Molecular docking studies, structural analysis, biological studies, and synthesis of certain novel Schiff base from benzohydrazide derivate

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Abstract

The research described involves the synthesis and characterization of a new benzohydrazide based Schiff base compound called 4-methyl-N'-(2,4,5-trimethoxybenzylidene)benzohydrazide (HL). The compound was synthesized by condensing a primary amine and an aldehyde functional group. The characterization of the compound was carried out using various spectroscopic techniques, including elemental analysis, Ultraviolet spectroscopy, nuclear magnetic resonance spectroscopy, and infrared spectroscopy. These techniques helped confirm the excellent quality of the synthesized molecules. In addition to the characterization, the Schiff base compound was subjected to docking studies and biological studies. The antibacterial activity of the compound were tested against three strains of bacteria, namely E. *faecalis*, B. *subtills*, and E.*coli*. The results of these tests provided information on the compound effectiveness against these bacterial strains. Furthermore, docking studies was performed to assess the interaction between the synthesized compound and three target enzymes like GlcN-6-p synthase, E. coli, and EGFR tyrosine kinase. Docking studies are computational simulations that

provide insights into the binding interactions between Schiff base ligand and target proteins. In this case, the docking studies helped understand the potential mechanisms of action of the synthesized compound by calculating binding constants and the number of binding modes. The biological activity studies revealed interesting fragmentation patterns, which could be further investigated to understand the compound modes of action. Moreover, the docking studies evaluated the compound's potential as a drug candidate by assessing its binding interactions with the target enzymes. The present study suggests that the synthesized benzohydrazide based Schiff base compound exhibits potential antibacterial activity and shows promising binding interactions with the target protein/ enzymes. This information is valuable for designing and developing more potent compounds in the future.

Keywords:

Schiff base, Spectral characterization, Biological activity, Infrared spectroscopy, Docking Studies.

1. Introduction:

Schiff bases, also known as imines or azomethines, are a class of chemical compounds that are synthesized by condensing a primary amine and a carbonyl functional group [1]. These compounds are named after the German chemist Hugo Schiff [2], who first synthesized them in 1864. Schiff bases have a wide range of applications in various fields, including medicine, pharmacology, and materials science, due to their diverse chemical properties and biological activities. The pharmacological properties of Schiff bases are attributed to the presence of aromatic rings with oxygen, sulfur, and nitrogen donor atoms, which contribute to their wide range of activities such as anti-inflammatory, antimalarial, antibacterial, antipyretic actions, and antifungal [3-12]. The type and position of substituents on the aromatic ring, nature of the

functional group on the imine moiety and as well as the type, influence the pharmacological properties of Schiff bases. This flexibility allows for the development of bioactive molecules with specific properties.

Schiff bases also have the ability to form stable complexes with metal ions, making them effective chelators. This property has been utilized in the development of quantify metal ions and chemo sensors that can selectively detect in various samples. The formation of metal complexes often leads to changes in the magnetic or electrochemical, optical properties of the compound, which can be exploited for selective and sensitive detection.

Heterocyclic-based Schiff bases have exhibited significant potential in combating pathogenic strains, demonstrating promising antimicrobial properties. Furthermore, these Schiff bases have been extensively studied for their potential in anti-inflammatory, antioxidant, and antitumor applications. In the field of coordination chemistry, they exhibit ligand behavior by forming complexes with metal ions. They are also used as building blocks in supramolecular chemistry due to their ability to chelate metal ions [14-15]. Triazole-based Schiff bases have demonstrated significant antibacterial properties. For example, in a study by Singh et al., a series of triazole-based Schiff bases were synthesized and evaluated for their antibacterial studies against Gram-negative and Gram-positive bacteria. Some of the compounds exhibited even better activity than standard antibiotics [16]. Overall, Schiff bases are versatile compounds with diverse applications in medicine, pharmacology, and materials science. Their chemical flexibility and biological activities make them promising candidates for the development of therapeutic agents and chemo sensors. Ongoing research continues to explore their potential in various fields. The synthesis and biological applications of Schiff base derived from amino acid is intriguing. The antibacterial study of these compounds, as demonstrated by Jha.et.al [17], highlights their

potential as novel antibacterial agents. It is promising to see that some of these Schiff bases exhibited even better activity than standard antibiotics. Regarding benzohydrazide-based Schiff bases, their unique properties, such as excellent biocompatibility, biodegradability, and the ability to selectively target biomolecules, make them attractive for various biomedical applications. These applications include drug delivery, bio-imaging, and tissue engineering [18-19]. The incorporation of different functional groups into hydrazide-based Schiff bases further expands the possibilities for creating biomaterials with specific properties and functions.

