Available free online at www.medjchem.com

Mediterranean Journal of Chemistry

Mediterranean Journal of Chemistry **2023**, 13(3), 248-262

Molecular Docking and Biological Activity of Pyridine and Thiosemicarbazide derived Schiff base Ligands

Karuna Chourasia [1,*], Himanshu Pandey [2], and Ritu Yadav [2]

[1] Department of Chemistry Dr. Harisingh Gour Vishwavidyalaya Sagar 470001 M.P. India
[2] Department of Chemistry Dr. Harisingh Gour Vishwavidyalaya Sagar 470001 M.P.

Abstract: Two Schiff base ligands KL-3 and KL-4 were synthesized using thiosemicarbazide with pyridine-4-carboxaldehyde and para-nitrobenzaldehyde, respectively. Further, the complexation of our synthesized Schiff bases with Co (II), Ni (II), Cu (II), and Zn (II) ions was carried out successfully. Our synthesized compounds KL-3 and KL-4 act as deprotonated tridentate ligands. Physical, spectral, and analytical data characterized Schiff bases and their complexes. Schiff bases and their complexes were screened for antibacterial activity against strains such as Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. The metal complexes were more potent in antibacterial activity against selected bacterial species than Schiff bases KL3 and KL4. Molecular docking studies of KL3 and KL4 were performed on protein chosen Penicillin-Binding Protein 6 (PBP6) from *E. coli*, Penicillin-Binding Protein 4 (PBP4) from *S. aureus* and Mycobacterium Tuberculosis Glutamine Synthetase protein (MTGS) from *Mycobacterium Tuberculosis*. Comparative analysis of docking scores with standard drugs showed good binding affinity in the active site of the selected protein. The molecular docking scores of the KL-3 and KL-4 Schiff bases confirm our in-vitro biological analysis results.

Keywords: Thiosemicarbazide; Pyridine; Molecular Docking; biologically active; Therapeutic agent; Protein Data Bank.

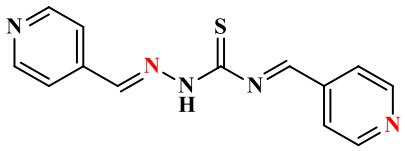
1. Introduction

During the year 1864, Hugo Schiff synthesized his first Schiff base. During the condensing reaction of the active carbonyl group with a primary amine compound, the Schiff base consists of functional carbonyl groups and imine compounds. The synthesis of a Schiff base was performed by Hugo Schiff in the year 1864. The synthesis of Schiff base was composed of an active carbonyl group and imine compounds formed by the condensation reaction of a primary amine compound. Antimicrobial activity is the foremost characteristic of Schiff bases [1]. Compounds based on Schiff bases that form complexes with metal ions act as chelating ligands [2,3]. Their work was combined with Dynamic Covalent Chemistry (DCC) of imine covalent bonds in metal-organic systems [4,5] By preparing certain chocolate-containing di-aldehydes [6,7], sulfur atoms have been successfully inserted into macrocyclic Schiff bases. Still, selectivity and center specificity must be considered when designing such ligands. Schiff base exhibits a wide range of pharmacological activities like antibacterial [8], antifungal [9], antioxidant activity [10], antitumor [11], and anti-inflammatory activity [12], etc. Schiff base metal complexes derived from transition metal elements [13] play crucial roles as bio-ligands in the biological system, and they have significant applications in the field of food chemistry, the dye industry, the field of catalysts [14-16], etc. Due to different ligand-metal interactions, their electronic properties change [17,18]. The change in their electronic properties via other ligands to metal signals from these sensors comes from solid interactions between metal ions and sample molecules [19-22]. Several variations of Schiff bases have been synthesized in the past. (L-M) and Metal to Ligand (M-L) (i.e., π–π*, n–π*) charge transfer processes produce the sensing signal [23-25]. Origen of signal from these sensors emerges a strong metal ion interaction with sample molecule [26,27]. Better solubility, lower cost, and lower cytotoxicity, and there have been many applications for them, including catalysis, enzymatic reactions, electronics, cosmetics, polymer manufacturing [28], luminescence materials, magnetism, and molecular design. Molecular Schiff bases represent a significant class of active molecules from a pharmacological perspective of luminescence materials, magnetism, and molecular design. Schiff bases (azomethine, –C=N–) show primarily antibacterial activity [29,30]. They are among the most efficient agents for increasing antimicrobial activities in many compounds and/or extending the spectrum of an antibiotic against a resistant bacterium to include a common bacterial pathogen [31-36]. Chemists have been interested in Schiff bases for decades because they represent an important class of active molecules in pharmacology [37]. Several studies underline the

*Corresponding author: Karuna Chourasia
Email address: Karuchem128@gmail.com
DOI: http://dx.doi.org/10.13171/mjc02309141705chourasia

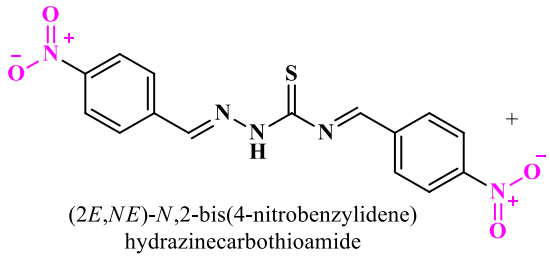
Received April 20, 2023
Accepted June 5, 2023
Published September 14, 2023

importance of Schiff bases as antimicrobial agents [38-40].



(2*E*,*NE*)-*N*,2-bis(pyridin-4-ylmethylene) hydrazinecarbothioamide

KL-3



(2*E*,*NE*)-*N*,2-bis(4-nitrobenzylidene) hydrazinecarbothioamide

KL-4

Figure 1. Structure of Antimicrobial Schiff bases

For the synthesis of bioactive intermediate compounds, thiosemicarbazides are often used [41-43]. The importance of carbazides, carbazones, and Thiosemicarbazone moieties plays a significant role in inhibiting these enzymes' thiosemicarbazide moieties capable of forming stable complexes with divalent metal ions through chelation. Despite their relatively short history, their transition metal complexes are being investigated for their potential to inhibit a range of pathogens, including flu, protozoa, variola (smallpox), and cancers [44-45] Due to these features, carbazides and thiosemicarbazide have become important topics of research for chemists and pharmacists. They attracted much attention. Their transition metal complexes have overgrown because of the discovery of their effects on flu, protozoa, variola (smallpox), certain types of tumors, and fungi [44-45]. Due to these features, carbazides and thiosemicarbazide have become noteworthy research topics for chemists and pharmacists. They attracted much attention, and the number of studies on these substances has increased rapidly.

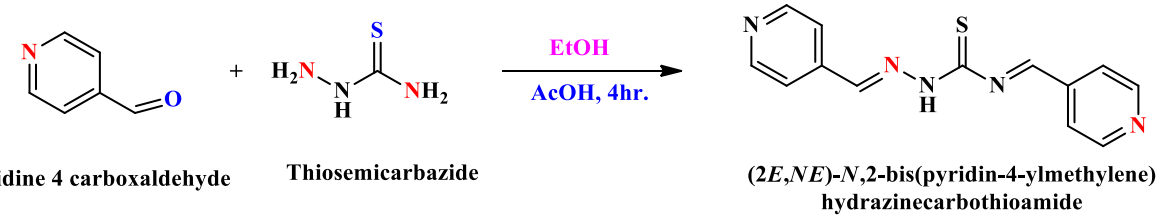
2-Experimental

2.1 Materials and Methods

Pyridine- 4 carboxaldehyde was purchased from Sigma Aldrich assay 97%, thiosemicarbazide (CH_5N_3S) assay 99%, and Merck TLC Silica Plates 20x20 cm^2 were purchased from Sigma-Aldrich. TLC paper, Acetic acid (CH_3COOH), Methanol (CH_3OH), and ethanol (CH_3CH_2OH) were purchased from HiMedia Laboratories Pvt. Ltd. Chemical solvents are used in analytical grades. All the salt and reactants were dried before use, and moisture was omitted from the glass apparatus using $CaCl_2$ drying tubes. The reagents and solvents which was used for synthesis were bought from Fluka, Loba-Chemie, Spectrochimica, etc., and these were used after further purification. Solvents are used in analytical grades. For the preparation of metal complexes of corresponding Schiff bases, the nitrate salts of metals were used to synthesize metal complexes. Silica-coated aluminum TLC plates determined the products' purity and reactions monitored under the U.V chamber. Open capillaries were used to determine the melting points of all compounds. A Bruker FT-IR spectrophotometer was used to record the spectra of all synthesized compounds. A Thermos Scientific Spectrophotometer (Multiskan Go) was used to measure the UV/VIS spectra of synthesized Schiff bases and their metal complexes under 200-800 nm wavelength using DMSO solventProton NMR [^{1}HNMR] spectra were recorded in DMSO-d_6 on a JEOL Delta-550 spectrometer (400 MHz). Mass spectra were learned on Bruker Compass Data Analysis 4.0.

