Synthesis, Characterization and in Silico Antibacterial Studies of p-nitrobenzohydrazide Derived Schiff Bases, A 1,3,4-Oxadiazole Derivative and a Cu(II) Complex

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ABSTRACT

The present study reports the successful synthesis and structural characterization of novel *p*-nitrobenzohydrazide derivatives, encompassing Schiff bases, a 1,3,4-oxadiazole derivative, and a corresponding Cu(II) complex. The target molecules were prepared via condensation, cyclization, and metal coordination pathways. Structural confirmation and purity were established using analytical and spectroscopic techniques, including FT-IR, UV–Visible spectrophotometry, and Thin Layer Chromatography (TLC). Molecular docking simulations against bacterial DNA gyrase (PDB ID: 5BS8) were subsequently conducted utilizing PyRx and AutoDock Vina. The Schiff base, 1,3,4-oxadiazole derivative, and Cu(II) complex exhibited favorable binding affinities of −7.2 kcal/mol, −7.5 kcal/mol, and −8.1 kcal/mol, respectively. Comparative evaluation demonstrated that structural cyclization and metal complexation effectively enhanced interaction parameters with the target protein. Among the tested compounds, the Cu(II) complex displayed the most prominent binding affinity. Overall, these findings indicate that *p*-nitrobenzohydrazide-derived scaffolds represent promising candidates for advanced antibacterial research and the development of novel antimicrobial agents.

Keywords: P-Nitrobenzaldehyde, Schiff Base, 1,3,4-Oxadiazole, Cu(II) Complex, Molecular Docking.

INTRODUCTION:

Nitrogen-oxygen heterocycles, particularly 1,3,4-oxadiazoles and Schiff bases, represent vital classes of compounds in medicinal chemistry due to their aromatic stability, favorable pharmacokinetic profiles, and broad spectrum of pharmacological activities [1,2]. Functioning as effective bioisosteres, 1,3,4-oxadiazoles enhance metabolic stability, while the incorporation of electron-withdrawing substituents—such as nitro and chloro groups—significantly improves lipophilicity, membrane permeability, and target interaction [3]. Furthermore, Schiff bases featuring azomethine linkages serve as versatile synthetic intermediates and effective ligands that readily form copper(II) complexes, boosting cellular penetration, DNA binding, and antimicrobial efficacy [4]. In the present study, a series of *p*-nitrobenzoic acid-derived Schiff bases, 2,5-disubstituted 1,3,4-oxadiazoles, and their Cu(II) complexes were synthesized and characterized using melting point determination and FT-IR spectroscopy. To complement the experimental findings, molecular docking investigations were performed targeting bacterial DNA gyrase (PDB ID: 5BS8) to evaluate binding affinities and structural interaction modes within the active site [5]. Together, these theoretical and experimental insights highlight these novel derivatives as promising candidates for combating antimicrobial resistance[1-5].

MATERIALS AND METHOD

Materials

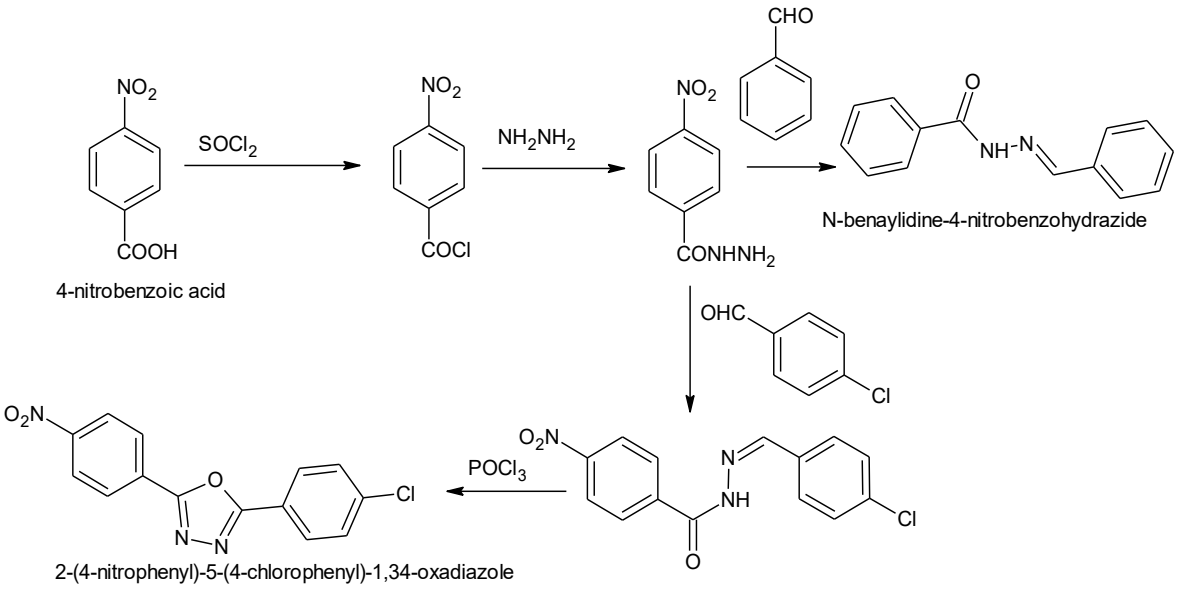
All chemicals used in the present work were of analytical reagent (AR) grade and were used without further purification.