In the present research, the use of 4-methylbenzohydrazide in the synthesis of Schiff base adds an interesting element. The presence of a methyl group in the para position of the benzene ring may contribute to enhanced biological activity of the compound. Characterizing the synthesized compound using spectroscopic techniques such as UV Visible spectrometry, FT-IR, and ^{1}H NMR is crucial for confirmed its chemical purity and structure. Furthermore, evaluating the antibacterial activity of the compound against strains of E. faecalis, B. subtills, and E. coli provides valuable insights into its potential as an antibacterial agent. Conducting docking studies with the synthesized compound against target enzymes such as EGFR tyrosine kinase, GlcN-6-p synthase, and E. coli is another significant aspect of your research. Docking studies offer insights into the binding interactions between the compound and target proteins, shedding light on its potential mechanism of action at the molecular level. This information can guide the design and development of more potent compounds in the future.

Overall, research combines the synthesis, characterization, biological activity studies, and docking studies of Schiff base compound derived from benzohydrazide derivative. This comprehensive approach provides a deeper understanding of their potential as antibacterial agents and their interactions with target proteins.

2. Experimental part

2.1 Materials and methods

The chemicals 4-methylbenzohydrazide and 2,4,5-trimethoxybenzaldehyde were obtained from Sigma-Aldrich and HIMEDIA, respectively. Methanol, diethyl ether, and sodium hydroxide were purchased from Sisco Research Laboratories (SRL), India. High-quality solvents of spectrometric grade were utilized without the need for additional purification.

2.2 Instrumentation

Infrared spectra were collected using a Perkin-Elmer Spectrum 100 spectrometer, which covered the wavelength range of 4000-400 cm^{-1}. KBr pellets were utilized as the medium for the measurements. The ligand ^{1}H NMR spectra were obtained at room temperature employing an AV-400 M-HZ NMR spectrometer and DMSO-D6 as the solvent. Furthermore, absorption spectra were recorded using a Perkin Elmer Lambda 25 UV-visible spectrophotometer.

2.3 Synthesis

The synthesis of the Schiff base compound followed a general procedure as outlined in Scheme-1.

2.3.1 Synthesis of 4-methyl-N'-(2,4,5-trimethoxybenzylidene)benzohydrazide (HL):

In a 50 ml round bottom refluxing flask, a solution was prepared by dissolving 0.01 moles of 4-methylbenzohydrazide and 2,4,5-trimethoxy benzaldehyde in 20 ml of methanol. A small amount of sodium hydroxide (NaOH) was added to the solution. The mixture was then refluxed on a water bath for 3 hours. After cooling, light yellow crystals of the newly synthesized azomethine compound, named HL, were obtained. The compound was further purified by recrystallization from methanol. The yield of the compound was determined to be 92%, and its

melting point was found to be in the range of 178-180 °C. The physical and analytical data of the Schiff base ligand can be found in Table 1.

2.4 Ligand preparation:

The chemical structure of the Schiff base ligand was visualized using ChemBioDraw Ultra 12.0. To optimize the ligand's geometry, molecular mechanics minimization was conducted using ChemBio 3D Ultra 12.0, following the prescribed procedure [20]. The resulting energy-minimized structures were employed in docking experiments with the target protein using the online tool Patch Dock.

2.5 Target protein identification and preparation:

The three-dimensional (3D) structures of GlcN-6-P synthase (PDB ID: 1P7T, resolution: 1.95 Å), EGFR tyrosine kinase (PDB ID: 4J97, resolution: 2.54 Å), and E. coli (PDB ID: 5I5H, resolution: 1.65 Å) were obtained from the Research Collaborator for Structural Bioinformatics (RCSB) Protein Data Bank (www.rcsb.org) [21]. Each protein chain was isolated by removing other chains (B, C, and D), as well as ligands and water molecules without hydrogen bonds. Protein preparation was conducted using UCSF Chimera software, which is available at www.cgi.ucsf.edu/chimera.

2.6 ADME analysis:

The Absorption, Distribution, Metabolism, and Excretion (ADME) analysis was performed using the Swiss ADME analysis method [21].