2.2-General synthetic procedure for ligand (KL-3)

The Schiff base was synthesized by the reported method [46-50]. by condensing pyridine- 4 carboxaldehyde and thiosemicarbazide **(**2.0mmol) in ethanol (10ml) with thiosemicarbazide (1mmol) in ethanol (10mL) dissolved separately an aldehyde was added drop by drop to the compound (amine) under stirring. The resulting mixture was mechanically stirred for 30 minutes at room temp. Further, a few drops of dilute acetic acid were added to the reaction mixture, followed by refluxing at 60-650°C. Silica-coated aluminum TLC plates monitored the progress of the reaction under the UV chamber. Hexane and ethyl acetate were taken (7:3v/v) as eluent.



Pyridine 4 carboxaldehyde **Thiosemicarbazide** **(2*E*,*NE*)-*N*,2-bis(pyridin-4-ylmethylene) hydrazinecarbothioamide**

Scheme 1. Synthesis of KL-3

2.2.1-Synthesis of (2E, NE)-N,2-bis(pyridine-4-ylmethylene)hydrazinecarbothioamide-[KL-3]- *Pyridine -4 Carboxaldehyde* derivatives, In 10 mL of ethanol, derived (1mmol) and thiosemicarbazide

(1mmol) were dissolved separately, and aldehydes were added, stirring. The resulting mixture was stirred for 30 minutes at rt. A few drops of dilute acetic acid were added to the reaction mixture, and the mixture was refluxed at 60-65 °C for 5-7 hr, and TLC monitored the completion of the reaction. Cooling the reaction mixture afforded precipitation, which was filtered, washed with DCM methanol (2:1), and dried under a vacuum. Dark orange amorphous powder; Yield: 0.572g (81**%**).; mp. 156 °C.

FTIR (selected vibrations; cm^{-1}); 1648 cm^{-1} (C=N),1582 cm^{-1} (CNO_2), 3257 cm^{-1}, 3493 cm^{-1} (N-H),

^{1}HNMR Spectra of KL-3 ^{1}HNMR (DMSO- d_6, δ, ppm); 9.9 (d, 1H, J = 9.0 Hz), 8.88 (d, 1H, J = 2.3 Hz), 8.069, 8.24 (m, 2H, CH), 8.06 (m, 1H), 7.0 (NH), 7.72 (m, 1H), 7.4 (m, 1H); (CDCl3, 400MHz)/∂ ppm as shown in Figures 2, 3 and 4.

Chemical Formula: $C_{13}H_{11}N_5S$ Exact Mass: 269.0735 Molecular Weight: 269.3249m/z: 269.0735 (100.0%), shown in Table 1.

Elemental: C, 57.97; H, 4.12; N, 26.00; S, 11.9.

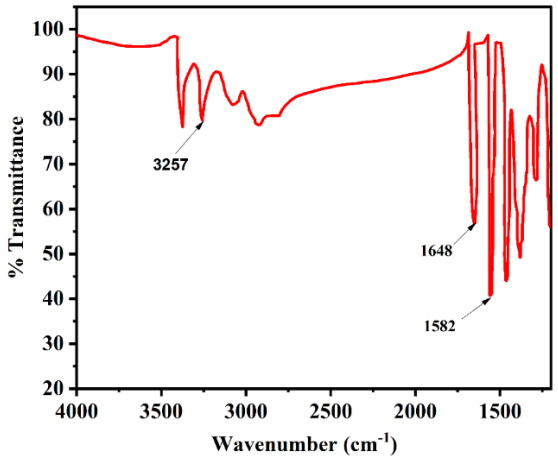


Figure 2. IR Spectra of KL-3

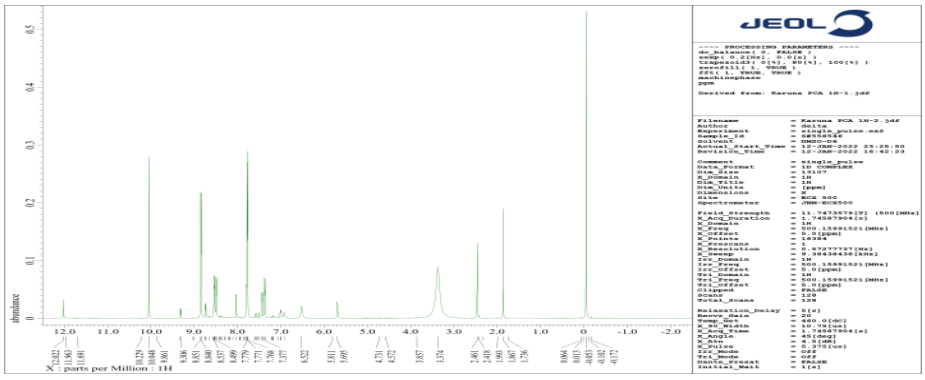


Figure 3. NMR spectra of KL-3

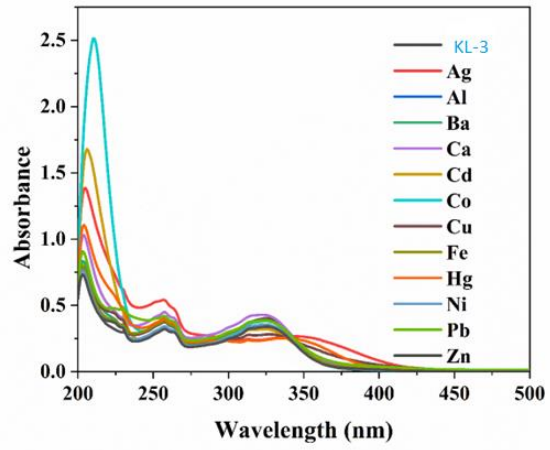


Figure 4. U.V. Visible spectra of KL-3

2.2.2. General synthetic procedure for [M(L-1)2]: Complexes of KL-3-have been synthesized using metal(II) nitrates. To a stirring methanolic solution of KL-3and KL-4 (1.0 mmol), 0.282g/0.327g) and KOH (1.0 mmol, 0.056g), a methanolic solution of metal(II) nitrates (1.0 mmol) were added dropwise in a methanolic solution of KL-3 and KL-4 respectively at room temperature. The resulting reaction mixture was further stirred for 30 minutes. Further, The reaction mixture was refluxed at 60-70 °C for 2-4 h, and TLC monitored the completion of the reaction. Cooling the reaction content resulted in a precipitate, which was filtered, washed with methanol (5 mL) followed by diethyl ether (3 × 5 mL), and dried in a vacuum.

2.2.3. Synthesis of (2E, NE)-N,2bis(pyridine-4-ylmethylene)hydrazinecarbothioamide, cobalt(II) salt-The reaction was carried out following general procedure by using KL-3 (1.0 mmol)and $Co(NO_3)_2$ (1.0 mmol) as metal (II) nitrate; Light pink, yield 1.145 g (81 %); FTIR (cm^{-1}): 1689 (C=N), 739 (C-H); Chemical Formula: $C_{13}H_{11}CoN_5S^{2+}$Exact Mass: 328.0056Molecular Weight: 328.2570 Elemental Analysis: C, 47.57; H, 3.38; Co, 17.95; N, 21.33; S, 9.77 Calculated:: C, 47.47; H, 3.32; Co, 17.91; N, 21.30; S, 9.74m/z: 164.0028 (100.0%), 164.5045 (14.1%), 165.0007 (4.5%), 164.5014 (1.8%).

2.2.4. Synthesis of (2E, NE)-N,2-bis(pyridine-4-ylmethylene)hydrazinecarbothioamide, copper(II) salt-The reaction was carried out following general procedure by using KL-3 (1.0 mmol)and $Cu(NO_3)_2$ (1.0 mmol) as metal (II) nitrate; Light green, yield 1.14g (81 %); Chemical Formula: $C_{13}H_{11}CuN_5S^{2+}$ Exact Mass: 332.0020Molecular Weight: 332.8698 m/z: 166.0010 (100.0%), 167.0001 (44.6%), 166.5027 (14.1%), 167.5018 (6.3%), 166.9989 (4.5%), 167.9980 (2.0%), 166.4996 (1.8%).
Elemental Analysis: C, 46.91; H, 3.33; Cu, 19.09; N, 21.04; S, 9.63 Calculated: C, 46.89; H, 3.31; Cu, 19.00; N, 21.00; S, 9.45.

2.2.5. Synthesis of (2E, NE)-N,2-bis(pyridine-4-ylmethylene) hydrazinecarbothioamide, nickel (II) salt-The reaction was carried out following general procedure by using KL-3 (1.0 mmol)and $Ni(NO_3)_2$ (1.0 mmol) as metal (II) nitrate; Dark green, yield (80%).
Chemical Formula: $C_{13}H_{11}N_5NiS^{2+}$ Exact Mass: 327.0078 Molecular Weight: 328.0172 m/z: 163.5039 (100.0%), 164.5016 (38.5%), 164.0056 (14.1%), 165.0033 (5.4%), 165.5004 (5.3%), 164.5018 (4.5%), 164.0024 (1.8%), 165.4995 (1.7%), 165.0018 (1.7%), 166.5002 (1.4%).
Elemental Analysis: C, 47.60; H, 3.38; N, 21.35; Ni, 17.89; S, 9.78 Calculated, 47.57; H, 3.37; N, 21.32; Ni, 17.82; S, 9.75.