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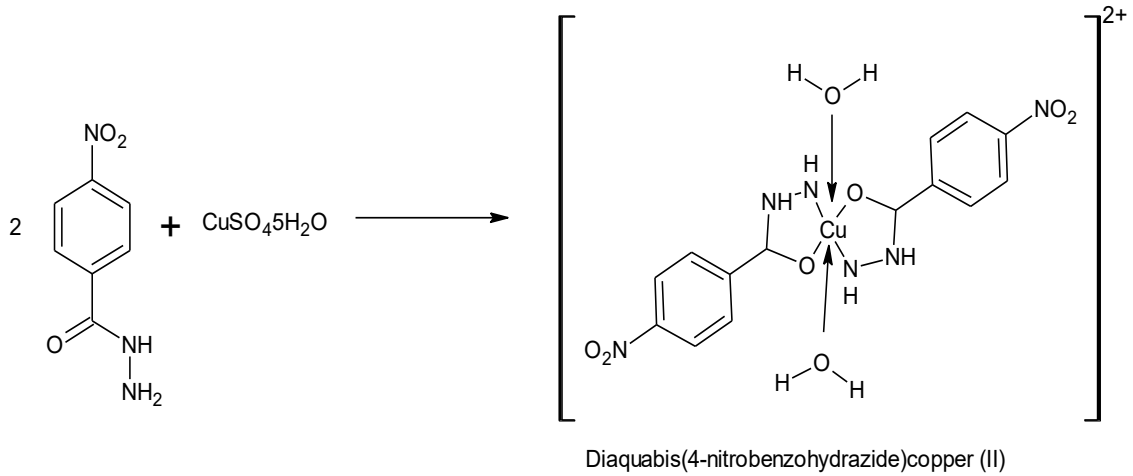
Method

The melting points of synthesized compounds were determined by open capillary method and are uncorrected. The progress of the reactions was monitored by thin layer chromatography (TLC) using suitable solvent systems. Purification of products was carried out by recrystallization using ethanol or methanol depending on solubility.

The synthesized compounds were characterized using melting point determination and Fourier Transform Infrared (FT-IR) spectral analysis from SAIF, Cochi and UV-Vis spectrophotometry for confirmation of functional groups and structural verification.



Scheme 1a: Synthetic pathway for the preparation of substituted 1,3,4-oxadiazole derivative and Schiff base derived from benzaldehyde



Scheme 1b: Synthetic pathway for the preparation of Cu(II) complex using hydrazide ligand

Step 1: Preparation of p-Nitro Benzo Hydrazide

p-Nitro benzoic acid was converted into p-nitro benzoyl chloride by treatment with thionyl chloride under reflux conditions. The reaction was carried out in two batches using 6 g of p-nitro benzoic acid in each batch, giving a total of 12 g. Equimolar quantity i.e 5.2 ml of thionyl chloride was added gradually, and the reaction mixture was refluxed for approximately 2–3 hours under warm conditions until the evolution of gases ceased, indicating completion of the reaction. The obtained p-nitro benzoyl chloride was used for the subsequent reaction without further purification.

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The synthesized p-nitro benzoyl chloride was then reacted with hydrazine hydrate to obtain p-nitro benzoic hydrazide. An excess amount of hydrazine hydrate was added slowly with continuous stirring, and the reaction mixture was heated gently on a heating mantle for about 1 hour. Formation of a yellow coloured product indicated successful conversion into the hydrazide derivative.