2.7 Docking studies:

Docking studies were carried out using the PatchDock online server available at http://bioinfo3d.cs.tau.ac.il/PatchDock. PatchDock utilizes a geometry-based molecular docking algorithm to calculate binding scores based on ligand atomic contact energy with the binding

residues. The docking results were received via email and included a URL that provided a table with the top 20 solutions. From these solutions, the highest-ranked one representing the docked protein-ligand complex was selected and downloaded in pdb file format. Subsequently, the binding site analysis was conducted using PyMOL software, accessible at www.pymol.org [21].

2.8 Invitro antimicrobial activities

In this study, the well diffusion method was utilized to evaluate the antibacterial effect of the ligand against *E. coli*, B. *subtillis*, and E. *faecalis* strains. Nutrient agar was used for bacterial cultures, while potato dextrose agar was employed for antifungal studies. The test solutions were spread onto the agar medium, leading to an impact on the growth of the inoculated microorganisms. The development of inhibition zones around the wells was correlated with the concentration of the samples. To establish a positive control for the antibacterial experiments, ciprofloxacin, a well-known antibacterial agent, was employed. For antifungal investigations, fluconazole served as the positive standard, while dimethylformamide (DMF) was used as the negative control. The minimal inhibitory concentration (MIC) was determined through a serial dilution method, enabling the calculation of the lowest ligand concentration that exhibited inhibitory effects against the tested microorganisms. By employing these methods and controls, the study provided a comprehensive analysis of the ligand antibacterial and antifungal activities.

3. **Results and Discussion:**

3.1 ***FT-IR spectra***

Figure 1 illustrates the spectra of the unbound Schiff base ligand HL, which displayed distinct azomethine ν(C=N) bands at 1631 cm^{-1}. The spectra of the free Schiff base ligand also exhibited bands at 1278 cm^{-1}, which were assigned to the phenolic C-O stretching vibrations of HL [22]. Furthermore, aromatic ν(C−H) bending vibrations in the Schiff base ligand HL were

observed within the approximate range of 875-636 cm^{-1} in previous studies [23,24,25].

3.2 ^{1}H NMR spectra:

Figure 2 displays the ^{1}H NMR spectra of the free Schiff base ligand HL, revealing singlet signals at δ 8.54 ppm assigned to the protons of the azomethine (−HC=N). The aromatic protons appeared as multiplets within the range of δ 7.28-7.30 ppm for HL [23,24,26]. The three methyl (-CH_3) group protons in HL were observed as sharp singlet signals at δ 2.43 ppm [27,28]. Furthermore, the three methoxy (-OCH_3) group protons in HL appeared as sharp singlet signals at δ 3.94 ppm.

3.3 Electronic spectra:

Figure 3 illustrates the electronic spectra of HL in aqueous solutions, capturing the graphical representation of the spectra. The UV spectra were recorded in the presence of DMF as the solvent, covering the range of 200-600 nm. Notably, the electronic spectrum of HL exhibited two distinct bands. The peak at 248 nm was attributed to the $\pi \rightarrow \pi^*$ transition, while the band at 356 nm corresponded to the $n \rightarrow \pi^*$ transition [25, 27, 29]. The provided details regarding the electronic spectra shed light on the ligand HL absorption properties and the specific transitions occurring within the compound. These spectroscopic investigations play a crucial role in understanding the electronic structure and behavior of the compounds, providing valuable insights for further analysis and characterization.

3.4 Docking Studies:

The present study focuses on Schiff base derivatives, as listed in Table 2. Prior to conducting docking studies, it is crucial to evaluate the physicochemical and drug-likeness properties of the Schiff base ligands. Lipinski's rule of five is commonly employed to assess the potential of lead compounds for oral drug development in humans. This rule considers factors such as molecular

weight (MW), lipophilicity (logP), the number of hydrogen bond acceptors and donors (N and O atoms), and the number of rotatable bonds. Violations of these criteria may indicate challenges in terms of oral bioavailability [30].

Table 3 in the study reveals that the Schiff base derivatives analyzed exhibited zero violations of Lipinski's rule of five. This suggests favorable physicochemical properties and drug-likeness characteristics, supporting their potential for further exploration in drug development. It is important to note that adherence to Lipinski's rule of five does not guarantee a compound's suitability as a drug candidate. Other factors, including target specificity, toxicity, and pharmacokinetics, must also be considered. Nevertheless, the absence of violations among the Schiff base derivatives in this study indicates their promising potential as orally active drug candidates.

