, 9H, Ar-H), 5.21 (s, 2H, PhCH_2O), 4.27 (t, J = 7.6 Hz, 2H), 4.23 (q, J = 6.8 Hz, 2H), 2.96 (t, J = 7.6 Hz, 2H), 1.37 (t, J = 6.4 Hz, 3H); ¹³C NMR (100 MHz, DMSO-d_6 , δ ppm): 193.47, 167.16, 150.70, 149.13, 138.10, 134.93, 133.92, 129.00, 127.11, 126.78, 125.12, 125.00, 119.83, 118.22, 117.20, 115.01, 69.16, 64.56, 45.74, 32.59, 15.06. Chemical Formula: $\text{C}_{27}\text{H}_{23}\text{Cl}_2\text{NO}_3\text{S}_2$ Molecular Weight: 544.51 Elemental Analysis: C, 59.56; H, 4.26; Cl, 13.02; N, 2.57; O, 8.81; S, 11.78

2.7 Antibacterial Protocol

The antibacterial activity of the synthesized ligands and their corresponding Ni(II), Cu(II), and Cr(III) complexes was evaluated against two Gram-positive bacterial strains, *Staphylococcus aureus* and *Bacillus subtilis*, and two Gram-negative bacterial strains, *Escherichia coli* and *Pseudomonas aeruginosa*, using the agar well diffusion method.

Mueller–Hinton agar was used as the culture medium. The test compounds were dissolved in dimethyl sulfoxide (DMSO), while ciprofloxacin was used as the reference antibacterial agent. Sterile wells were made in the inoculated Mueller–Hinton agar plates and filled with the respective test solutions. The plates were incubated at 37 °C for 24 h, and antibacterial activity was determined by measuring the diameter of the inhibition zones surrounding the wells in millimetres.

We determined the minimum inhibitory concentration (MIC) by broth microdilution. We prepared serial dilutions of each test compound in nutrient broth and inoculated them with the respective bacterial cultures. The inoculated samples were incubated at 37 °C for 24 h. The MIC was recorded as the lowest concentration of the test compound that completely inhibited visible bacterial growth. All antibacterial experiments were performed in triplicate, and results were expressed as mean $\pm$ standard deviation (SD) [26–29].

2.8 Molecular docking protocol

Molecular docking studies were performed using AutoDock Vina and AutoDock4. The synthesized rhodanine derivatives 8a–8c and their corresponding metal complexes MC4–MC6 were prepared as ligand structures for docking analysis. The three-dimensional structures of *Staphylococcus aureus* DNA gyrase (PDB ID: 2XCT) and *S. aureus* dihydrofolate reductase (DHFR; PDB ID: 2W9S) were obtained from the Protein Data Bank. We prepared the protein structures by removing non-essential molecules and adding the required hydrogen atoms and partial charges before docking.

The rhodanine derivatives 8a–8c were docked against both 2XCT and 2W9S, whereas the metal complexes MC4–MC6 were docked against 2XCT. Ciprofloxacin and trimethoprim were used as reference inhibitors for 2XCT and 2W9S, respectively. Docking calculations were performed within the defined binding sites of the respective proteins, and the resulting poses were ranked by predicted binding energy. The compounds' interactions with the target proteins were then analyzed in terms of hydrogen-bonding and hydrophobic interactions.[30–33]

2.9 ADME and drug-likeness properties

The predicted ADME and drug-likeness properties of the synthesized compounds 8a–8c were evaluated using the SwissADME web-based platform. We submitted the molecular structures of compounds 8a–8c individually to the SwissADME platform and obtained the corresponding physicochemical and pharmacokinetic parameters. The evaluated parameters included molecular weight (MW), molecular volume (MV), topological polar surface area (TPSA), predicted gastrointestinal absorption, MLOGP, number of rotatable bonds, hydrogen-bond donors and acceptors, and Lipinski's rule-of-five parameters. We also assessed lead-

likeness using the criteria provided by the SwissADME platform. We used the computational predictions to compare the physicochemical, absorption, and drug-likeness profiles of compounds 8a–8c. [34–35]

3.0 Thermogravimetric (TG) and differential thermogravimetric (DTG)

We performed thermogravimetric (TG) and differential thermogravimetric (DTG) analyses to investigate the thermal stability and decomposition behaviour of the synthesized Cr(III), Cu(II), and Ni(II) metal complexes. The TG/DTG curves were recorded over approximately 35–1000 °C under the experimental conditions used. The onset temperature, DTG peak temperature(s), end temperature, experimental weight loss, and final residue were determined from the corresponding TG/DTG curves. We used the observed mass-loss steps to assess the thermal decomposition pattern of the complexes and identify the temperature ranges associated with successive decomposition events. The experimental thermal data were then compared with the calculated mass losses, using the established molecular formulae of the complexes, to support the proposed compositions and thermal decomposition behaviour.[36–38]

3.1. Powder X-ray diffraction (PXRD)

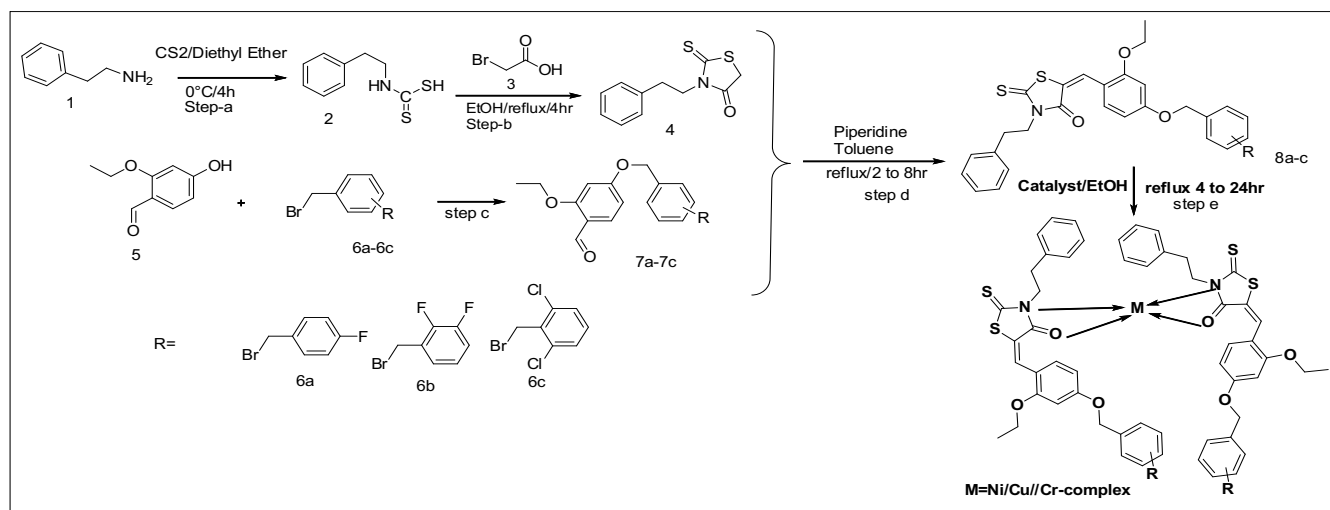
PXRD analysis was performed to investigate the crystalline nature and structural ordering of the synthesized Ni(II), Cu(II), and Cr(III) complexes. The diffraction patterns were recorded over the appropriate 2θ range under the experimental conditions employed. We analyzed the observed diffraction peaks in terms of their 2θ positions, d-spacing, and full width at half maximum (FWHM). The interplanar spacing was determined from the observed diffraction angles, and the crystallite size was estimated from the broadening of the diffraction peaks using the Scherrer equation. The PXRD patterns were subsequently used to assess the relative degree of crystallinity and structural ordering of the synthesized metal complexes.[39–41]