2.2.6. Synthesis of (2E, NE)-N,2-bis(pyridine-4-ylmethylene)hydrazinecarbothioamide, zinc(II) salt -The reaction was carried out following general procedure by using KL-3 (1.0 mmol)and $Zn(NO_3)_2$ (1.0 mmol) as metal (II) nitrate; Dark green, yield **(**78 %);
Chemical Formula: $C_{13}H_{11}N_5SZn^{2+}$ Exact Mass: 333.0016 Molecular Weight: 334.7038 m/z: 166.5008 (100.0%), 167.4993 (57.4%), 168.4987 (38.6%), 167.0025 (14.1%), 167.9998(8.4%), 168.0009 (8.1%), 169.0003 (5.4%), 167.4987 (4.5%), 168.4971 (2.6%), 166.9993.

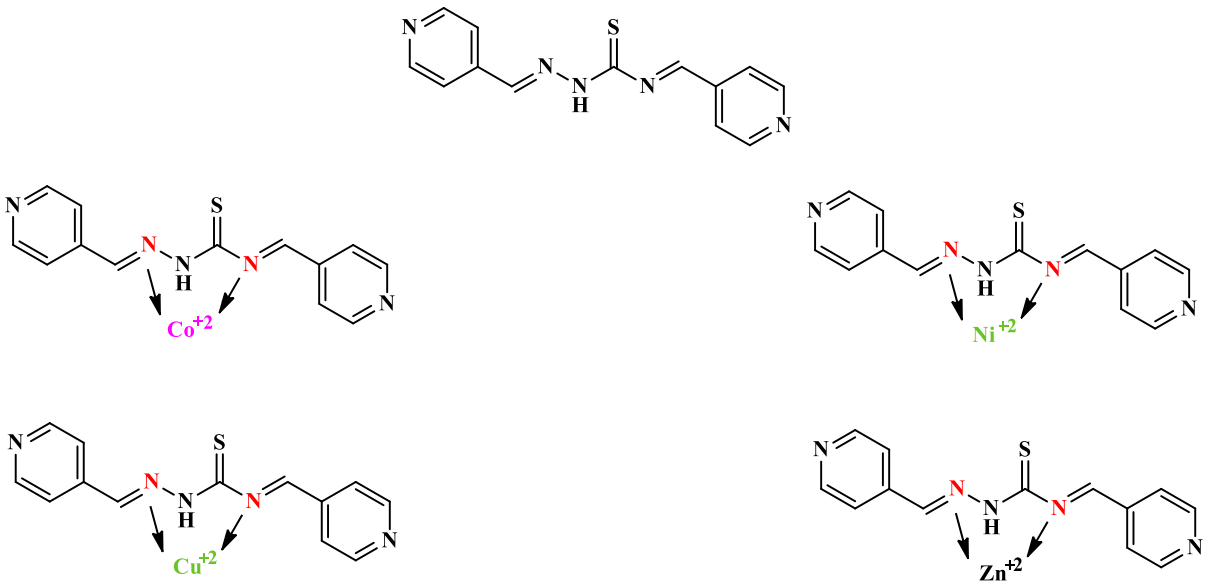


Figure 5. Proposed structures of KL-3 ligands and their metal complexes

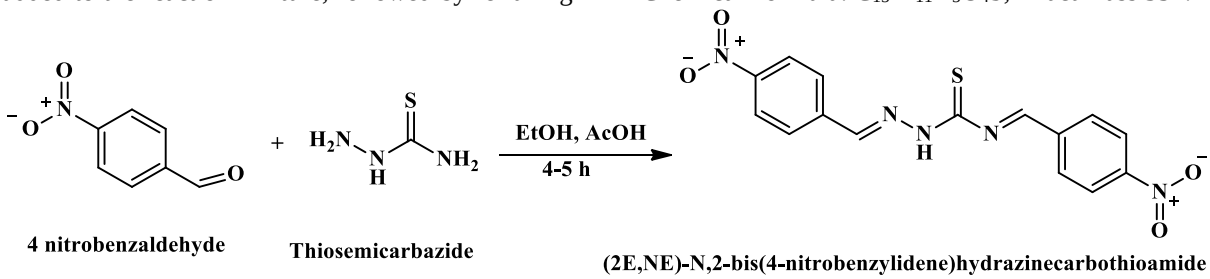
3. General synthetic

procedure for ligand (KL-4)- The Schiff base was synthesized by the reported procedure [47-50] by condensing pyridine- 4 carboxaldehyde **(**2.0mmol) in ethanol (10 ml) with thiosemicarbazide (1mmol) in ethanol (10 ml) were dissolved separately, and aldehyde was added to the amines under stirring. The resulting mixture was stirred for 30 minutes at room temp. Further, a few drops of dilute acetic acid were added to the reaction mixture, followed by refluxing at 70-75°C. Hexane and ethyl acetate were taken (7:3v/v) as eluent.

FTIR (selected vibrations; cm^{-1}); 1670 cm^{-1} (C=N),1520 cm^{-1} (C-NO_2), 2982 cm^{-1}, 3493 cm^{-1}, (N-H) as shown in Figure 6.

^{1}H NMR Spectra of KL-4 ^{1}HNMR (DMSO- d_6, δ, ppm); 9.9 (d, 1H, J = 9.0 Hz), 8.88 (d, 1H, J = 2.3 Hz), 8.069, 8.24 (m, 2H CH), 8.06 (m, 1H), 7.0 (NH), 7.72 (m, 1H), 7.4 (m, 1H); (CDCl3, 400MHz)/∂ ppm as shown in Figure 7.

Chemical Formula: C_{15} $H_{11}N_5O_4S$, Exact mass 357.