Table 4 in the study presents the drug-likeness scores of the Schiff base derivatives, particularly in relation to their bioactivity as enzyme inhibitors. The scoring system used indicates whether a compound is active, moderately active, or inactive based on the drug-likeness score range. A drug-likeness score greater than 0 suggests activity, a score between 5.0 and -0.0 indicates moderate activity and a score less than -5.0 indicates inactivity [31].Based on the provided information, the Schiff base derivative demonstrates moderate activity bioactivity scores in the enzyme inhibitor descriptor, indicating a reasonable likelihood of significant activity as enzyme inhibitors. However, it is important to acknowledge that the drug-likeness score is just one factor to consider when evaluating a compound's potential as a drug candidate.

In the drug discovery and development process, multiple factors need to be taken into account to determine the suitability of a compound for further investigation and eventual use as a

drug. In addition to the drug-likeness score, considerations such as target specificity, toxicity, pharmacokinetics, and efficacy must be thoroughly assessed. Therefore, while the Schiff base derivative in Table 4 shows moderate activity bioactivity scores as enzyme inhibitors, further studies and analyses are required to fully evaluate their overall potential as viable drug candidates. In the early stages of drug discovery, drug screening, and drug design, it is crucial to assess the absorption, distribution, metabolism, and excretion (ADME) profile of potential drug candidates before conducting docking studies [32-33]. ADME prediction plays a significant role in understanding the characteristic nature of compounds and their potential as drugs.

Table 4 in the study provides the ADME profile of the Schiff base derivative, indicating that the ligand in the study is predicted to have a high gastrointestinal (GI) absorption effect. This suggests that this compound was likely to be effectively absorbed through the gastrointestinal tract, which is a desirable property for orally administered drugs. Assessing the ADME profile of compounds is essential for evaluating their pharmacokinetic properties and understanding their behavior in the human body. It provides valuable insights into compound absorption, distribution to target tissues, metabolism by enzymes, and elimination from the body. However, it is crucial to acknowledge that ADME prediction serves as an initial assessment and requires further experimental studies to confirm and refine these predictions. The ADME profile is just one aspect of drug development, and thorough investigations of safety, efficacy, and target specificity are also necessary. In summary, the ADME prediction results presented in Table 5 indicate that the Schiff base derivative exhibits a high GI absorption effect, suggesting their potential as orally administered drug candidates. However, additional experimental investigations are needed to validate these predictions and comprehensively evaluate the overall

ADME properties of the compound. In our study, the Schiff base derivative was employed for molecular docking analysis with specific antibacterial and antimicrobial proteins.

The docking study and binding free energy calculations revealed that HL showed strong binding affinity with the target protein/enzyme GlcN-6-p synthase (1P7T), as indicated by the highest interaction energy (-254.39 kcal/mol). Similarly, for the target protein/enzyme EGFR tyrosine kinase (4J97), HL exhibited a significant interaction energy (+9.33 kcal/mol), suggesting a strong binding affinity. HL demonstrated interactions with specific amino acid residues of GlcN-6-p synthase and EGFR tyrosine kinase, providing insights into the binding sites and molecular interactions. More information regarding these interactions can be found in Figure 4 and 5, Table 6, and Table 7.

Overall, the findings highlight the potential of HL as a promising candidate for antibacterial and antimicrobial applications, with the highest interaction energies observed for GlcN-6-p synthase and EGFR tyrosine kinase. In the context of antibacterial studies, E. coli was chosen as an additional target protein/enzyme. The docking studies and binding free energy calculations demonstrated a strong binding affinity between HL and E. coli, with the highest interaction energy recorded (-246.90 kcal/mol). Notably, HL interacted with specific amino acid residues of E. coli, providing insights into potential binding sites and enhancing our understanding of ligand-protein interactions. Detailed visualizations of these interactions can be found in Figure 6 and Table 8.

These findings suggest that HL has the potential to effectively target E. coli in antibacterial studies, supported by its high interaction energy and strong binding affinity. However, it is important to note that these results are specific to the docking studies and binding free energy

calculations conducted in this study. Further experimental investigations are necessary to validate and confirm the antibacterial activity of HL against E. coli. The docking studies contribute to our understanding of ligand-protein interactions in the context of antibacterial studies, emphasizing the potential of HL as an effective ligand for targeting E. coli.These findings suggest that HL has the potential to effectively target E. coli in antibacterial studies, supported by its high interaction energy and strong binding affinity. However, it is important to note that these results are specific to the docking studies and binding free energy calculations conducted in this study. Further experimental investigations are necessary to validate and confirm the antibacterial activity of HL against E. coli. The docking studies contribute to our understanding of ligand-protein interactions in the context of antibacterial studies, emphasizing the potential of HL as an effective ligand for targeting E. coli.