4.0 Results and Discussion:

The target compounds (E)-5-(4-(substituted)-2-ethoxybenzylidene)-3-phenethyl-2-thioxothiazolidin-4-one (8a–8c) were synthesized through a multistep route, as outlined in Scheme 1. We first prepared phenethylcarbamodithioic acid (2) from readily available 2-phenylethanamine (1). Cyclization of intermediate 2 with 2-bromoacetic acid (3) afforded 3-phenethyl-2-thioxothiazolidin-4-one (4), the key building block for the subsequent synthesis of the target compounds. In the final step, compound 4 underwent a Knoevenagel condensation with the appropriate substituted aromatic aldehydes (7a–7c) to furnish the corresponding (E)-5-(4-(substituted)-2-ethoxybenzylidene)-3-phenethyl-2-thioxothiazolidin-4-one derivatives (8a–8c). Using different substituted aromatic aldehydes enabled the preparation of the desired analogues with varying

substituents on the benzylidene moiety. Scheme 1 presents the detailed synthetic sequence and reaction

conditions.



Scheme 1: Synthesis of (E)-5-(4-(substituted)-2-ethoxybenzylidene)-3-phenethyl-2-thioxothiazolidin-4-one (8a-8c) derivatives:

Reagents and conditions: (Step-a): CS₂, (Et)₂O, 0°C – RT, 4 h; (Step-b): EtOH, 70°C, 4 h; (Step-c): K₂CO₃, DMF, RT, 6h; (Step-d): Piperidine, toluene, 80°C, 4h; (Step-e): MCl₂, EtOH, 70°C, 24 h;

In step (a), phenethylcarbamodithioic acid (2) was synthesized from 2-phenylethanamine (1) by treatment with carbon disulfide in diethyl ether at 0 °C for 4 h, affording the corresponding carbamodithioic acid in good yield. The reaction introduced the characteristic –NHC(=S)SH functionality, which serves as an appropriate sulfur-containing precursor for the subsequent construction of the thioxothiazolidinone ring. Similar approaches to prepare carbamodithioic acid derivatives using solvent-free conditions, copper-mediated three-component coupling, and visible-light or copper catalysis have been reported.

In step (b), phenethylcarbamodithioic acid (2) was cyclized with bromoacetic acid (3) in ethanol under reflux for 4 h to afford 3-phenethyl-2-thioxothiazolidin-4-one (4) in good yield. The formation of the thioxothiazolidinone ring was supported by ¹H NMR spectroscopy, which showed a characteristic singlet at δ 2.33 ppm corresponding to the methylene protons of the –CO–CH₂– group. The observed spectral feature is consistent with formation of the expected heterocyclic product. Related synthetic procedures for 2-thioxothiazolidin-4-one derivatives have been reported under comparatively higher-temperature conditions [28–30].

In step (c), 2-ethoxy-4-hydroxybenzaldehyde (5) was subjected to O-alkylation with the appropriate substituted benzyl bromides (6a–6c) in the presence of potassium carbonate to afford the corresponding O-alkylated aldehydes (7a–7c) in good yields. Incorporating substituted benzyl groups provided structural variation in the aldehyde component and enabled preparation of a series of related derivatives. Subsequently, aldehydes 7a–7c were reacted with 3-phenethyl-2-thioxothiazolidin-4-one (4) under Knoevenagel condensation conditions using piperidine in toluene under reflux to furnish the corresponding arylidene-3-phenethyl-2-thioxothiazolidin-4-one derivatives (8a–8c) [31–34].

NMR spectral data supported the formation of the condensed product. For the representative compound 8a, the ¹H NMR spectrum displayed a singlet at δ 7.73 ppm integrating for one proton, which was assigned to the newly formed benzylidene –CH= proton. The aromatic protons appeared as multiplets at δ 7.55–7.48 ppm (2H) and δ 7.37–7.18 ppm (10H). A singlet at δ 5.19 ppm integrating for two protons was assigned to the benzylic Ar–O–CH₂–Ph group. The triplet at δ 4.26 ppm (J = 7.6 Hz, 2H) was attributed to the NCH₂ protons of the phenethyl substituent, while the quartet at δ 4.21 ppm (J = 6.8 Hz, 2H) and triplet at δ 1.37 ppm (J = 6.4 Hz, 3H) corresponded to the OCH₂CH₃ group. The triplet at δ 2.96 ppm (J = 7.6 Hz, 2H) was assigned to the benzylic CH₂ protons of the phenethyl group. The appearance of the benzylidene proton signal together with the loss of the aldehydic proton signal of precursor 7a supports the occurrence of the Knoevenagel condensation.

The ¹³C NMR spectrum of 8a further supported the proposed structure. Signals at δ 193.43 and 167.15

ppm were assigned to the thiocarbonyl (C=S) and carbonyl (C=O) carbons, respectively. The signals in the δ 163.33–114.58 ppm region were attributed to the aromatic and unsaturated carbons. The resonances at δ 69.70, 64.51, 45.73, 32.59, and 15.07 ppm were assigned to Ar–O–CH₂–Ph, OCH₂CH₃, NCH₂, CH₂Ph, and OCH₂CH₃ carbons, respectively. These ¹H and ¹³C NMR data are consistent with the formation of the expected arylidene-2-thioxothiazolidin-4-one structure.

To establish suitable conditions for the Knoevenagel condensation, we performed a systematic optimization study using 2-ethoxy-4-((4-fluorobenzyl)oxy)benzaldehyde (7a) and 3-phenethyl-2-thioxothiazolidin-4-one (4) as model substrates in equimolar proportions. We investigated different catalytic systems, amines, solvents, temperatures, and reaction times, and summarized the yields in Table 1. Initial experiments using pyridinium chromate/sodium citrate dihydrate and ammonium ferrous sulfate/sodium citrate dihydrate at room temperature for 24 h afforded the desired product in 26% and 30% yields, respectively. These conditions therefore gave relatively low conversion to the desired condensation product.

We then examined cyclic secondary amines. Piperidine in ethanol at 70 °C afforded the product in 48% yield, while THF at the same temperature provided 55% yield. Piperidine in acetonitrile at 100 °C afforded the product in 60% yield. In contrast, piperidine in toluene under reflux at approximately 100 °C afforded the desired product in 85% yield. Pyrrolidine was also investigated under comparable conditions, affording yields of 38%, 32%, 50%, and 55% in ethanol, THF, toluene, and acetonitrile, respectively. Among all investigated conditions, piperidine in toluene under reflux gave the highest yield (85%) and was therefore selected as the optimized condition for the synthesis of derivatives 8a–8c.

The synthesized arylidene-2-thioxothiazolidin-4-one derivatives (8a–8c) were subsequently used as ligands to prepare Ni(II), Cu(II), and Cr(III) complexes. The corresponding metal complexes were prepared by reacting the ligands with the appropriate metal salts in ethanol under reflux conditions, as represented in step (e) of the synthetic scheme.