Scheme 2. IR Spectra of KL-4

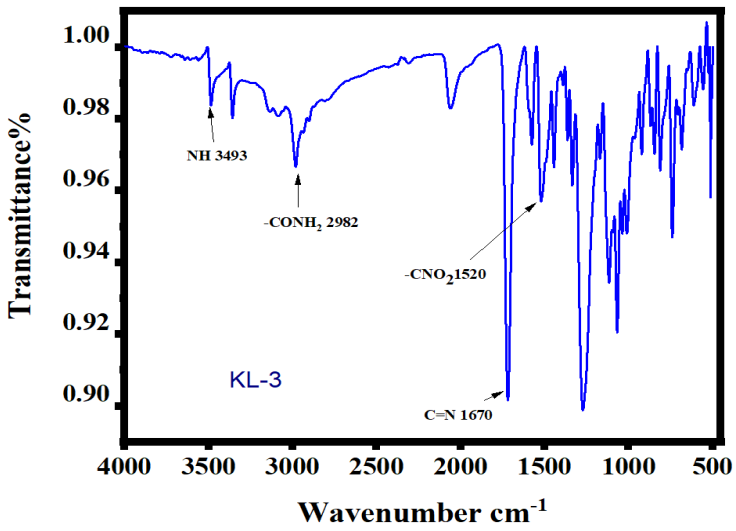


Figure 6. NMR Spectra of KL-4

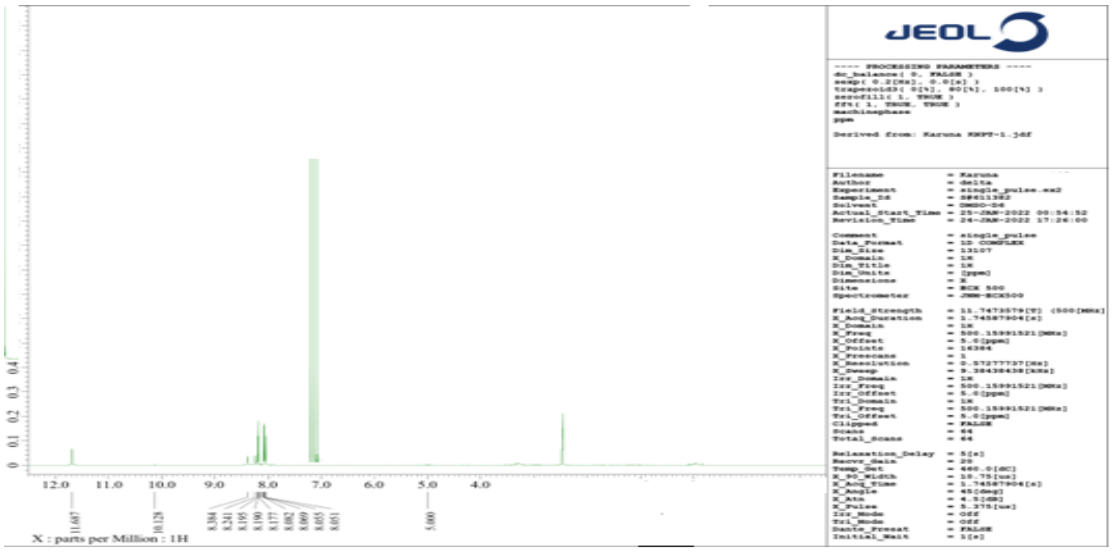


Figure 7. NMR Spectra of KL-4

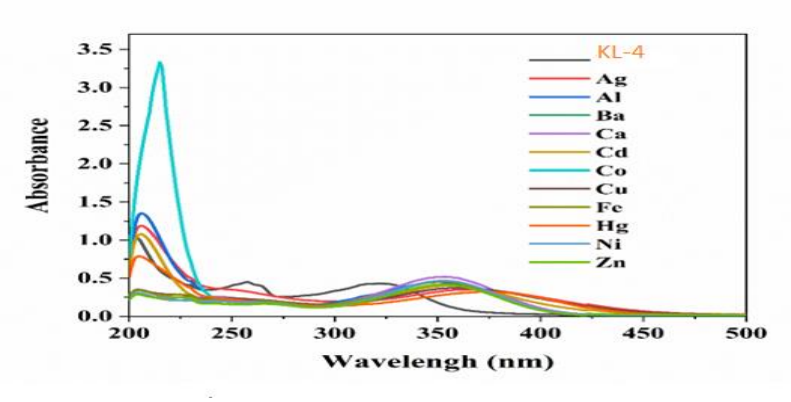
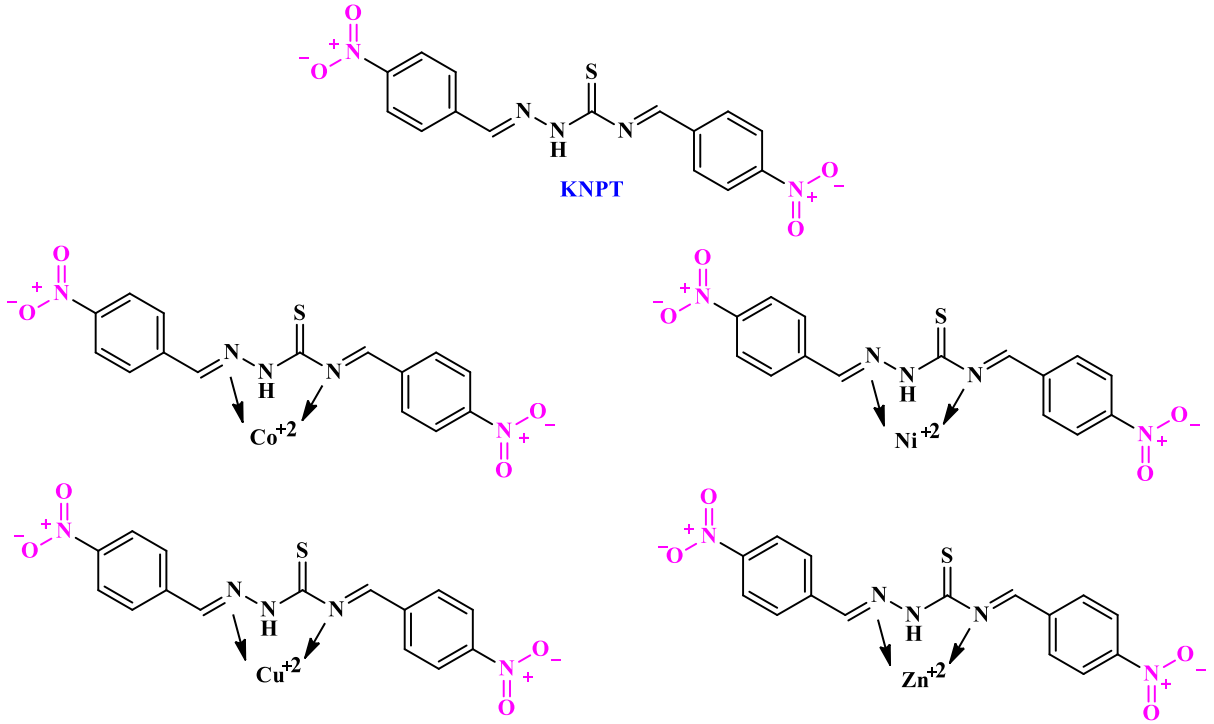


Figure 8. UV/VIS spectra of the compound KL-4 and its metal complexes



Proposed structures of ligand KL-4 and their metal complexes

3.1-Synthesis of (2E, NE)-N,2-bis(4-nitro benzylidene) hydrazinecarbothioamide, cobalt (II) salt-The reaction was carried out following general procedure by using KL-4 (1.0 mmol) and$(NO_3)_2$ (1.0 mmol) as metal (II) nitrate; pink, yield **(**78 %**)**; Chemical Formula: $C_{15}H_{11}CoN_5O_4S^{2+}$ Exact Mass: 415.9853 Molecular Weight: 416.2760 m/z: 207.9927 (100.0%), 208.4943 (16.2%), 208.9906 (4.5%), 208.4912 (1.8%), 208.9960 (1.2%)
Elemental Analysis: C- 43.28; H, 2.66; Co, 14.16; N, 16.82; O, 15.37; S, 7.70, Calculated: C- 43.27; H, 2.64; Co, 14.16; N, 16.80 2; O, 15.37; S, 7.70.

3.2-Synthesis of (2E, NE)-N,2-bis(4-nitro benzylidene) hydrazinecarbothioamide, nickel (II) salt--The reaction was carried out following general procedure by using KL-4 (1.0 mmol)and $Co(NO_3)_2$ (1.0 mmol) as metal (II) nitrate; Dark green, yield (83%); 1): Chemical Formula: $C_{15}H_{11}N_5NiO_4S^{2+}$ Exact Mass: 414.9874Molecular Weight: 416.0362m/z: 207.4937 (100.0%), 208.4915 (38.5%), 207.9954 (16.2%), 208.9931 (6.2%), 209.4902 (5.3%), 208.4916 (4.5%), 207.9923 (1.8%), 209.4894 (1.7%), 208.9916 (1.7%), 210.4900 (1.4%), 208.4971 (1.2%).
Elemental Analysis: C, 43.30; H, 2.66; N, 16.83; Ni, 14.11; O, 15.38; S, 7.71Calculated: C, 43.30; H, 2.66; N, 16.83; Ni, 14.11; O, 15.38; S, 7.71.

3.4-(2E, NE)-N,2-bis(4-nitrobenzylidene) hydrazinecarbothioamide, copper (II) salt
The reaction was carried out following general procedure by using KL-4 (1.0 mmol) and $(NO_3)_2$ (1.0 mmol) as metal (II) nitrate; green, yield (81 %); FTIR (cm^{-1}): Chemical Formula: $C_{15}H_{11}CuN_5O_4S^{2+}$, Exact Mass: 419.9817Molecular Weight:

420.8888m/z: 209.9909 (100.0%), 210.9900 (44.6%), 210.4925 (16.2%), 211.4916 (7.2%), 210.9888 (4.5%), 211.9879 (2.0%), 210.4894 (1.8%), 210.9942 (1.2%).
Elemental Analysis: C, 42.80; H, 2.63; Cu, 15.10; N, 16.64; O, 15.21; S, 7.62.

3.5-(2E,NE)-N,2-bis(4-nitrobenzylidene) hydrazinecarbothioamide Zinc (II) salt
The reaction was carried out following general procedure by using KL-4 (1.0 mmol) and Cu $(NO_3)_2$ (1.0 mmol) as metal (II) nitrate; White, yield (68%);): Chemical Formula: $C_{15}H_{11}N_5O_4SZn^{2+}$Exact Mass: 420.9812Molecular Weight: 422.7228m/z: 210.4906 (100.0%), 211.4891 (57.4%), 212.4885 (38.6%), 210.9923 (16.2%), 211.9908 (9.3%), 211.9896 (8.4%), 212.9902 (6.3%), 211.4885 (4.5%), 212.4870 (2.6%), 210.9892 (1.8%), 213.4864 (1.7%), 212.4913 (1.4%), 213.4887 (1.3%), 211.4940 (1.2%), 211.9876 (1.1%)
Elemental Analysis: C, 42.62; H, 2.62; N, 16.57; O, 15.14; S, 7.59; Zn, 15.47

Table 1. The ESI-MS spectral data of ligand KL-3, KL-4, and metal complexes.

S.N.	SAMPLE	*m/z*[M+]$^+$	*m/z*[M+1] $^+$
1.	**KL-3**	269	270
2.	**KL-4**	357	358.00
3.	**KL-3 Zn**	333	334
4.	**KL-4 Cu**	209	210.

4. Molecular docking study of the synthesized compounds.
In order to predict the interaction of our synthesized compounds with selected macromolecular targets, AutoDock Vina software is used. The motivation of our molecular docking analysis is to dock our synthesized compound in the binding site of the chosen protein Penicillin-Binding Protein 6 (PBP6) from *E. coli*, Penicillin-Binding Protein 4 (PBP4) from *S. aureus* and Mycobacterium Tuberculosis Glutamine Synthetase protein from *Mycobacterium Tuberculosis* and compared the docking scores with standard drugs used against the selected pathogen. Auto Dock Vina is an automated module virtual software that offers a faster search method and scoring function. It provides reproducible results with upwards of 20 flexible bonds for larger systems [51-55] PYRAZINAMIDE(Z) and CIPROFLOXACIN are used as standard Drugs. As shown in Table 2.