3.5 ***Biological activity studies:***

Table 9 presents the antibacterial activity of Schiff base ligand HL against different bacterial strains, with the highest activity observed against B. *subtillis* and the lowest activity against E. *coli*. When comparing our study with the literature, Bhaskar et al. [34] investigated the bacterial activity of common medications such as Clotrimazole and Ciprofloxacin. According to the comparison data in Table 9, Clotrimazole Complex exhibited no activity, while Ciprofloxacin Complex showed the highest activity against E. *faecalis*. Similarly, M.A. Ashraf et al. [35] reported on the bacterial activity of Amoxycillin, which displayed the highest activity against E. *coli* and the lowest activity against B. *subtillis*, with no activity observed against E. *faecalis*.

It is important to consider various factors that can impact antibacterial activity, including the geometry of the ligands, parameters, presence of co-ligands, lipophilicity, coordinating sites, concentration, and the nature of the ligands themselves. These factors collectively contribute to the observed antibacterial activity. Although the examined Schiff base compounds demonstrated improved antibacterial activity, their activity levels were lower compared to the reference standards. In the near future, there is hope to utilize the synthesized Schiff base compounds as therapeutic molecules to treat infections caused by these bacterial strains.

4 **Conclusions:**

The present study demonstrates that three Schiff base derivatives have the potential to effectively dock and bind with all four targeted enzymes, including GlcN-6-p synthase, EGFR tyrosine kinase, and E. coli. These docking studies provide strong evidence supporting the potential of these derivatives as inhibitory agents in antibacterial and antimicrobial activities. The ability of the Schiff base derivatives to dock and bind with the targeted enzymes suggests that they have the capability to inhibit the activities of these enzymes. This finding is particularly significant in the context of antibacterial and antimicrobial applications. The docking studies conducted in this study strongly support the notion that these Schiff base derivatives hold promise as effective agents for antibacterial and antimicrobial activities. The results provide valuable insights into the interactions and binding modes between these derivatives and the targeted enzymes. However, it is important to emphasize those further experimental investigations and studies are necessary to validate and confirm the antibacterial and antimicrobial activities of these Schiff base derivatives. Additionally, comprehensive evaluations of aspects such as toxicity, pharmacokinetics, and target specificity are required to determine their overall potential as therapeutic agents. In summary, the findings of this study highlight the

potential of this Schiff base derivative as inhibitory agents against the targeted enzymes, indicating their potential utility in antibacterial and antimicrobial applications.

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Tables:

Table 1: Physical and analytical data of Schiff base ligand.

Compound	**Color**	**Empirical formula weight**	**M.P. (^{0}C)**	**Elemental Analysis Found and Calculated (%)**				**Yield %**
				C	H	N	O	
$C_{18}H_{20}N_2O_4$ (HL)	328.39	Light Yellow	178-180	65.67 (65.77)	6.05 (6.09)	8.46 (8.52)	19.39 (19.49)	92

Table 2: Structure of the Selected Organic ligand and its SMILES

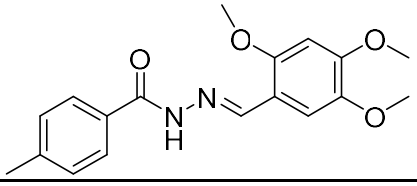
Ligand Name	Structure of Ligand	SMILES
HL		O=C(N/N=C/C1=CC(OC)=C(OC)C=C1OC)C2=CC=C(C)C=C2

Table 3: Molecular physicochemical descriptors analysis of Schiff base derivative using Molinspiration online software tool.

Schiff base derivative	Log A [a]	TPSA[b]	Natoms [c]	MW [d]	No N [e]	nOH NH [f]	Nviolations[g]	Nrotb[h]	Volume[i]
HL	3.18	69.16	24	328.37	6	1	0	6	303.30

[a] Octanol-Water partition coefficient, [b] Polar surface area, [c] Number of non-hydrogen atoms, [d] Molecular weight, [e] Number of hydrogen bond acceptors [O and N atoms], [f] Number of hydrogen bond donors [OH and NH groups], [g] Number of Rule of 5 violations, [h] Number of rotatable bonds, [i] Molecular volume.

Table 4: Drug-likeness property analysis of Schiff base derivative using Molinspiration online software tool.