Step 2: Preparation of Schiff Base (using p-Chloro Benzaldehyde)

Schiff base formation was carried out by condensation of p-nitro benzoic hydrazide with benzaldehyde in the presence of glacial acetic acid as catalyst. p-Nitro benzoic hydrazide (2 g, 0.011 mol) was dissolved in ethanol, followed by the addition of equimolar benzaldehyde (1.12 mL, 0.011 mol). A few drops of glacial acetic acid were added to facilitate the condensation reaction. The reaction mixture was heated gently with continuous stirring for about 30–45 minutes.

Formation of the Schiff base was indicated by the appearance of a yellow-coloured product. The obtained product was collected and combined from multiple reaction batches to obtain sufficient quantity of the Schiff base derivative. The reaction involves formation of an azomethine (–C=N–) linkage through condensation between the amino group of the hydrazide and the carbonyl group of benzaldehyde.

Step 3: Preparation of Another Schiff Base (using Benzaldehyde)

The Schiff base derivative was synthesized by condensation of p-nitro benzoic acid hydrazide with p-chlorobenzaldehyde in ethanolic medium using glacial acetic acid as catalyst. p-Nitro benzoic acid hydrazide (6 g, 0.033 mol) was dissolved in ethanol and subjected to continuous stirring in a round bottom flask. Equimolar quantity of p-chlorobenzaldehyde (4.64 g, 0.033 mol), dissolved in a small quantity of ethanol, was added slowly to the reaction mixture through a burette under constant stirring conditions.

A few drops of glacial acetic acid were added to catalyze the condensation reaction. The reaction mixture was stirred and heated gently for about 1–2 hours, resulting in the formation of the corresponding Schiff base derivative. Formation of the product was indicated by development of a coloured precipitate. The obtained product was filtered, washed with cold ethanol and dried.

Step 4: Preparation of 2-(4-nitrophenyl)-5-(4-chlorophenyl)-1,3,4-oxadiazole

The oxadiazole derivative was synthesized by cyclization of the Schiff base derived from p-chlorobenzaldehyde using phosphoryl chloride ($POCl_3$) as the cyclizing agent. The synthesized Schiff base (6 g) was taken in a round bottom flask and subjected to continuous stirring under slightly warm conditions. Phosphoryl chloride was added slowly through a burette over a period of time in order to maintain controlled reaction conditions.

The reaction mixture was then refluxed for approximately 3 hours. During the course of the reaction, the yellow needle-shaped crystalline Schiff base gradually transformed into a brown coloured precipitate, indicating formation of the oxadiazole derivative through cyclization. After completion of the reaction, excess $POCl_3$ was carefully quenched using ice-cold water, resulting in evolution of HCl fumes.

The obtained precipitate was neutralized using sodium bicarbonate solution. Neutralization of the reaction mixture was monitored using litmus paper until neutral pH was attained. The precipitate was subsequently filtered, washed thoroughly with cold water to remove impurities and dried to obtain the final oxadiazole derivative.

Step 5: Preparation of Cu(II) Complex

A Cu (II) complex of p-nitro benzoic acid hydrazide was synthesized using copper sulphate pentahydrate as the metal precursor. p-Nitro benzoic acid hydrazide (2 g, 0.011 mol) was dissolved in ethanol, while copper sulphate pentahydrate (1.37 g, 0.0055 mol) was dissolved separately in distilled water. The aqueous copper sulphate solution was added slowly to the ethanolic ligand solution under constant stirring conditions.

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The reaction mixture was stirred continuously for about 1 hour, resulting in formation of the Cu(II) complex as a coloured precipitate. Formation of the complex was indicated by change in colour of the reaction mixture due to coordination between Cu(II) ion and the hydrazide ligand. The obtained precipitate was filtered, washed thoroughly with distilled water to remove unreacted impurities and dried.

Protein Preparation

The three-dimensional crystal structure of DNA gyrase was retrieved from the RCSB Protein Data Bank with PDB ID: 5BS8, corresponding to DNA gyrase from *Mycobacterium tuberculosis* H37Rv.

The protein structure was prepared for molecular docking using UCSF ChimeraX. Water molecules, heteroatoms, and co-crystallized ligands present in the protein structure were removed to obtain a clean protein model. Unwanted chains were deleted and polar hydrogen atoms were added to stabilize the protein structure prior to docking analysis. The cleaned protein was then saved in the appropriate format for further molecular docking studies.