4.1. Selection of the Protein

We have selected three types of protein for our study, obtained from the Protein Data Bank.

4.1.1. Crystal structure of Penicillin-Binding Protein 6 (PBP6) from *E. Coli* **[PDB ID: 3ITA], Figure 9.**

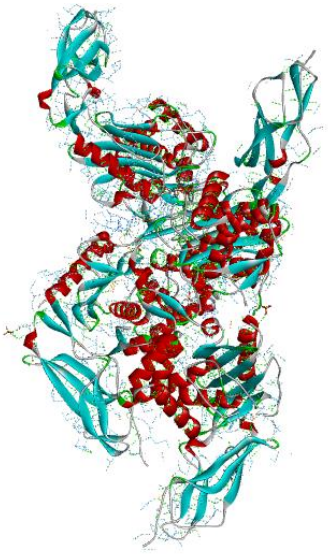


Figure 9. Structure of Protein [PDB ID: 3ITA]

4.1.2. Crystal Structure of Penicillin-Binding Protein **4** (PBP**4**) from ***Staphylococcus Aureus*** **[PDB ID: 3HUN], Figure 10.**

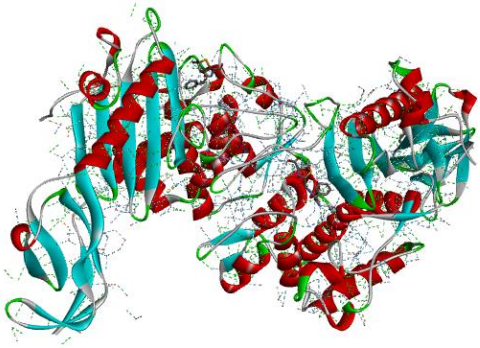


Figure 10. Structure of Protein [PDB ID: 3HUN]

4.1.3. Crystal structure of Mycobacterium Glutamine Synthetase from *Mycobacterium Tuberculosis* **[PDB ID: 3ZXR].**

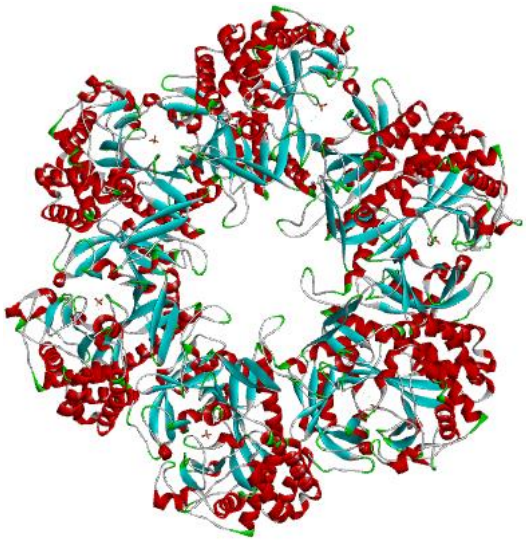


Figure 11. Structure of Protein **[PDB ID: 3ZXR]**

Ligands

We have selected our synthesized KL3 and KL4 ligands for the molecular docking analysis. The structure was prepared with Chembiodraw 3D Ultra 12, and energy was minimized.

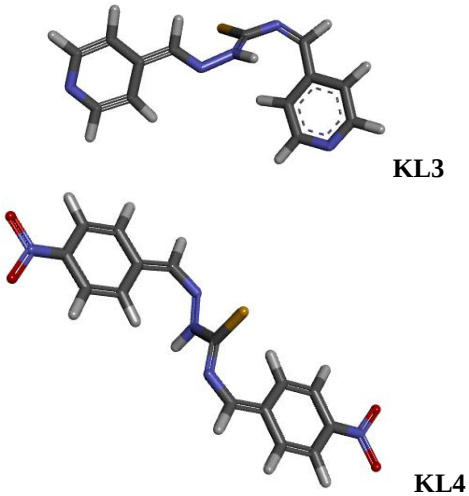


Figure 12. KL3 and KL4 structure

4.2. Results of Molecular Docking-

Our ligand KL-4 exhibited excellent docking results with Penicillin-Binding Protein 6 (PBP6) from *E. coli*. The 2D binding pose of the KL-4 ligand shows five conventional hydrogen bonds with SER10, SER94, LYS250, GLN92, and ASP91, as highlighted by green colored bond. These are very crucial for a prominent docking score. Pi-pi T-shaped interaction with TRP254. Van der waals interaction with ASN187 and GLU7. Pi-anion interaction with ASP97.

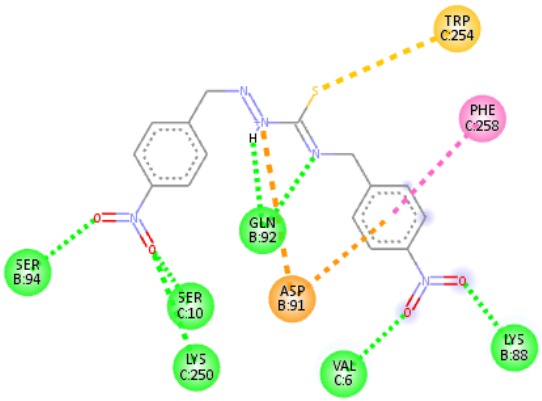


Figure 13. 2D pose of KL-4 with PBP6 PDB ID-3ITA

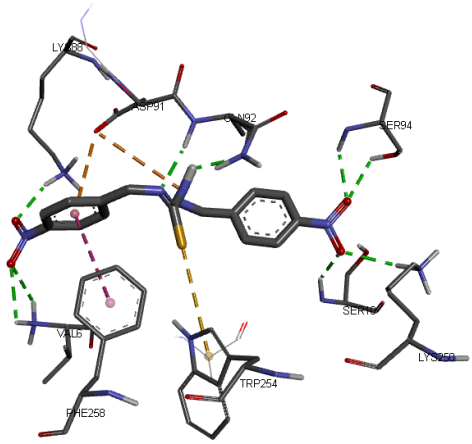


Figure 14. 3D poses of KL-4 with PBP6 PDB ID-3ITA

Our ligand KL4 exhibited excellent docking results with **Penicillin-Binding Protein** 4 (PBP4) from S. aureus PDB ID – 3HUN. Conventional hydrogen bond with LYS221. Pi-sulfur interaction with TRP254. Pi-pi stacked interaction with PHE225. Pi-alkyl interaction with ALA129. Electrostatic interaction with ASP130.

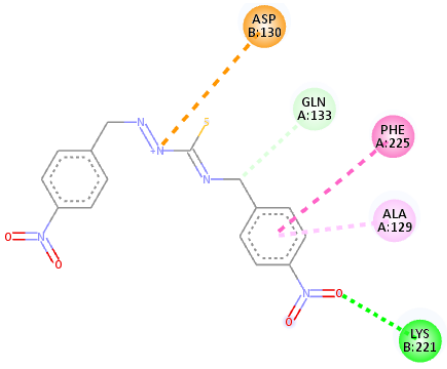


Figure 15. 2D pose of KL-4 with PBP4 PDB ID-3HUN

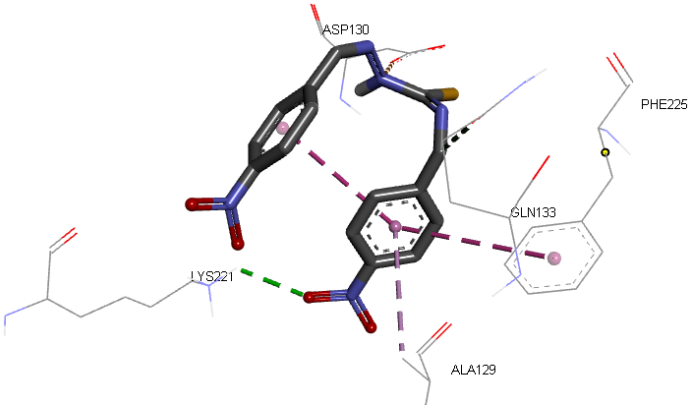


Figure 16. 3D pose of KL-4 with PBP4 PDB ID-3HUN

Mycobacterium tuberculosis glutamine synthetase (MtGS) is a promising target for antituberculosis drug discovery. We docked both the ligands KL-3 and KL-4 against Mycobacterium tuberculosis glutamine synthetase.

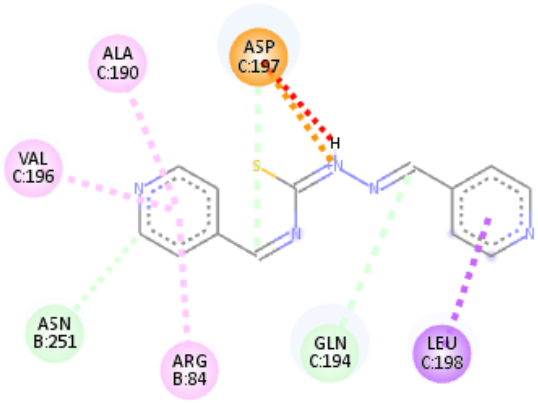


Figure 17. 2D pose of ligand KL-3 in the binding site of Mycobacterium tuberculosis glutamine synthetase protein

Pi-sigma interaction with LEU198. Pi-alkyl interaction with ALA190, VAL196, ARG84. Carbon hydrogen bonds with ASN251 and GLN194.