Schiff base derivative	GPCR* ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
HL	-0.42	-0.93	-0.46	-0.54	-0.58	-0.49

*GPCR- G Protein coupled receptors

Table 5: ADME analysis of Schiff base derivatives using Swiss ADME online tool.

Schiff base derivative	GI[#]	BBB[##]	P-gp[###]	CYP1A2*	CYP219**	CYP2C9***	CYP2D6****	CYP3A4*****	Log Kp[####]
HL	High	Yes	No	Yes	Yes	No	Yes	Yes	-6.09

[#]-Gastrointestinal, [##]-Blood-brain barrier permeant, [###]-P-gp-P-glycoprotein substrate, **- *****-Cytochrome P450 Inhibitors, [####]-Skin Permeation (cm/s).

Table 6: Binding energy analysis of Schiff base derivative with antimicrobial enzymes (GlcN-6-p synthase) using PatchDock.

Schiff Base Derivative	-ACE (-kcal/mol)*	Interaction of amino acid residue	Bond distance (Å)
HL	254.39	No Interaction	-

* - Atomic contact energy

Table 7: Binding energy analysis of Schiff base derivative with antimicrobial enzymes (EGFR tyrosine kinase) using PatchDock.

Schiff Base Derivative	-ACE (-kcal/mol)*	Interaction of amino acid residue	Bond distance (Å)
HL	+ 9.33	No Interaction	-

* - Atomic contact energy

Table 8: Binding energy analysis of Schiff base derivative with antibacterial enzymes (E.Coli) using PatchDock.

Schiff Base Derivative	-ACE (-kcal/mol)*	Interaction of amino acid residue	Bond distance (Å)
HL	246.90	No Interaction	-

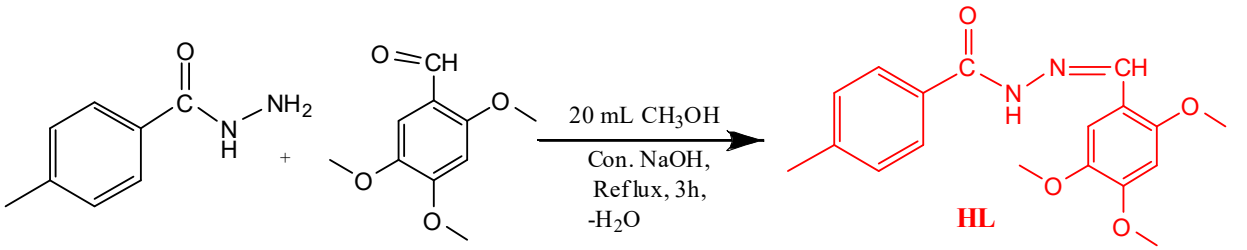
* - Atomic contact energy

Table 9: Antimicrobial activity of schifff base ligand (zoning in mm).

Compound	*Escherichia coli*	*Bacillus subtills*	*Enterococcus faecails*
HL	10	14	13
Ciprofloxacin*	13	14	15
Clotrimazole*	-	-	-
Amoxycillin**	18	12	-

Comparison data, Bhaskar *et al*. [34]*, Muhammad Aqeel Ashraf *et al* [35]**

Scheme 1: Schematic representation of synthesis of Schiff base ligand (HL).



4-methylbenzohydrazide 2,4,5-trimethoxybenzaldehyde (*Z*)-4-methyl-*N*'-(2,4,5-trimethoxybenzylidene)benzohydrazide

Figures:

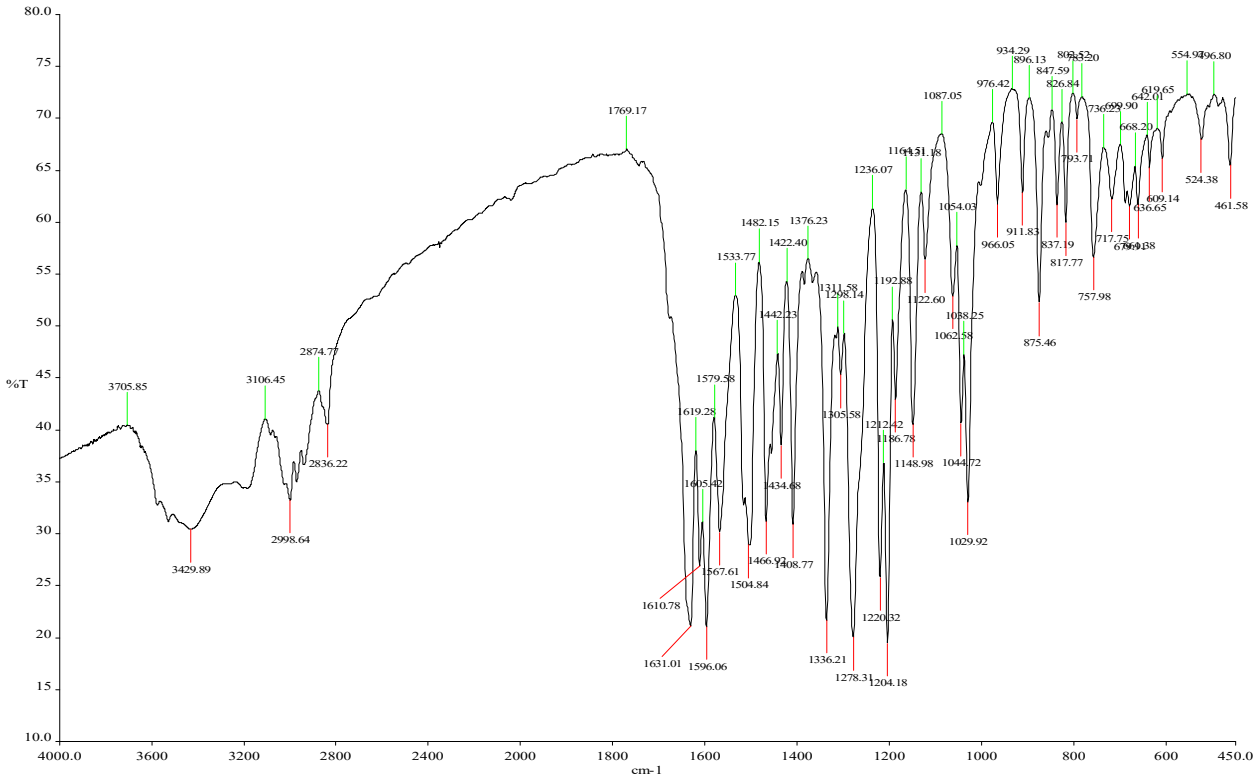


Figure 1: IR spectra of HL Schiff base Ligand.