Ligand Preparation

The synthesized compounds, namely the p-nitrobenzohydrazide-derived Schiff base, 1,3,4-oxadiazole derivative, and Cu(II) complex, were selected as ligands for the molecular docking study. The chemical structures of the compounds were drawn using Avogadro software and subjected to geometry optimization to obtain stable conformations. The optimized structures were saved in PDB format and subsequently converted into PDBQT format using the appropriate docking tools.

Prior to docking, the ligands were prepared by assigning proper atom types, adding hydrogen atoms, and defining rotatable bonds where applicable. The prepared ligand structures were then used for docking against the active site of DNA gyrase (PDB ID: 5BS8) to evaluate their binding affinities and interaction patterns.

Molecular Docking Procedure

Molecular docking studies were performed using PyRx employing the AutoDock Vina algorithm to evaluate the binding interactions between the synthesized Schiff base, 1,3,4-oxadiazole derivative, Cu(II) complex and the target protein, DNA gyrase.

The prepared protein and optimized ligand were imported into the PyRx workspace for docking analysis. Blind docking was carried out by enlarging the grid box to encompass the entire protein structure, allowing the ligand to explore all possible binding regions within the target protein. The docking simulation generated multiple binding conformations with different binding affinities. The docked complexes obtained from AutoDock Vina were analyzed based on binding energy values and intermolecular interactions. The best docking pose exhibiting the lowest binding energy was selected for further visualization and interaction studies.

RESULTS AND DISCUSSION

Table-1: Elemental analysis, yield and physical constant of synthesized products

Sl.No.	Compound name	C (%) T(E)	H (%) T(E)	O(%) T(E)	N(%) T(E)	Practical yield	Percentage yield
1	p-nitro benzoic hydrazide $C_7H_7N_3O_3$	50.3	3.0	38.2	8.4	10.14g	78%
2	Schiff Base of Benzaldehyde $C_{13}H_{11}N$	86.2	6.12	0	7.73	2.23g	75%
3	Schiff Base of p-Chlorobenzaldehyde $C_{13}H_{10}ClN$	72.39	4.67	0	6.49	8.13g	80.8%
4	Oxadiazole Derivative $C_{14}H_8ClN_3O_3$	55.73	2.67	15.91	13.93	4.09g	72.2%
5	Cu (II) Complex	--	--	--	--	5.0 g	85 %