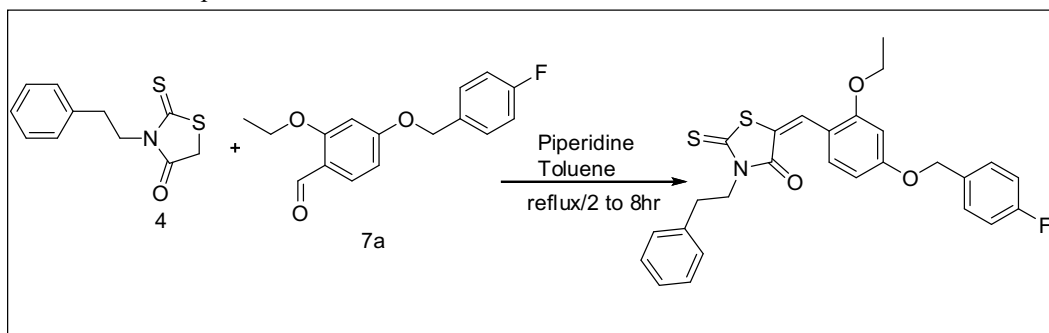


Table 1: Screening of various catalysts for the synthesis of (E)-5-(4-(4-fluorobenzyl)-2-ethoxybenzylidene)-3-phenethyl-2-thioxothiazolidin-4-one (8a): Piperidine, Pyrrolidine, TES, EtOH, DCE, THF, ACN

Sr No	Reaction Condition	Yields (%)
1	Pyridinium chromate, sodium citrate dihydrate, RT, 24 h	26
2	Ammonium ferrous sulfate, sodium citrate dihydrate, RT, 24 h	30
3	Piperidine, EtOH, 70°C, 2-8h	48
4	Piperidine, toluene, 100°C, 2-8h	85
5	Piperidine, THF, 70°C, 2-8h	55
6	Piperidine, ACN, 100°C, 2-8h	60
7	Pyrrolidine, EtOH, 70°C, 2-8h	38
8	Pyrrolidine, THF, 70°C, 2-8h	32
9	Pyrrolidine, toluene, 100°C, 2-8h	50
10	Pyrrolidine, ACN, 100°C, 2-8h	55

The synthesized arylidene-2-thioxothiazolidin-4-one derivatives (8a–8c) were subsequently reacted with Ni(II), Cu(II), and Cr(III) metal salts in ethanol under reflux conditions to afford the corresponding metal complexes. The resulting complexes were isolated as solid products and characterized by FT-IR, UV–

Visible spectroscopy, powder X-ray diffraction (PXRD), thermogravimetric (TG) analysis, and elemental analysis. The rhodanine framework contains several potential donor atoms, including the ring nitrogen, carbonyl oxygen, and sulfur atoms associated with the thioxo group. These donor sites

can facilitate bidentate or tridentate coordination with transition-metal ions, depending on the metal ion and its coordination environment. Metal–ligand coordination can modify the electronic and physicochemical properties of the parent ligands.

Detailed synthetic procedures and characterization data are provided in the Experimental section. The proposed structures of the resulting Ni(II), Cu(II), and Cr(III) complexes are presented in Figure 1.

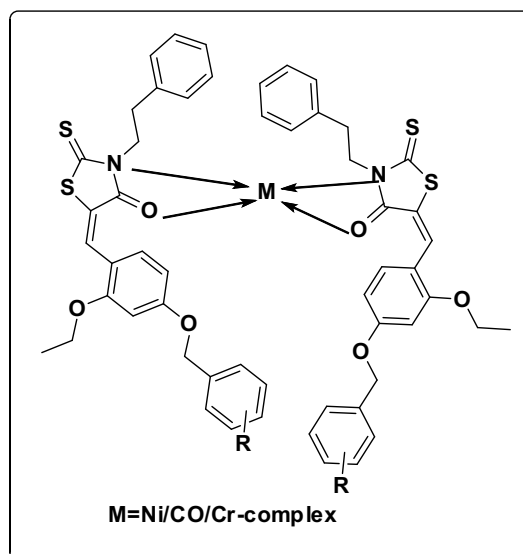


Figure-1: Metal complex of (E)-5-(4-(substitued)-2-ethoxybenzylidene)-3-phenethyl-2-thioxothiazolidin-4-one (8a-8c) derivatives.

4.1 Antibacterial activity

The antibacterial activities of the synthesized ligands (8a–8c) and their corresponding Ni(II), Cu(II), and Cr(III) complexes were evaluated against two Gram-positive bacterial strains, *Staphylococcus aureus* and *Bacillus subtilis*, and two Gram-negative bacterial strains, *Escherichia coli* and *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) values are presented in Table 2.

The free ligands 8a–8c exhibited MIC values ranging from 25 to 100 $\mu\text{g mL}^{-1}$ against the tested bacterial strains. In comparison, their corresponding Ni(II), Cu(II), and Cr(III) complexes generally exhibited lower MIC values, ranging from 6.25 to 50 $\mu\text{g mL}^{-1}$, indicating improved antibacterial activity following metal complexation. Among the synthesized compounds, the Ni(II) complexes exhibited MIC values of 6.25–25 $\mu\text{g mL}^{-1}$ across the tested bacterial strains. Ni(8b)₂ and Ni(8c)₂ showed

MIC values of 6.25 $\mu\text{g mL}^{-1}$ against *B. subtilis*, while Ni(8b)₂ also exhibited an MIC of 6.25 $\mu\text{g mL}^{-1}$ against *S. aureus*. The Cu(II) and Cr(III) complexes also displayed lower MIC values than their respective free ligands for several bacterial strains. However, activity varied with both ligand structure and bacterial strain.

Ciprofloxacin, used as the reference antibacterial agent, showed MIC values of 6.25, 12.5, 12.5, and 25 $\mu\text{g mL}^{-1}$ against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively. Several synthesized metal complexes showed MIC values within the same range as ciprofloxacin against individual bacterial strains. Overall, the MIC results indicate that coordination with Ni(II), Cu(II), and Cr(III) was associated with enhanced antibacterial activity for the synthesized compounds.

Table 2. Antibacterial activity of the synthesized ligands and metal complexes.

Compound	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
8a	50	50	100	100
Ni(8a) ₂	12.5	6.25	25	25
Cu(8a) ₂	25	12.5	50	50
Cr(8a) ₂	12.5	12.5	25	25
8b	25	25	50	100
Ni(8b) ₂	6.25	6.25	12.5	25
Cu(8b) ₂	12.5	12.5	25	50
Cr(8b) ₂	12.5	6.25	25	25
8c	50	50	100	100
Ni(8c) ₂	12.5	12.5	25	25
Cu(8c) ₂	25	25	50	50
Cr(8c) ₂	25	12.5	25	50

Ciprofloxacin	6.25	12.5	12.5	25
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Figure 1. Antibacterial activity of the synthesized ligands and metal complexes.