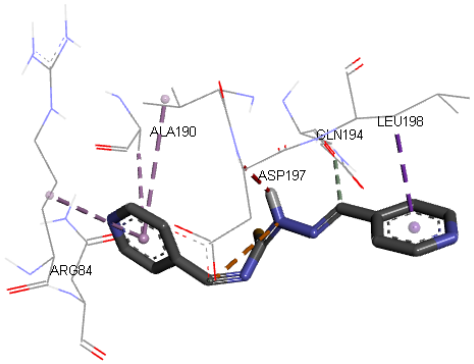


Figure 18. 3D pose of ligand KL-3 in the binding site of Mycobacterium tuberculosis glutamine synthetase protein

KL-3 ligand interaction affinity (kcal/mol) =-6.0
KL-4 ligand interaction with Mycobacterium tuberculosis glutamine synthetase protein.-
Our ligand KNPT showed excellent docking results in the binding site of Mycobacterium tuberculosis glutamine synthetase protein, Depicted in Figure 20. Conventional hydrogen bond with ARG176, SER173, GLN255. Pi-sigma interaction with ILE158. Pi-alkyl interaction with VAL196 and ALA190, Depicted in Figure 19.

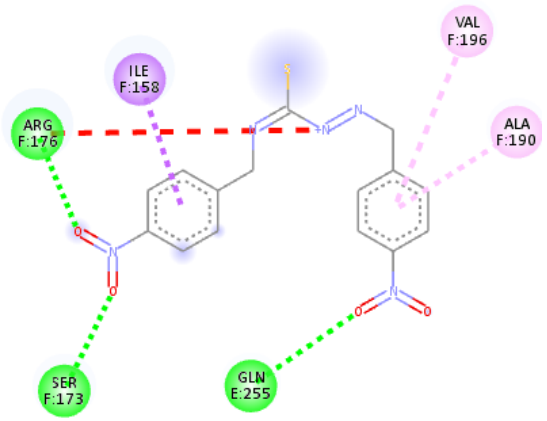


Figure 19. 2D pose of ligand KL4 in the binding site of Mycobacterium tuberculosis glutamine synthetase protein

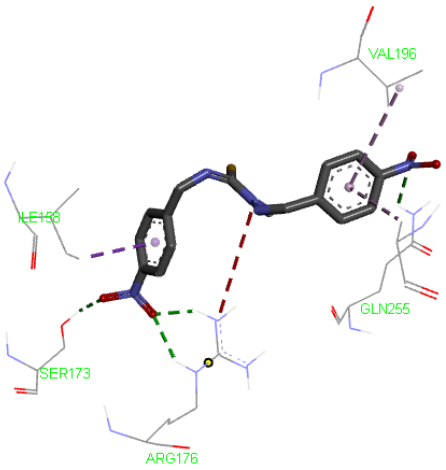


Figure 20. 3D poses of ligand KL-4in the binding site of Mycobacterium tuberculosis glutamine synthetase protein

Table 2. Comparison of docking scores of KL-3 and KL-4 Ligands with STD DRUGS.

Compound	DOCKING SCORE in Kcal/mol
KL-3	-6.0
KL-4	-7.1
PYRAZINAMIDE(Z)	-4.69
CIPROFLOXACIN	-6.45

5. Antimicrobial Activity

5.1. Antifungal activity- (in vitro)

For the experimental purpose, the fungus**, *Candida albicans***wastaken from the Department of Microbiology of the Harisingh Gour University in Sagar, MP was recorded using KBr pellets at 28° Chotometer. Antifungal activity of all compounds was conducted on a solid Czapek dox medium using a suitable diffusion method against A. Niger. Using a wet cotton swab, all the Spores of A. Niger was collected, followed by spreading these on a Czapek dox medium. Using a sterilized cork borer, six-mm-diameter wells are created in solid agar plates containing Czapek dox medium. After pouring 100µL of sample into a well, the plates incubate at 30°C for 48 hours. All values in mm and the activity against ***Candida albicans*** of both ligands and their metal complexes are shown in Figures 21 and 22.

5.2. Antibacterial activity

Similarly, the antibacterial activity does assess against ***Bacillus subtillis S. aureus*** grew and maintained on a nutrient agar medium. Mukherjee et al. described an in vitro method for measuring the antibacterial activity of compounds [51-53]. 10 minutes of centrifugation, the overnight old bacterial culture was centrifuged at 10000 g. The bacterial pellet was washed by suspension in sterile distilled water (20 mL) followed by centrifugation at 10000 g, then resuspended in sterilized distilled water and used for the assay. Solid nutrient agar plates were prepared by spreading a bacterial suspension and creating wells

(6mm in diameter) using a sterilized cork borer. After pouring 100 µ litters of test samples into wells, plates were placed in an incubator at 37°C for 24 hours. After incubation, the inhibition zones were recorded as the total diameter of the zone of inhibition (mm) minus the diameter of the well. All test runs were conducted in triplicate, and the average inhibition value was presented as an activity.

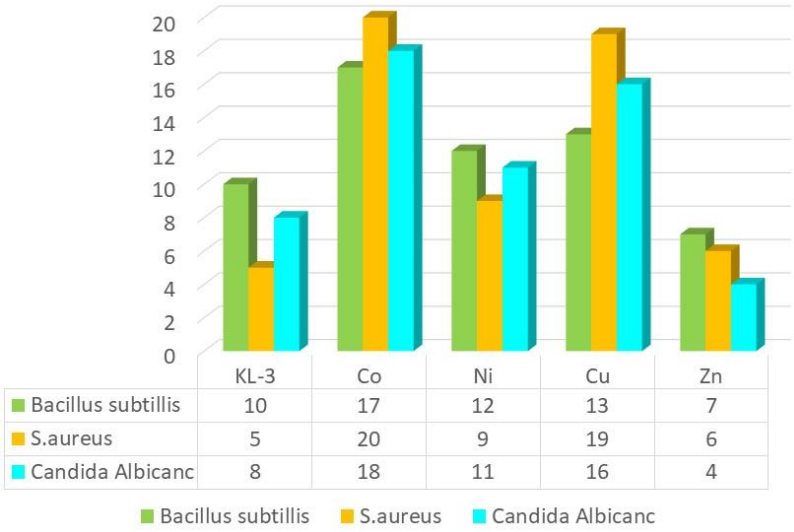


Figure 21. Antimicrobial study of KL-3 and its metal complexes
Zone of inhibition in mm]

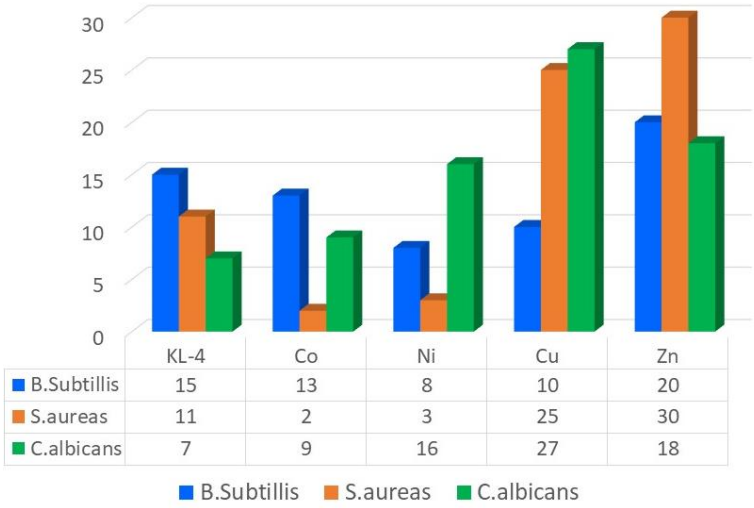


Figure 22. Antimicrobial study of KL-4 and its metal complexes

6. Results and Discussion

The Schiff bases and their metal complexes were synthesized using the two-step reaction protocols outlined in Scheme-I and Scheme-II. (KL-3 and KL-4) analyzed the compounds using FT-IR, ^{1}HNMR, UV-VIS, and HRMS spectra to confirm their formation of synthesized mixed ligand complexes (Schiff bases) KL-3 and KL-4 by reacting Para Nitrobenzaldehyde and Pyridine -4 carboxaldehyde with thiosemicarbazide in a 2:1 ratio in ethanol, with the help of a small amount of dilute acetic acid as a catalyst (Scheme 1). Metal complexes of the corresponding Schiff bases were prepared using nitrate salt. The resulting metal complexes were neutral, colored, air-stable, and highly soluble in DMF and DMSO. Several reports have described the effect of ligands with protein preparation, including so many steps of molecular docking efficiency; the recognition of ligands by proteins depends on both their shape (3D structure) and electrostatic complementarities. We adjusted protein and ligand databases protonation, tautomeric, and stereoisomeric states using Ligand Protein Preparation Wizard and SPORES. In addition, ligand conformation sampling is equally important to correct ligand preparation. FRED and HYBRID Glide's docking, ensemble docking, and induced-fit docking (IFD) modules, PLANTS, and POSIT,

feature a conformational search algorithm. PDB structures used in this study are cheque for integrity, especially in the active site region, and any structures with gaps in the binding site region are discarded. As protein conformational changes occur upon ligand binding, ignoring protein flexibility during molecular docking may be inaccurate - The active site is defined by amino acid residues surrounding the bound ligand. In order to include protein flexibility in docking, there are several approaches. Here, we concluded that all the synthesized complexes of Schiff base compounds exhibited significant antimicrobial activities against Bacterial and fungi organisms.