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Spectral Analysis

FTIR Analysis of Schiff Base Derived from Benzaldehyde

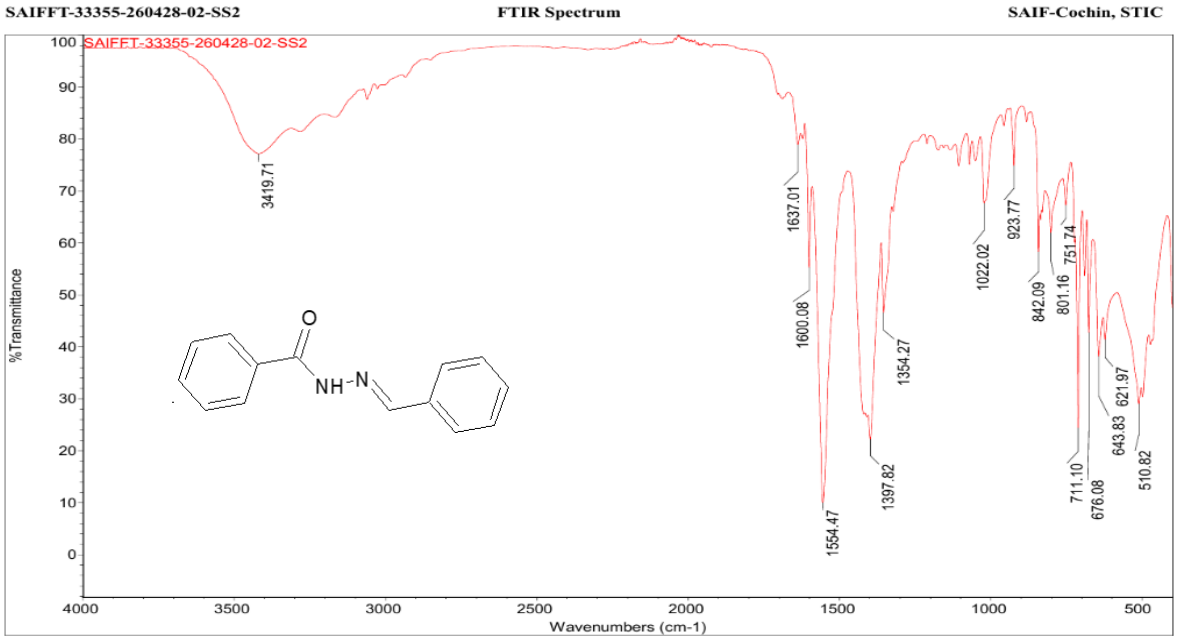


Figure 1.1: FTIR spectrum of Schiff base derived from benzaldehyde

Table 2: IR interpretation table of Schiff Base Derived from Benzaldehyde

Sl. no.	Peak (cm^{-1})	Functional Group	Assignment
1	3419.71	N-H	N-H stretching
2	1637.01	C=N	Azomethine stretching
3	1600.08	C=C	Aromatic stretching
4	1554.47	NO_2	Asymmetric stretching
5	1354.27	NO_2	Symmetric stretching
6	842.09	Ar-H	Aromatic C-H bending

FTIR Analysis of Oxadiazole Derivative

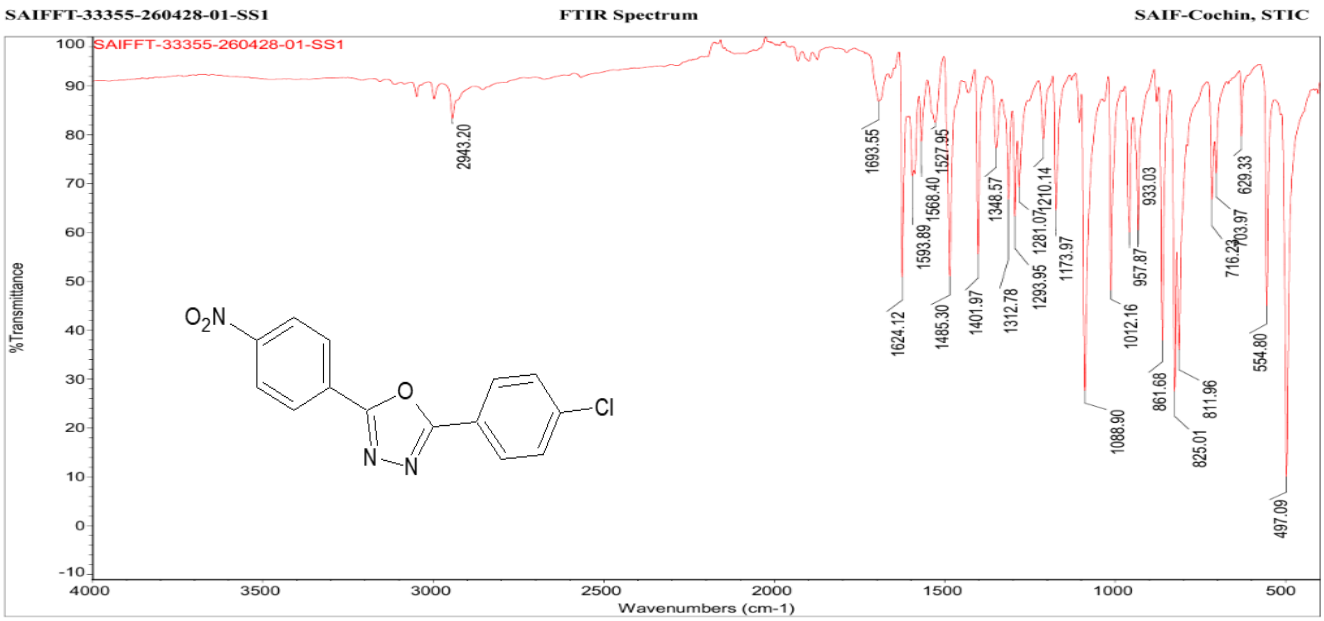


Figure 1.2: FTIR spectrum of synthesized oxadiazole derivative

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Table 3: IR interpretation table of Oxadiazole Derivative

Sl no	Peak (cm^{-1})	Functional Group	Assignment
1	2493.20	C-H	Aromatic/aliphatic C-H stretching
2	1693.55	C=N/C=O	Ring associated stretching vibration
3	1624.12	C=N	Oxadiazole ring stretching
4	1593.89	C=C	Aromatic stretching
5	1527.95	NO_2	Asymmetric stretching
6	1348.57	NO_2	Symmetric stretching
7	1281.07	C-O-C	Oxadiazole ring vibration
8	1088.90	C-N	Stretching vibration
9	825.01	Ar-H	Aromatic C-H bending