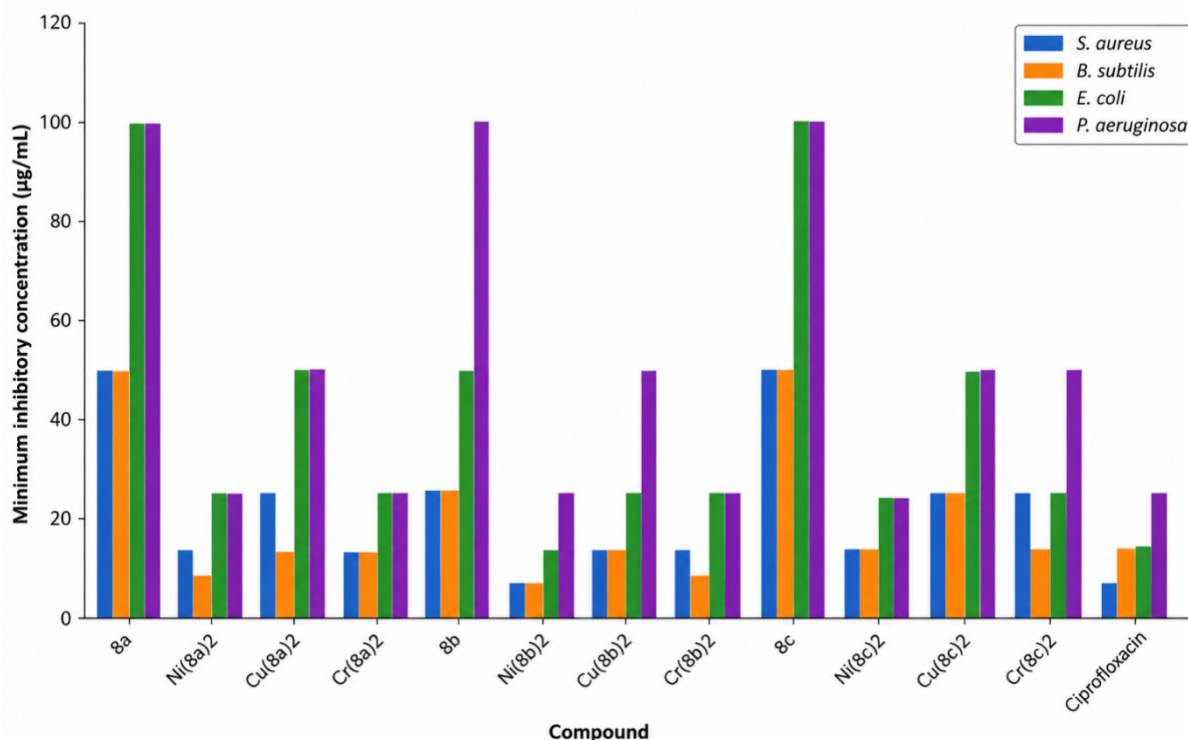


Figure 1. Antibacterial activity of the synthesized ligands and Ni(II), Cu(II), and Cr(III) metal complexes.

4.2. Molecular Docking Studies

Molecular docking studies were performed using AutoDock Vina and AutoDock4 to investigate the predicted binding affinities and interaction profiles of the synthesized ligands 8a–8c against *Staphylococcus aureus* DHFR (PDB ID: 2W9S) and gyrase (PDB ID: 2XCT). We used trimethoprim and ciprofloxacin as reference inhibitors for 2W9S and 2XCT, respectively.

Against 2W9S, ligands 8a, 8b, and 8c showed docking scores of -9.634 , -10.400 , and -9.783 kcal/mol, respectively. Ligand 8b exhibited the most negative docking score and formed 12 hydrophobic interactions along with two hydrogen bonds involving THR46 and GLN95. Ligand 8a showed 13 hydrophobic interactions, while 8c exhibited six hydrophobic interactions involving residues within the binding region. Trimethoprim showed a docking score of -7.609 kcal/mol.

Against 2XCT, ligands 8a, 8b, and 8c exhibited docking scores of -6.321 , -6.817 , and

-7.127 kcal/mol, respectively. Ligand 8c showed the most negative docking score and formed four hydrophobic interactions involving GLU435, LYS460, ILE516, and LEU583, together with one hydrogen bond involving GLY1082. Ligands 8a and 8b predominantly showed hydrophobic interactions with residues in the binding site. Ciprofloxacin exhibited a docking score of -4.307 kcal/mol.

Overall, ligands 8b and 8c showed the most negative predicted binding scores against 2W9S and 2XCT, respectively. The interaction analysis indicates that hydrophobic contacts were predominant, with additional hydrogen-bonding interactions observed for 8b and 8c. These computational results suggest favourable ligand–protein interactions and provide supportive structural information for interpreting the experimental antibacterial activity. However, docking scores are predictive and should not be interpreted as a direct quantitative measure of antibacterial potency.

Table 3. Molecular docking affinities and protein–ligand interactions of ligands, metal complexes, and reference compounds against 2W9S and 2XCT.

Compound	Affinity (kcal/mol)	Interaction_Type	Interaction_Count	Residues
2W9S_8a	-9.634	Hydrophobic	13	ILE5, LEU20, LEU28, ILE31, THR46, ILE50, LEU54, PHE92
2W9S_8b	-10.4	Hydrophobic	12	VAL6, ILE14, LEU20, ILE31, ALA43, LYS45, THR46, PHE92, THR96

		Hydrogen_Bond	2	THR46, GLN95
2W9S_8c	-9.783	Hydrophobic	6	LYS45, THR46, ILE50, LEU54, PHE92, THR121
2W9S_Trimethoprim	-7.609	Hydrophobic	2	ILE14, LEU20
		Hydrogen_Bond	4	ASN18, THR46, GLN95, TYR98
2XCT_8a	-6.321	Hydrophobic	7	GLU435, ASP512, ILE516, LEU583, LYS1043, VAL1045, HIS1081
2XCT_8b	-6.817	Hydrophobic	5	ARG1033, ALA1034, HIS1081, GLU1088, ALA1089
2XCT_8c	-7.127	Hydrophobic	4	GLU435, LYS460, ILE516, LEU583
		Hydrogen_Bond	1	GLY1082
2XCT_Ciprofloxacin	-4.307	Hydrogen_Bond	1	SER442
2XCT_MC4	-7.6	Hydrophobic	4	LYS1043, PHE1097, GLN1267
		Pi_Cation	2	LYS1043, ARG1092
		Metal	2	UNL1
2XCT_MC5	-6.65	Hydrophobic	8	TYR1087, GLU1088, VAL1091, PHE1097, ALA1118, ILE1175, ASN1269
		Metal	2	UNL1
2XCT_MC6	-9.07	Hydrophobic	5	GLU1088, THR1220, PHE1266, ASN1269
		Salt_Bridge	1	GLU1088
		Pi_Stacking	1	PHE1097
		Halogen	1	SER1085
		Metal	2	UNL1