7. Conclusion

Two new Schiff base ligands (**KL-3 and KL-4**) derived from thiosemicarbazide with pyridine-4-carboxaldehyde and 4- nitrobenzaldehhyde have been synthesized and characterized. Further, a series of metal complexes of KL-3 and KL-4 comprising these ligands have been prepared and characterized by FTIR, ^{1}HNMR, Mass, and UV/vis spectral studies. The coordination behavior of these ligands has been explored, and it has been found that these prefer to bind with metal Centers in 2:1 (ligand: metal) stoichiometry. These Schiff bases behave like a suitable π-donor ligand, nitrogen in thiosemicarbazide, coordinate with metal, and form complexes with transition metals (Cu, Co, Ni, and Zn, (II)). IR spectra determined the formation of new C=N bond stretching frequency, NMR data defined the presence of aromatic and corresponding proton in a synthesized compound, and Mass-Spectra show the actual weight of the synthesized compound as projected for synthesis.

Molecular docking results of our ligands KL-3 and KL-4 showed excellent binding affinity in the active site of the selected protein. The docking scores of both our ligands are comparable to standard drugs. The binding pose of ligands with proteins contains conventional hydrogen bonding for efficient docking and high docking scores. The docking scores of both ligands confirm the results that we obtained from our in vitro screening results.

References

1- N.E. Borisova, M.D. Reshetova, Y.A. Ustynyuk, Metal-Free Methods in the Synthesis of Macrocyclic Schiff Bases, Chem. Rev., **2007**, 107 (1), 46–79.

2- C. Jiang, Z. Song, L. Yu, S. Ye, H. He, Fluorescent Probes Based on Macrocyclic Hosts: Construction, Mechanism and Analytical Applications, TrAC Trends Anal. Chem., **2020**, 133, 116086.

3- A.J. Gallant, M.J. MacLachlan, Ion-induced Tubular Assembly of Conjugated Schiff-base Macrocycles, Angew. Chemie, **2003**, 115 (43), 5465–5468.

4- S. Tariq, P. Kamboj, M. Amir, Therapeutic Advancement of Benzothiazole Derivatives in the Last Decennial Period, Arch. Pharm., **2019**, 352 (1), 1800170.

5- B. Vavaiya, S. Patel, V. Pansuriya, V. Marvaniya, P. Patel, Synthesis, Antitubercular Evaluation and Molecular Docking Studies of Nitrogen-Rich Piperazine-Pyrimidine-Pyrazole Hybrid Motifs, Curr. Chem. Lett., **2022**, 11 (1), 95–104.

6- P.A. Vigato, S. Tamburini, L. Bertolo, The Development of Compartmental Macrocyclic Schiff Bases and Related Polyamine Derivatives, Coord. Chem. Rev., **2007**, 251 (11–12), 1311–1492.

7- G. Yakalı, Examination of Aggregation-Induced Enhanced Emission in a Propeller-Shaped Chiral Non-Conjugated Blue Emitter from Restricted Intramolecular Rotation and J-Type Π… π Stacking Interactions, Phys. Chem. Chem. Phys., **2021**, 23 (19), 11388–11399.

8- A.B. Deshmukh, R. Sharma, P. Jain, P.K. Upadhyay, P.P. Timbadia, V. Kshirsagar, M.V. Bramhe, M.V. Sarode, L.G. Malik, R.K. Watile, Engineering and Technology (ET), Recent Advances in Science, Management, Engineering and Technology, 2**015**.

9- A.L. Berhanu, I. Mohiuddin, A.K. Malik, J.S. Aulakh, V. Kumar, K.H. Kim, A Review of the Applications of Schiff Bases as Optical Chemical Sensors, TrAC Trends Anal. Chem., **2019**, 116, 74–91.

10- M.T. Ali, N. Blicharska, J.A. Shilpi, V. Seidel, Investigation of the Anti-TB Potential of Selected Propolis Constituents Using a Molecular Docking Approach, Sci. Rep., **2018**, 8 (1), 1–8.

11- A.M. Abu-Dief, R.M. El-khatib, S.M. El Sayed, S. Alzahrani, F. Alkhatib, G. El-Sarrag,M. Ismael, Tailoring, Structural Elucidation, DFT Calculation, DNA Interaction and Pharmaceutical Applications of Some Aryl Hydrazone Mn (II), Cu (II) and Fe (III) Complexes, J. Mol. Struct., **2021**, 1244, 131017.

12- B. Manjunatha, Y.D. Bodke, H.M. Kumaraswamy, K.M.M. Pasha, N. Prashanth, Synthesis, Computational, Hepatoprotective, Antituberculosis and Molecular Docking Studies of Some Coumarin Derivatives, J. Mol. Struct., **2022**, 1254, 132410.

13- S. Malek-Ahmadi, A. Abdolmaleki, Synthesis, and Characterization of New Azo Containing Schiff Base Macrocycle, Chinese Chem. Lett., **2011**, 22 (4), 439–442.

14- S.K. Miryala, S. Basu, A. Naha, R. Debroy, S. Ramaiah, A. Anbarasu, S. Natarajan, Identification of Bioactive Natural Compounds as Efficient Inhibitors against Mycobacterium