UV-Visible Analysis of Cu(II) Complex

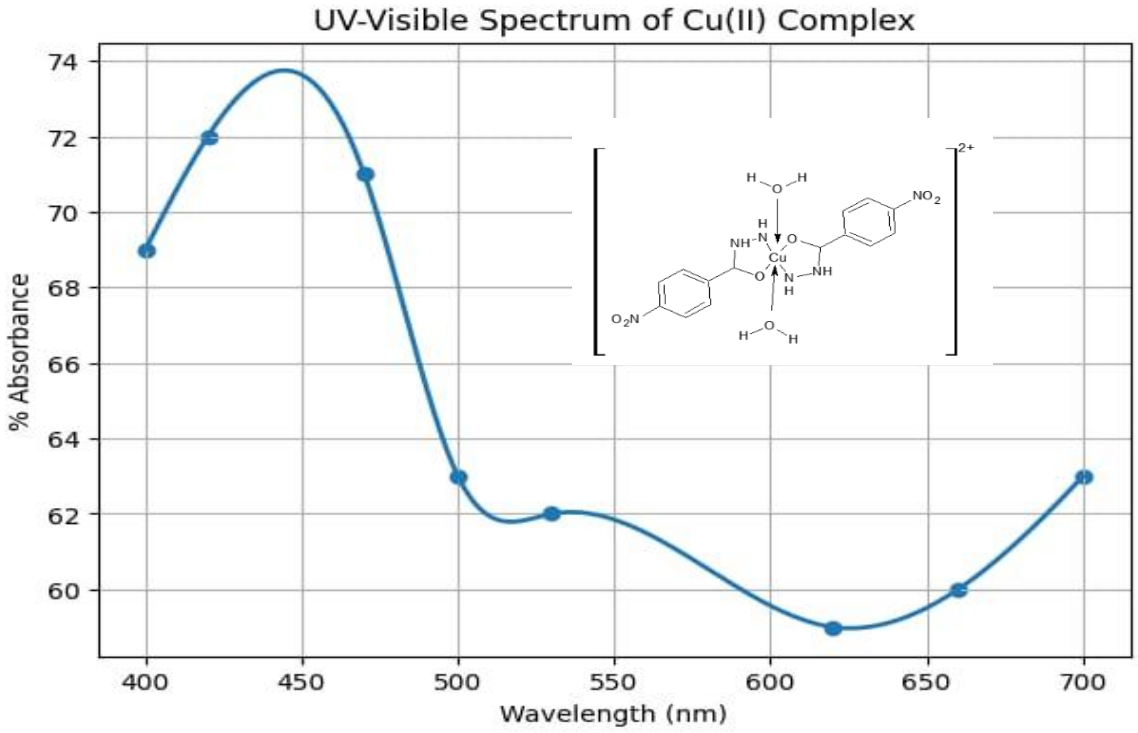


Figure 1.3: UV-Visible spectrum of synthesized Cu(II) complex

The absorption behaviour observed in the visible region indicates interaction between the metal ion and the ligand system through coordination. The presence of broad absorption bands is characteristic of Cu(II) complexes due to partially filled d-orbitals and ligand field interactions. Such broad transitions are commonly associated with distorted geometries exhibited by Cu(II) coordination compounds.

The shoulder peak observed in the spectrum may be attributed to overlapping electronic transitions occurring within the coordination environment of the metal center. In Cu(II) complexes, Jahn–Teller distortion often leads to splitting of energy levels, resulting in broad or shoulder-type absorption bands instead of sharp peaks.

The observed spectral pattern supports the proposed octahedral geometry of the synthesized Cu(II) complex. Coordination of ligand donor atoms with the Cu(II) ion modifies the electronic environment around the metal center, thereby influencing the electronic transition behaviour observed in the UV-Visible spectrum.

UV-Visible spectroscopy therefore served as an important analytical tool for confirming complex formation and studying the electronic properties of the synthesized coordination compound. The obtained spectral features were found to be consistent with reported Cu(II) hydrazide-based coordination complexes.

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Interaction Analysis

The molecular docking study revealed favorable interactions between the synthesized Schiff base, 1,3,4-oxadiazole derivative and Cu(II) complex with the target protein DNA gyrase. Docking simulations were carried out to evaluate the binding affinities and interaction patterns of the synthesized compounds within the active site of the protein.