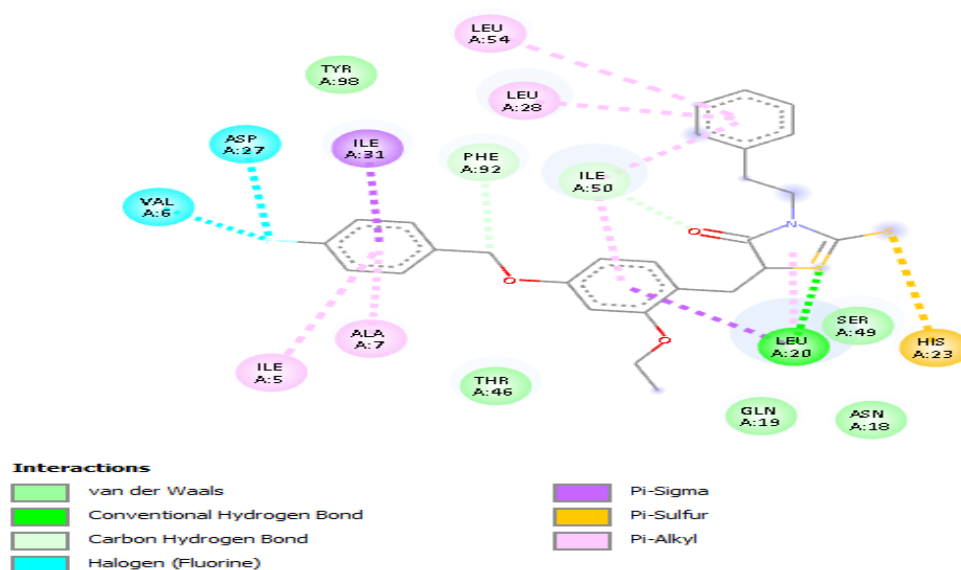


Figure 2- 2D Docking interaction of 8a with 2XCT

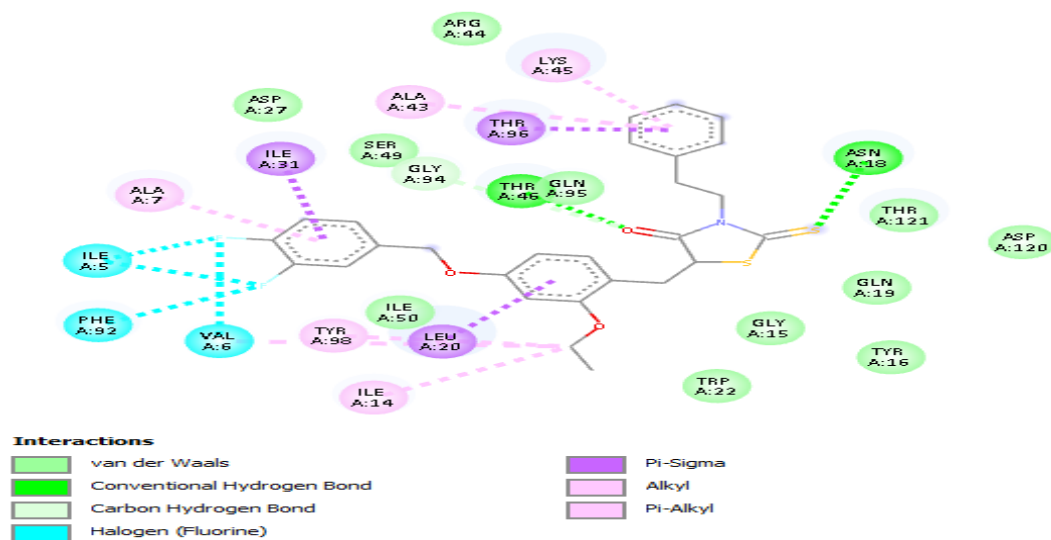
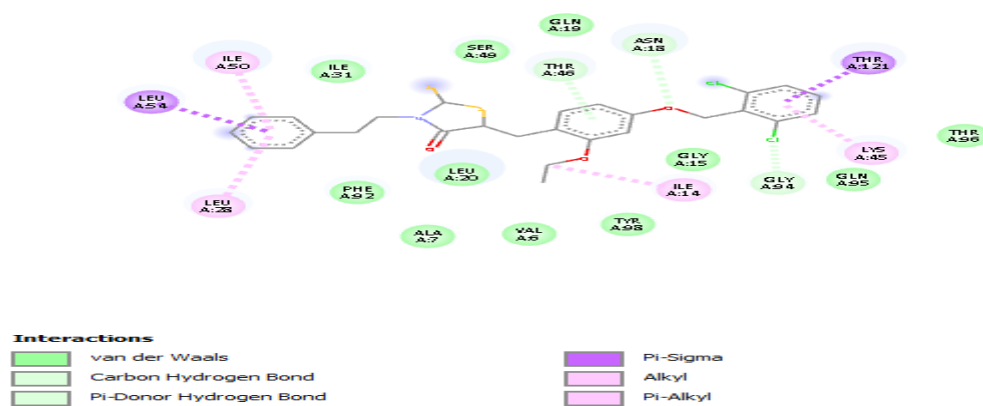