Tuberculosis Protein-Targets: A Molecular Docking and Molecular Dynamics Simulation Study, J. Mol. Liq., **2021**, 341, 117340.
15- T. Khan, S. Vaidya, D.S. Mhatre, A. Datta, The Prospect of Salophen in Fluorescence Lifetime Sensing of Al^{3+}, J. Phys. Chem. B, **2016**, 120 (39), 10319-10326.
16- F.F. Chang, L. Zhang, P.C. Zhao, W. Huang, Transmetalation for Flexible Pendant-Armed Schiff-Base Macrocyclic Complexes Influenced by Halide Effects, Inorg. Chem., **2018**, 57 (3), 1438–1448.
17- N.K. Abdulla, S.I. Siddiqui, B. Fatima, R. Sultana, N. Tara, A.A. Hashmi, R. Ahmad, M. Mohsin, R.K. Nirala, N.T. Linh, Silver Based Hybrid Nanocomposite: A Novel Antibacterial Material for Water Cleansing, J. Clean. Prod., **2021**, 284, 124746.
18- M.J. MacLachlan, Conjugated Shape-Persistent Macrocycles via Schiff-Base Condensation: New Motifs for Supramolecular Chemistry, Pure Appl. Chem., **2006**, 78 (4), 873–888.
19- R. Golbedaghi, A.M. Tabanez, S. Esmaeili, R. Fausto, Biological Applications of Macrocyclic Schiff Base Ligands and Their Metal Complexes: A Survey of the Literature (2005–2019), Appl. Organomet. Chem., **2020**, 34 (10), e5884.
20- M. Cleiton, L. Daniel, L.V. Modolo, R.B. Alves, M.A. Resende, C.V.B. Martins, Schiff bases: A short review of their antimicrobial activities, Journal of Advanced Research, **2011**, 2(1), 1-8.
21- M.A. Arafath, F. Adam, M.R. Razali, L.E. Ahmed Hassan, M.B.K. Ahamed, A.M.S.A. Majid, Synthesis, characterization, and anticancer studies of Ni(II), Pd(II), and Pt(II) complexes with Schiff base derived from N-methylhydrazinecarbothioamide and 2-hydroxy-5-methoxy-3-nitrobenzaldehyde, J. Mol. Struct., **2017**,1130, 791–8.
22- T. Khan, R. Yadav, An Ef fi client Synthesis and Antibacterial Activity of Some Novel 2-Azetidinone Derivativesof 4H-1, 2, 4-Triazoles Under Mild Conditions, Journal of heterocyclic chemistry, **2018**, 55(4), 1042-1047.
23- S.N. Manjula, N. Malleshappa Noolvi, K. Vipan Parihar, Synthesis and antitumor activity of optically active thiourea and their 2-amino benzothiazole derivatives: A novel class of anticancer agents, Eur. J. Med. Chem., **2009**, 44, 2923–2929.
24- A.A.S. Al-Hamdani, W. Al Zoubi, New metal complexes of N3 tridentate ligand: Synthesis, spectral studies, and biological activity, Spectrochim Acta - Part A Mol Biomol Spectrosc, **2015**, 137, 75–89.
25- S. Ramezani, A. Nakhaei, Synthesis, absorption, and adsorption properties, and DFT calculations of two new palladium (II) complexes of new fluorescence Schiff-bases, Inorg. Nano-Metal Chem., **2021**, 51, 560–568.
26- R.S. Josephus, M.S. Nair, Antibacterial and Antifungal Studies on Some Schiff Base Complexes of Zinc (II), Mycobiology, **2008**, 36, 93-98.
27- S. Parham, A.K. Kharazi, H.R. Bakhsheshi-Rad, Antioxidant, antimicrobial and antiviral properties of herbal materials, Antioxidants, **2020**, 9, 1309.
28- D. Jothi, S. Munusamy, S. Swaminathan, S. Kulathu Iyer, Highly sensitive naphthalimide-based Schiff base for the fluorimetric detection of Fe^{3+}, RSC Adv., **2021**, 11, 11338–11346.
29- K.S. Parikh, S.P. Vyas, Synthesis and spectral studies of some novel Schiff base derived with pyrimidines, Der Pharm. Lett., **2012**, 4, 638–640.
30- S.M. Essayag, S. Magaldi, C.H.D. Capriles, L. Deibis, G. Verde, C. Perez, "In vitro" Antifungal activity of protease inhibitors, Mycopathologia, **2001**, 152, 135–142.
31- M. Sahu, A.K. Manna, K. Rout, Inorganica Chimica Acta A highly selective thiosemicarbazone based Schiff base chemo sensor for colorimetric detection of Cu^{2+} and Ag^{+} ions and turn-on fluorometric detection of Ag^{+} ions, Inorganica Chim Acta, **2020**, 508, 119633.
32- V.V. Raju, K.P. Balasubramanian, Synthesis, spectral characterization, catalytic and biological studies of new Ru (II) carbonyl Schiff base complexes of active amines, Natural Science, **2011**, 3, 542–550.
33- D. DemirciGültekin, A. AlaylıGüngör, H. Önem, Synthesis of Copper Nanoparticles Using a Different Method: Determination of Its Antioxidant and Antimicrobial Activity, J. Turkish Chem Soc Sect A Chem., **2016**, 3, 623-636.
34- K. Chourasia, B.P. Prajapati, M. Kori, K. Kumar, Synthesis, Characterization, and Antimicrobial studies of Schiff base Ligand from the amino acid L-arginine and its Cu (II), Ni (II), Co (II) complexes, Oriental Journal of Chemistry, **2022**, 38, 1–8.
35- L. Tiancheng, G. Ranran, Z. Qida, Application of molecular docking in elaborating molecular mechanisms and interactions of supramolecular cyclodextrin, Carbohydrate Polymers, **2022**, 276, 118644.
36- L.H. Abdel-Rahman, A.M. Abu-dief, R.M. El-khatib, S.M. Abdel-fatah, Bioorganic Chemistry Some new nano-sized Fe (II), Cd (II), and Zn (II) Schiff base complexes as a precursor for metal oxides : Sonochemical synthesis, characterization, DNA interaction, in vitro antimicrobial and anticancer activities, Bioorg Chem., **2016**, 69, 140–152.
37- F.S. Tokalı, P. Taslimi, H. Usanmaz, Synthesis, characterization, biological activity, and molecular docking studies of novel Schiff bases derived from thiosemicarbazide: Biochemical and computational approach, J Mol Struct., **2021**, 1231, 129666.

38- N. Mishra, K. Kumar, H. Pandey, Synthesis, characterization, optical and antibacterial properties of benzothiazole Schiff bases and their lanthanide (III) complexes, J. Saudi. Chem. Soc., **2020**, 24, 925–933.
39- B.T. Vhanale, N.J. Deshmukh, A.T. Shinde, Heliyon Synthesis, characterization, spectroscopic studies and biological evaluation of Schiff bases derived from 1 – hydroxy-2-acetonapthanone, Heliyon, **2019**, 5, e02774.
40- K. Sunil, T.P.P. Kumara, B.A. Kumar, S.B. Patel, Synthesis, Characterization and Antioxidant Activity of Schiff Base Compounds Obtained Using Green Chemistry Techniques, Pharm Chem J., **2021**, 55, 46–53.
41- M.S. Kasare, P.P. Dhavan, B.L. Jadhav, S.D. Pawar, Synthesis of Azo Schiff Base Ligands and Their Ni (II), Cu (II) and Zn (II) Metal Complexes as Highly-Active Antibacterial Agents, ChemistrySelect, **2019**, 4, 10792–10797.
42- U. Kumar, S. Chandra, Synthesis, spectroscopic characterization of some Schiff base complexes derived from 2-methyl cyclohexanone and their activity against some fungi, J Saudi Chem Soc., **2011**, 15, 19–24.
43- N. Mishra, K. Kumar, H. Pandey, Synthesis, characterization, optical and antibacterial properties of benzothiazole Schiff bases and their lanthanide (III) complexes, J Saudi Chem Soc., **2020**, 24, 925–933.
44- M. Shih, Y. Xu, Y. Yang, T. Lin, Syntheses of Nickel (II) Complexes from Novel Semicarbazone Ligands with Chloroformylarylhydrazine, Benzimidazole, and Salicylaldehyde Moieties, Molecules, **2015**, 20, 5184–5201.
45- T. Mangamamba, M.C. Ganorkar, G. Swarnabala, Characterization of Complexes Synthesized Using Schiff Base Ligands and Their Screening for Toxicity Two Fungal and One Bacterial Species on Rice Pathogens, International Journal of Inorganic Chemistry, **2014**.
46- H.M.A. Hasan, H.A.A. Khalid, Infrared (IR) Characterizations and physicochemical properties of Schiff base compound obtained by the reaction between 4- Hydroxy-3-methoxy benzaldehyde and 2- Amino-3- methyl butanoic acid, Journal of Research in Pharmaceutical Science, **2021**, 7, 8–12.
47- J.H. Han, S.M. Lee, Y.S. Kim, Effects of Schiff base formation and aldol condensation on the determination of aldehydes in rice wine using GC-MS, Molecules, **2017**, 22, 618.
48- D.S. Black, Biomolecular Chemistry, Chemistry International-Newsmagazine for IUPAC, **2004**, 26(6), 31-32. https://doi.org/10.1515/ci.2004.26.6.31.
49- K. Avasthi, R. Yadav, T. Khan, Greener and Efficient Synthesis of Some Novel Substituted Azitidinones With 4- Amino Pyridine via Heterogenous Catalyst, Oriental Journal of Chemistry, **2018**, 38.
50- P. Kosti, G. Ahmad, N. Ratnesh, Biological and electrochemical studies of Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} metal complexes of Schiff base ligand derived from 4-amino benzoic acid and isonicotinic hydrazide, J. Iran. Chem. Soc., **2021**, 18, 1773–1780.
51- N. Mishra, S.S. Gound, R. Mondal, Synthesis, characterization, and antimicrobial activities of benzothiazole-imino-benzoic acid ligands and their Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes, Results Chem, **2019**. 1, 100006.
52- L.Y. Sang, X.S. Lin, L.F. Zou, R.F. Jin, X.H. Zhang, Y.H. Liu, Syntheses, crystal structures and antibacterial activities of mononuclear nickel (II) complexes with similar Schiff bases, Journal of Structural Chemistry, **2022**, 63, 956–963.
53- A.M. Abu-Dief, Azomethine Metal Chelates as an Efficient Catalyst for Oxidation of Organic Compounds, Ann Chem Sci Res., **2019**, 1, 2–4.
54- P.R. Chetana, M.N. Somashekar, B.S. Srinatha, Synthesis, Crystal Structure, Antioxidant, Antimicrobial, and Mutagenic Activities and DNA Interaction Studies of Ni (II) Schiff Base 4-Methoxy-3-benzyloxybenzaldehyde Thiosemicarbazide Complexes, ISRN Inorg Chem., **2013**, 1–11.
55- Y. Luo, S. Zhang, Z.J. Liu, Synthesis and antimicrobial evaluation of a novel class of 1,3,4-thiadiazole: Derivatives bearing 1,2,4-triazolo[1,5-a] pyrimidine moiety, Eur J Med Chem., **2013**, 64, 54–61.
56- A.I. Qbal, L.S. Siddiqui, M.A. Sharaf, Synthesis, Spectroscopic and Cytotoxic Studies of Biologically Active New Schiff Bases Derived from p –Nitrobenzaldehyde, **2007**, 55, 1070–1072.
57- P. Biçer, G. Taslimi, İ. Yakalı, M.S. Gülcin, G.T. Gültekin, Synthesis, Characterization, Crystal Structure of Novel Bis Thiomethylcyclohexanone Derivatives and Their Inhibitory Properties against A Some Metabolic Enzymes, Bioorg. Chem., **2019**, 82, 393- 404.
58- M. Huseynova, A. Medjidov, P. Taslimi, M. Aliyeva, Synthesis, Characterization, Crystal Structure of the Coordination Polymer Zn(II) with Thiosemicarbazone of Glyoxalic Acid and Their Inhibitory Properties Against Some Metabolic Enzymes, Bioorg. Chem., **2019**, 83, 55-62.