Among the investigated compounds, the Schiff base exhibited a binding affinity of −7.2 kcal/mol, while the 1,3,4-oxadiazole derivative showed a slightly improved binding affinity of −7.5 kcal/mol. The Cu(II) complex displayed the highest binding affinity of −8.1 kcal/mol, indicating stronger predicted ligand–protein interactions compared to the other synthesized compounds.

Visualization of the docked complexes using UCSF ChimeraX confirmed proper accommodation of all compounds within the binding pocket of DNA gyrase. The stabilization of the docked complexes may be attributed to hydrogen bonding, hydrophobic interactions, van der Waals forces and other non-covalent interactions between the ligands and amino acid residues present in the active site region.

The comparative docking results suggest that structural modification of the Schiff base into the oxadiazole derivative and subsequent complexation with Cu(II) enhanced the binding affinity towards DNA gyrase. Among the synthesized compounds, the Cu(II) complex exhibited the most favorable interaction profile and may possess improved antibacterial potential, warranting further investigation.

Table 4-Protein binding affinity values of synthesized compounds against DNA gyrase (PDB ID: 5BS8)

Compound	**Binding Affinity (kcal/mol)**
Schiff Base	-7.2
1,3,4-Oxadiazole Derivative	-7.5
Cu(II) Complex	-8.1

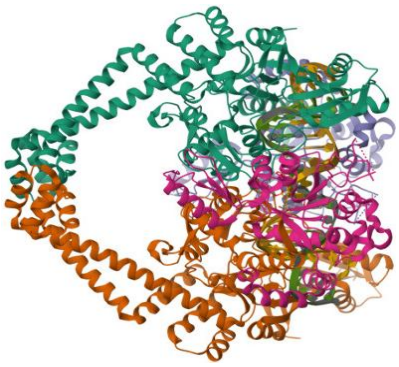


Fig 1.4: Overall ribbon representation of DNA gyrase showing the ligand binding pocket.

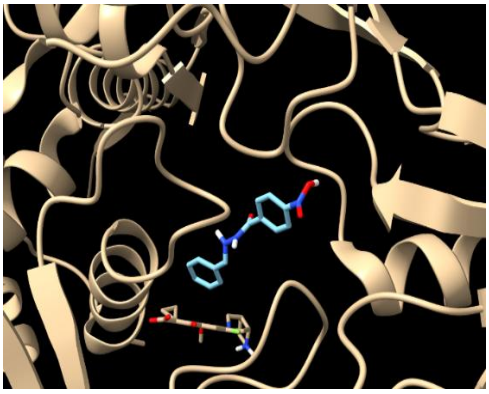


Fig 1.5: Schiff Base – Close-up ribbon view.

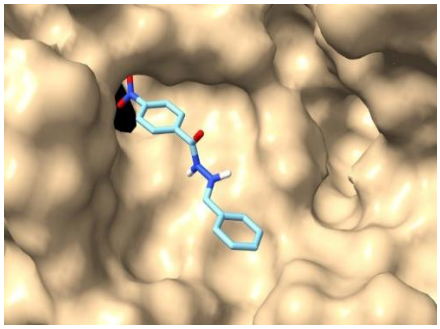


Fig 1.6: Schiff Base – Close-up surface view.

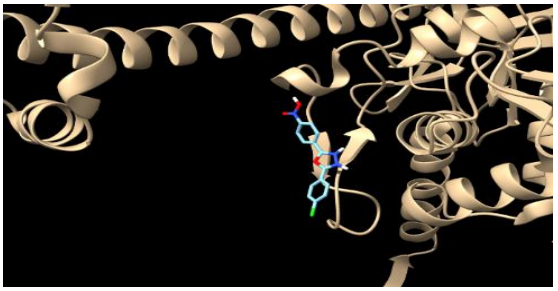


Fig 1.7: 1,3,4-Oxadiazole Derivative – Close-up ribbon view.