Figure 3- 2D Docking interaction of 8b with 2XCT



4- 2D Docking interaction of 8c with 2XCT

Figure

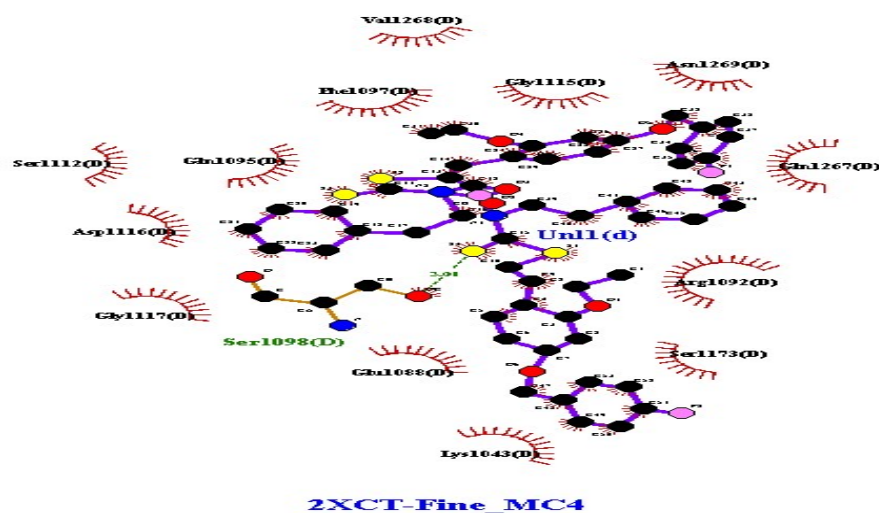


Figure 5- LigPlot interaction of metal complex of Ni(8a)₂ with 2XCT

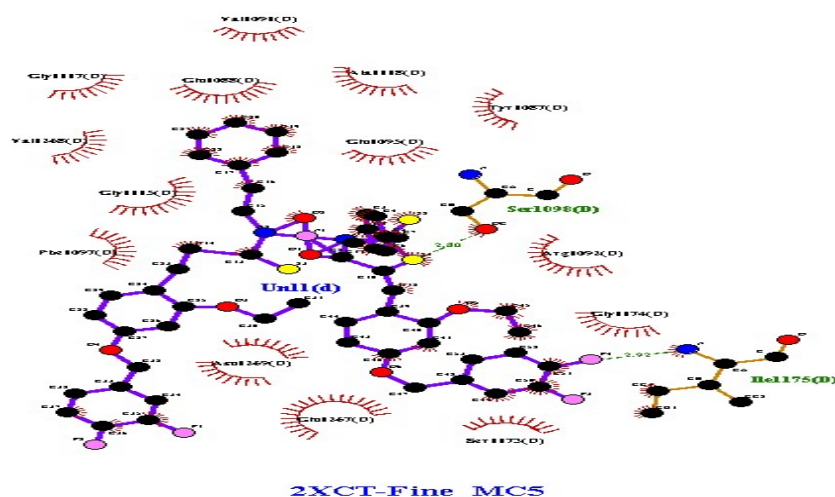


Figure 6- LigPlot interaction of metal complex of Cu(8b)₂ with 2XCT

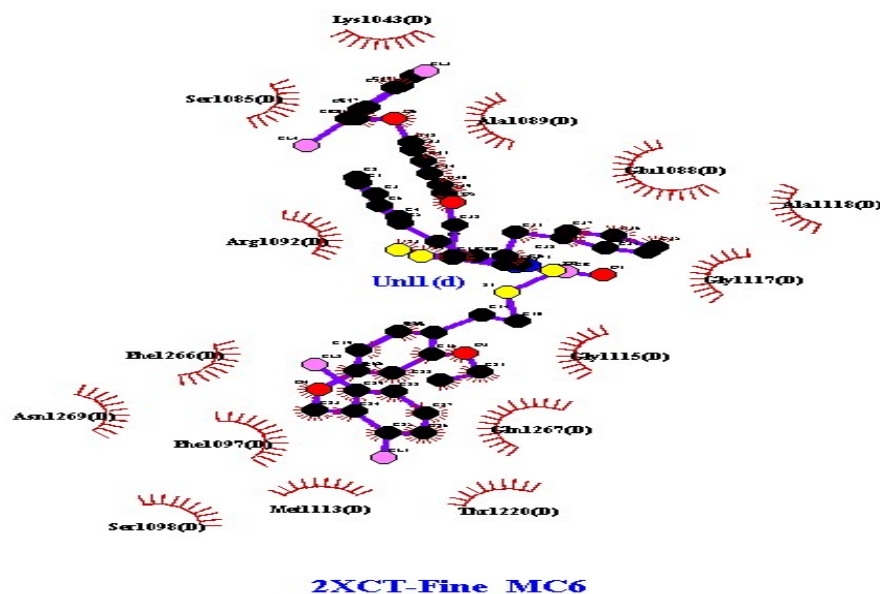


Figure 7- LigPlot interaction of metal complex (Cr(8c)₂) with 2XCT

4.3. ADME and Drug-Likeness Analysis

We evaluated the predicted ADME and drug-likeness properties of compounds 8a–8c using the SwissADME platform. The molecular weights (MW) ranged from 493.61 to 544.51 g/mol, while the molecular volumes (MV) ranged from 490.14 to 514.49. All three compounds showed an identical TPSA value of 96.16 Å² and a predicted percentage absorption (%ABS) of 75.82%. The MLOGP values increased from 4.08 for 8a to 4.44 for 8b and 4.64 for 8c, indicating increasing predicted lipophilicity across the series. The compounds possessed nine rotatable bonds, while the number of hydrogen-bond acceptors varied from 3 to 5. No compound was

predicted to have hydrogen-bond donors. Regarding Lipinski's rule of five, 8a showed no violations, whereas 8b and 8c each showed two violations. All three compounds exhibited three lead-likeness violations. Overall, compounds 8a–8c showed similar predicted polarity and absorption, with the main differences occurring in molecular weight, molecular volume, lipophilicity, hydrogen-bond acceptor count, and drug-likeness parameters. These computational predictions provide preliminary information on the physicochemical and ADME characteristics of the synthesized compounds and should be complemented by experimental pharmacokinetic and biological studies.

Table 4. Physicochemical and drug-likeness properties of compounds 8a–8c.

Compounds	MW	MV	TPSA	% ABS	MLOGP	Rotatable bonds	H-bond acceptors	H-bond donors	Lipinski violations	Leadlikeness violations
8a	493.61	490.14	96.16	75.82	4.08	9	4	0	0	3
8b	511.60	496.21	96.16	75.82	4.44	9	5	0	2	3
8c	544.51	514.49	96.16	75.82	4.64	9	3	0	2	3

4.4 Thermogravimetric and Differential Thermogravimetric (TG/DTG) Analysis

TG/DTG analysis was carried out to investigate the thermal stability and decomposition behaviour of the synthesized Ni(II), Cu(II), and Cr(III) complexes. The TG curves show multistep mass-loss processes for all the complexes, while the corresponding DTG curves display several distinct thermal events. The initial mass losses observed below approximately 150 °C may be attributed to the removal of physically retained moisture and/or volatile solvent molecules, where present. The subsequent mass-loss steps are mainly associated with the progressive thermal decomposition of the coordinated organic ligand framework. This multistage thermal decomposition is common for transition-metal complexes and can vary with the metal ion and ligand environment.

The DTG profiles revealed major high-temperature decomposition events at approximately 539 °C for Ni(1), 655 °C for Cu(2), 731 °C for Cu(3), 747 °C for Cu(1), 859 °C for Cr(1), 870 °C for Ni(2), 947 °C for Ni(3), and 973 °C for Cr(3). These high-temperature events indicate substantial degradation of the coordinated organic framework. The complexes therefore undergo successive thermal decomposition rather than a single-step degradation process, with the decomposition behaviour differing among the Ni(II), Cu(II), and Cr(III) complexes.

The total experimental mass losses were 88.55% for Cr(1), 65.86% for Cr(2), 99.10% for Cr(3), 82.59% for Cu(1), 76.50% for Cu(2), 80.20% for Cu(3), 87.92% for Ni(1), 99.80% for Ni(2), and 86.60% for Ni(3). The corresponding residues were 11.45%, 34.14%, 0.90%, 17.41%, 23.50%, 19.80%, 12.08%, 0.20%, and 13.40%, respectively. These residual masses represent the material remaining after extensive thermal decomposition of the organic components and are expected to contain the metal-containing inorganic fraction; however, TG/DTG data alone cannot establish their exact composition.

Among the investigated complexes, Cr(2) showed the highest residual mass (34.14%), whereas Cr(3) and Ni(2) exhibited very small apparent residues of 0.90% and 0.20%, respectively. For Cr(3) and Ni(2), the TG traces approach the baseline at the upper temperature limit; therefore, these near-zero residues should be interpreted cautiously. Baseline deviation and instrumental effects at the end of the temperature program can influence the apparent residual mass. Accordingly, the very small residues observed for Cr(3) and Ni(2) should not be regarded as definitive chemical residues without appropriate baseline correction and, preferably, independent characterization of the final solid products. Overall, the TG/DTG results show multistage thermal decomposition and substantial high-temperature stability of the synthesized Ni(II), Cu(II), and Cr(III) complexes before extensive degradation of their organic frameworks.

Table 5. TG/DTG thermal decomposition parameters of the synthesized Ni(II), Cu(II), and Cr(III) complexes

Complex	Onset (°C)	DTG peak(s) (°C)	End (°C)	Experimental weight loss (%)	Residue (%)
Cr(1)	34	68, 123, 859	900	88.55	11.45
Cr(2)	35	93, 135, 160, 365, 814	900	65.86	34.14
Cr(3)	35	99, 587, 620, 973	1000	99.10*	0.90*
Cu(1)	35	86, 223, 265, 747	900	82.59	17.41
Cu(2)	35	70, 259, 387, 655	900	76.50	23.50
Cu(3)	35	85, 262, 407, 731	900	80.20	19.80

Ni(1)	34	54, 163, 281, 539	900	87.92	12.08
Ni(2)	35	85, 151, 870	1000	99.80*	0.20*
Ni(3)	35	97, 138, 158, 388, 947	1000	86.60	13.40

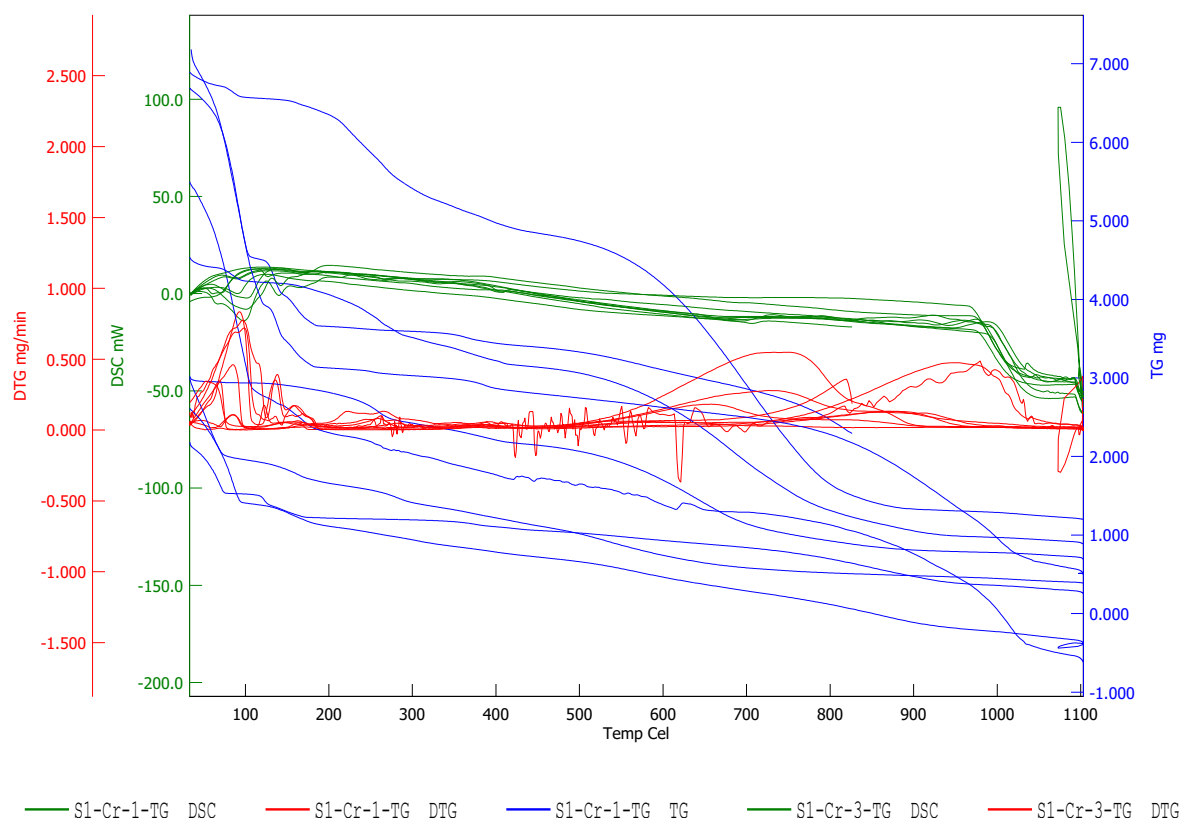


Figure 8. TG/DTG curves of the synthesized metal complexes:

4.5 Powder X-ray Diffraction (PXRD) Analysis

Powder X-ray diffraction (PXRD) analysis was performed to evaluate the crystallinity and structural ordering of the synthesized Ni(II), Cu(II), and Cr(III) complexes. The Ni(II) complexes (Ni-1–Ni-3) exhibited multiple distinct diffraction reflections, indicating crystalline/polycrystalline characteristics. Ni-1 showed well-defined reflections at 15.83° , 16.42° , 19.70° , 19.98° , 30.79° , 32.39° , 32.92° , 36.62° , 40.64° , and 42.69° , along with additional reflections at higher diffraction angles. Ni-2 displayed numerous relatively sharp reflections over a broad 2θ range, whereas Ni-3 also exhibited several distinct diffraction maxima with comparatively narrow peak widths. The relatively low FWHM values observed for several reflections indicate the presence of well-developed coherent diffraction domains.

The Cu(II) complexes (Cu-1–Cu-3) also exhibited multiple distinct diffraction reflections, consistent with crystalline/polycrystalline materials. Cu-1 showed reflections distributed over a broad 2θ range, while Cu-2 and Cu-3 displayed several prominent diffraction maxima together with additional weaker reflections. The variation in FWHM values among the Cu(II) complexes indicates differences in their apparent coherent-domain sizes. The domain sizes calculated using the Scherrer equation should be regarded as apparent coherent-domain sizes because diffraction-peak broadening may also be influenced by instrumental broadening and microstrain.

The Cr(III) complex Cr-2 exhibited broad and less-resolved diffraction features at approximately 12.60° , 33.80° , and 45.60° . The corresponding FWHM values ranged from 9.0° to 12.9° , indicating substantial peak broadening. The calculated apparent

coherent-domain sizes were very small, ranging from 6.7 to 9.4 Å, suggesting comparatively poor crystallinity and lower structural ordering. Overall, the PXRD results demonstrate differences in solid-state structural ordering among the investigated metal complexes. The Ni(II) and Cu(II) complexes exhibited multiple well-defined diffraction

reflections characteristic of crystalline/polycrystalline materials, whereas Cr-2 displayed broad diffraction features and very small apparent coherent-domain sizes. These results indicate comparatively greater crystalline ordering in the Ni(II) and Cu(II) complexes than in Cr-2.

Table 6. PXRD parameters of Ni(II), Cu(II), and Cr(III) complexes

Complex	Principal observed 2θ (°)	d-spacing (Å)	FWHM (°)	Apparent coherent-domain size (Å)	PXRD interpretation
Ni-1	15.83, 16.42, 19.70, 19.98, 30.79, 32.39, 32.92, 36.62, 40.64, 42.69, 52.97, 55.21, 56.54	5.593–1.626	0.24–5.80	14.4–392	Crystalline/polycrystalline pattern with several well-defined reflections; variation in peak width indicates different coherent-domain sizes.
Ni-2	6.40, 13.21, 15.76, 16.35, 18.18, 19.90, 20.49, 30.63, 32.53, 36.60, 40.63, 41.60, 42.64, 48.93, 52.86, 55.07, 56.29, 60.71	13.80–1.524	0.16–1.34	70–511	Highly crystalline/polycrystalline pattern with numerous relatively sharp reflections and comparatively narrow FWHM values.
Ni-3	13.22, 16.24, 18.12, 19.85, 20.44, 29.98, 31.19, 32.89, 34.02, 35.09, 36.56, 40.67, 42.79, 44.83, 46.18, 49.12, 52.83	6.691–1.731	0.06–0.85	102–1471	Crystalline/polycrystalline material with numerous distinct reflections and several narrow peaks indicating well-developed coherent diffraction domains.
Cu-1	6.29, 7.77, 8.70, 10.68, 12.91, 15.66, 18.69, 19.84, 23.99, 25.28, 32.17, 35.44, 39.43, 39.93, 45.20, 76.70	14.05–1.242	0.11–1.80	60–840	Crystalline/polycrystalline pattern with several distinct reflections; relatively sharp peaks indicate appreciable crystalline ordering.
Cu-2	6.73, 7.53, 11.36, 12.76, 15.65, 31.30, 32.17, 35.37, 39.91, 45.06, 48.90, 51.34, 54.76, 56.99	13.12–1.615	0.14–0.83	100–651	Crystalline/polycrystalline material with distinct diffraction maxima and comparatively narrow peaks indicating ordered coherent domains.
Cu-3	7.42, 8.03, 10.60, 12.89, 15.69, 17.97, 20.73, 25.80, 32.24, 35.52, 40.02, 45.07, 46.90, 49.06	11.90–1.855	0.15–3.40	25.3–592	Crystalline/polycrystalline material with several distinct reflections; variation in peak width indicates differences in apparent coherent-domain size.
Cr-2	12.60, 33.80, 45.60	7.02, 2.65, 1.989	9.0, 12.9, 9.6	9.3, 6.7, 9.4	Poorly crystalline/highly disordered material with broad diffraction features and very small apparent coherent-domain sizes.