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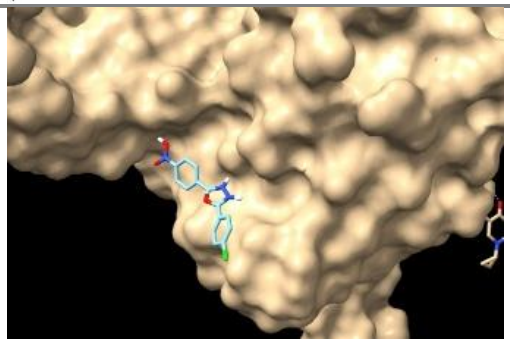


Fig 1.8: 1,3,4-Oxadiazole Derivative – Close-up surface view

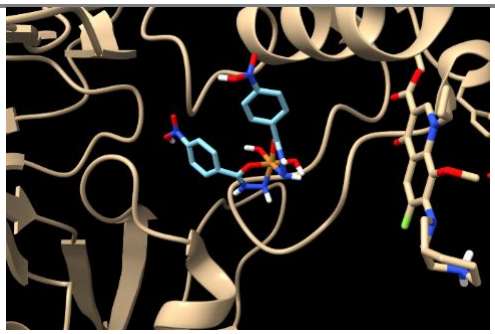


Fig 1.9: Cu(II) Complex – Close-up ribbon view.

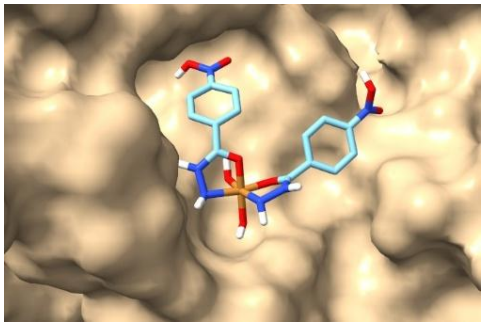


Fig 1.10: Cu(II) Complex – Close-up surface view.

CONCLUSION

The present study successfully achieved the synthesis, characterization and in silico antibacterial evaluation of p-nitrobenzohydrazide-derived compounds. p-Nitrobenzohydrazide was synthesized and subsequently converted into Schiff base derivatives through condensation reactions with aromatic aldehydes. Cyclization of the Schiff base led to the formation of a 1,3,4-oxadiazole derivative, which was further utilized for comparative studies. In addition, a Cu(II) complex of p-nitrobenzohydrazide was synthesized successfully.

The synthesized compounds were characterized using spectroscopic and analytical techniques including FT-IR, UV–Visible spectrophotometry and Thin Layer Chromatography (TLC). The spectral data confirmed the formation of the desired compounds and provided evidence for the proposed structures. The UV–Visible spectral studies and physicochemical observations further supported the successful synthesis of the Cu(II) complex.

Molecular docking studies against DNA gyrase (PDB ID: 5BS8) were carried out using PyRx integrated with AutoDock Vina. The Schiff base, 1,3,4-oxadiazole derivative and Cu(II) complex exhibited favorable binding affinities of −7.2 kcal/mol, −7.5 kcal/mol and −8.1 kcal/mol, respectively. Comparative analysis indicated that structural modification through cyclization and subsequent metal complexation enhanced the predicted interaction of the compounds with the target protein. Among the investigated compounds, the Cu(II) complex displayed the most favorable binding affinity toward DNA gyrase.

Overall, the results of the present investigation demonstrate that p-nitrobenzohydrazide-derived Schiff bases, 1,3,4-oxadiazole derivatives and their metal complexes represent promising scaffolds for further antibacterial research. The study provides a useful foundation for future biological evaluation and the development of novel antimicrobial agents.

REFERENCE

1 Blower, T. R., Williamson, B. H., Kerns, R. J., & Berger, J. M. (2016). Crystal structure and stability of gyrase–fluoroquinolone cleaved complexes from Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences, 113(6), 1706–1713.

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2 Gökçe, C., & Gup, R. (2013). Synthesis, characterization, and antimicrobial activity of new Cu(II) complexes with hydrazide derivatives. Journal of Coordination Chemistry, 66(14), 2530–2542.
3 Glomb, T., & Świątek, P. (2021). Antimicrobial and anti-inflammatory activity of 1,3,4-oxadiazole derivatives. Materials, 14(19), Article 5831.
4 Patel, N. B., & Patel, J. C. (2010). Synthesis and antimicrobial activity of novel 1,3,4-oxadiazole and Schiff base derivatives. Medicinal Chemistry Research, 19(9), 1141–1154.
5 Sharma, P., & Agarwal, S. (2023). Nitro- and chloro-substituted 1,3,4-oxadiazoles: Synthesis, spectral characterization, and enhanced antimicrobial evaluation. Journal of Molecular Structure, 1274, Article 134450.