Synthesis, Structural Characterization, and Antibacterial Evaluation of Novel Rhodanine-Based Ni(II), Cu(II), and Cr(III) Complexes Bearing Substituted Thiazolidinone Moieties

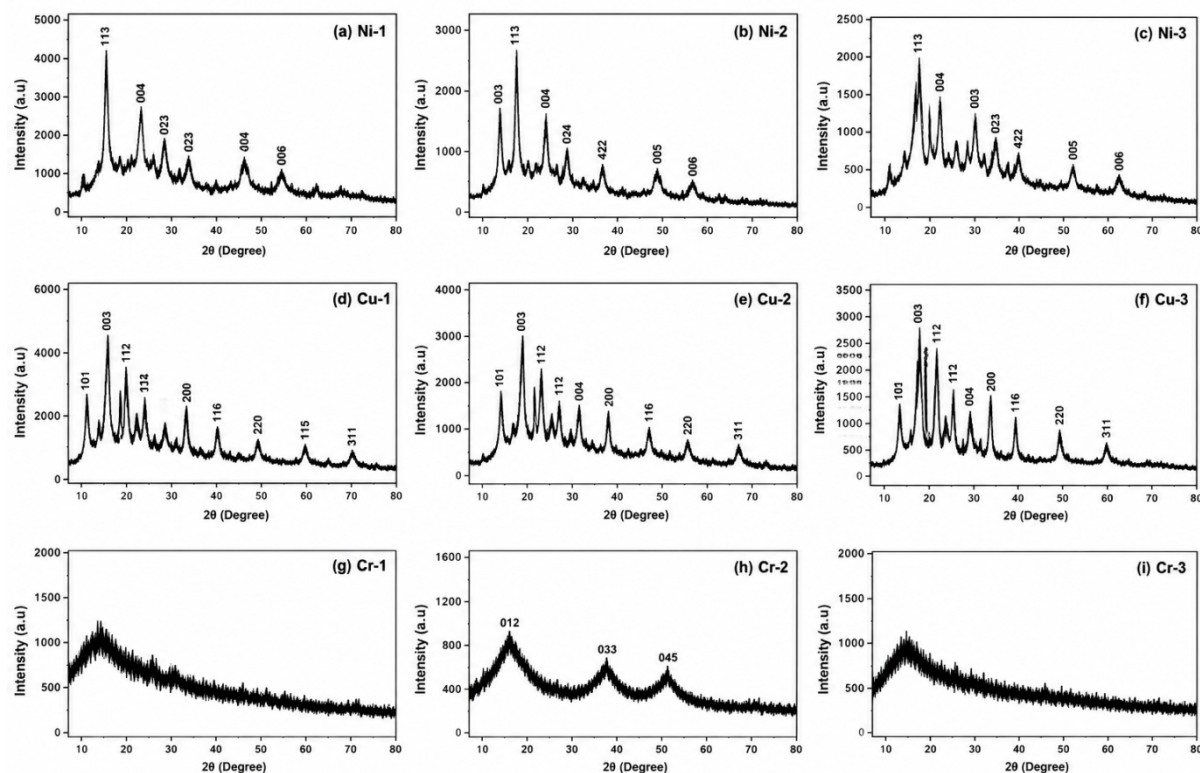
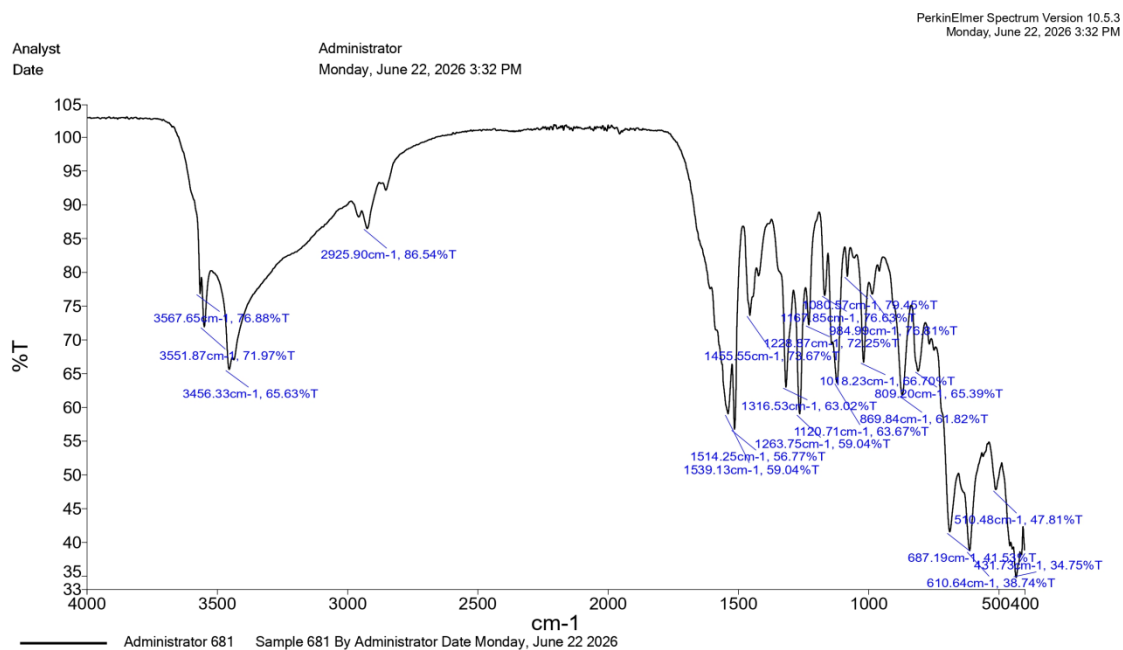


Figure 9. Powder X-ray diffraction (PXRD) patterns of the synthesized Ni(II), Cu(II), and Cr(III) complexes

4.6. Infrared studies



The FT-IR spectrum of Ni-2 showed characteristic bands at 3567–3456 cm^{-1} (O–H/N–H), 2925.90 cm^{-1} (aliphatic C–H), and 1539.13 and 1514.25 cm^{-1} (aromatic/C=N or amide-related vibrations). Bands

in the 1455–985 cm^{-1} region correspond to various C–C, C–N, C–O, and aromatic vibrations. The low-frequency bands at 687.19, 610.64, 510.48, and 431.73 cm^{-1} may be associated with metal–ligand

vibrations, supporting coordination of the ligand to Ni(II).

5.0 Conclusion

A novel series of 3-phenethyl-5-(substituted benzylidene)-2-thioxothiazolidin-4-one derivatives (8a–8c) and their Ni(II), Cu(II), and Cr(III) complexes were successfully synthesized through dithiocarbamate formation, rhodanine-ring cyclization, and C-5 Knoevenagel condensation. We characterized the compounds using spectroscopic and analytical techniques, and PXRD and TG/DTG analyses provided information on crystallinity and thermal stability.

The synthesized ligands and metal complexes exhibited antibacterial activity against the tested Gram-positive and Gram-negative bacteria, with several complexes showing improved activity compared with the corresponding free ligands. Molecular docking indicated favourable predicted interactions with selected bacterial targets, while ADME analysis provided preliminary information on drug-likeness. Overall, these rhodanine-based Ni(II), Cu(II), and Cr(III) compounds represent useful candidates for further investigation in coordination chemistry, antimicrobial research, and medicinal chemistry.

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