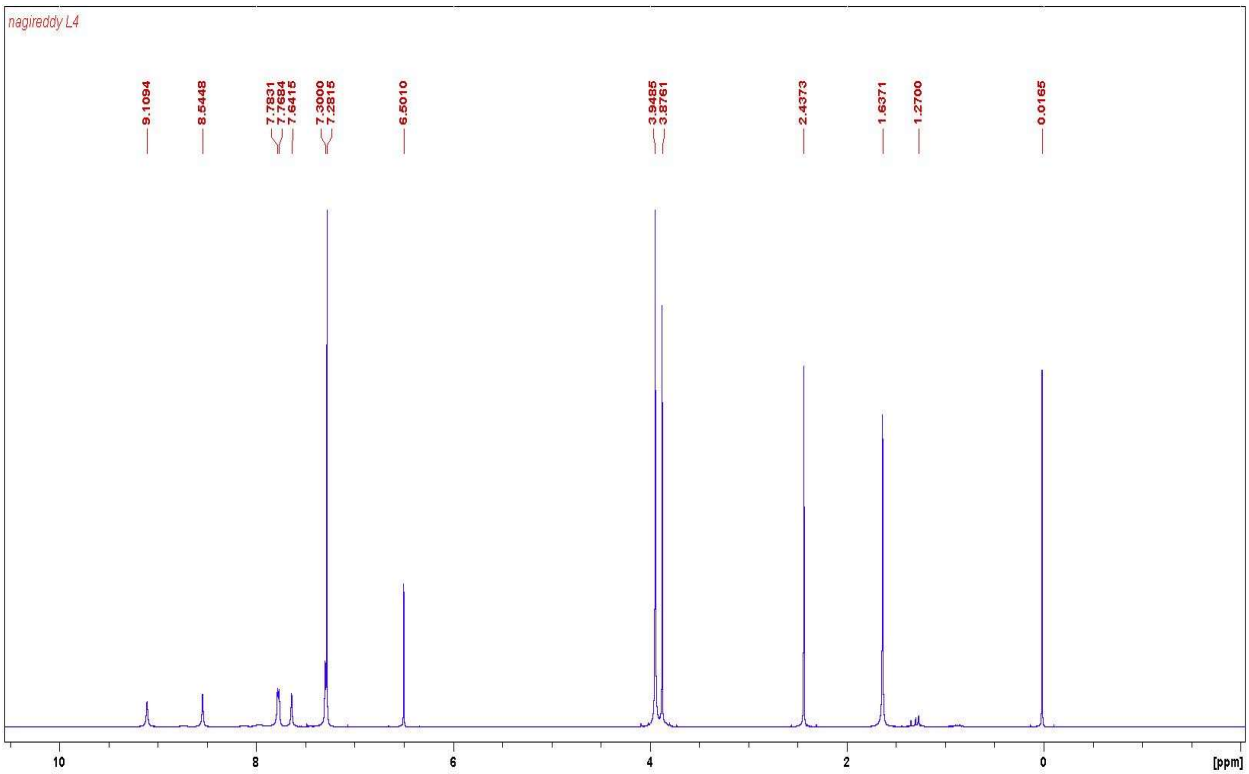


Figure 2: NMR spectra of HL Schiff base ligand.

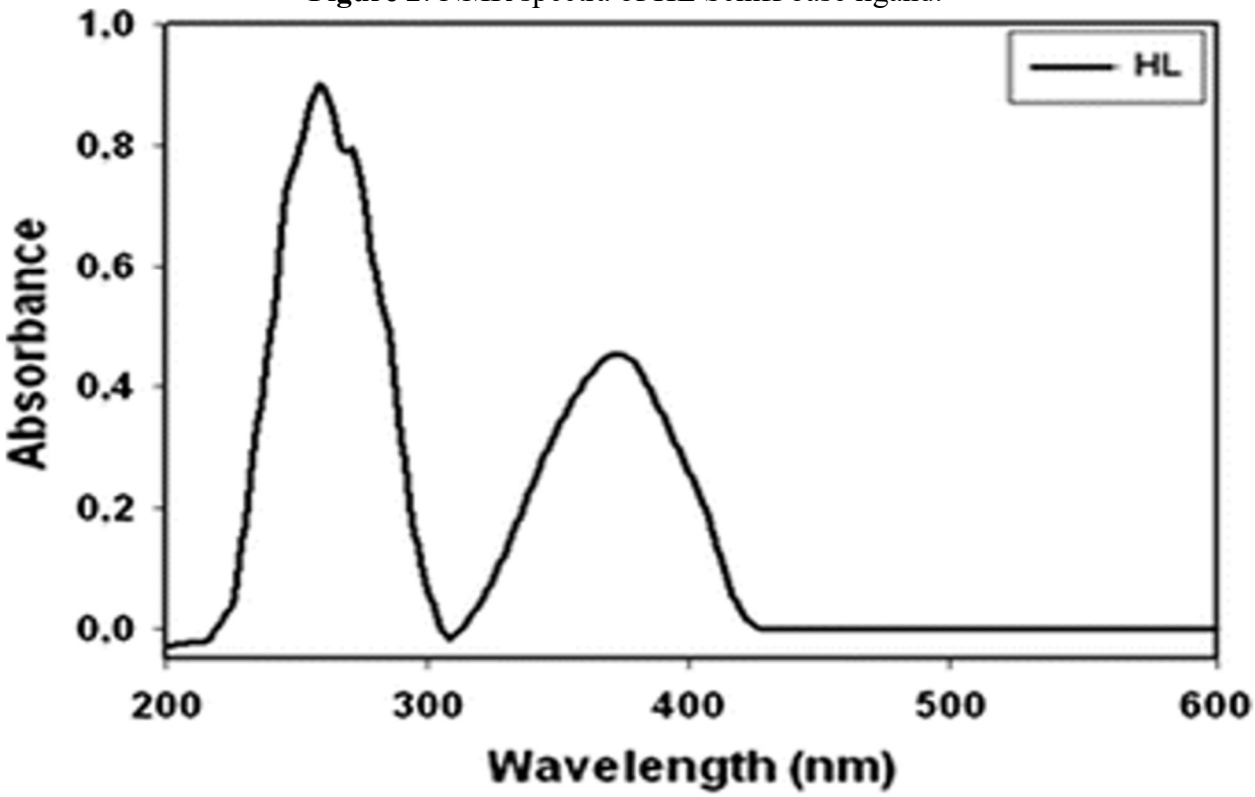


Figure 3: UV-Vis spectra of HL Schiff base ligand.

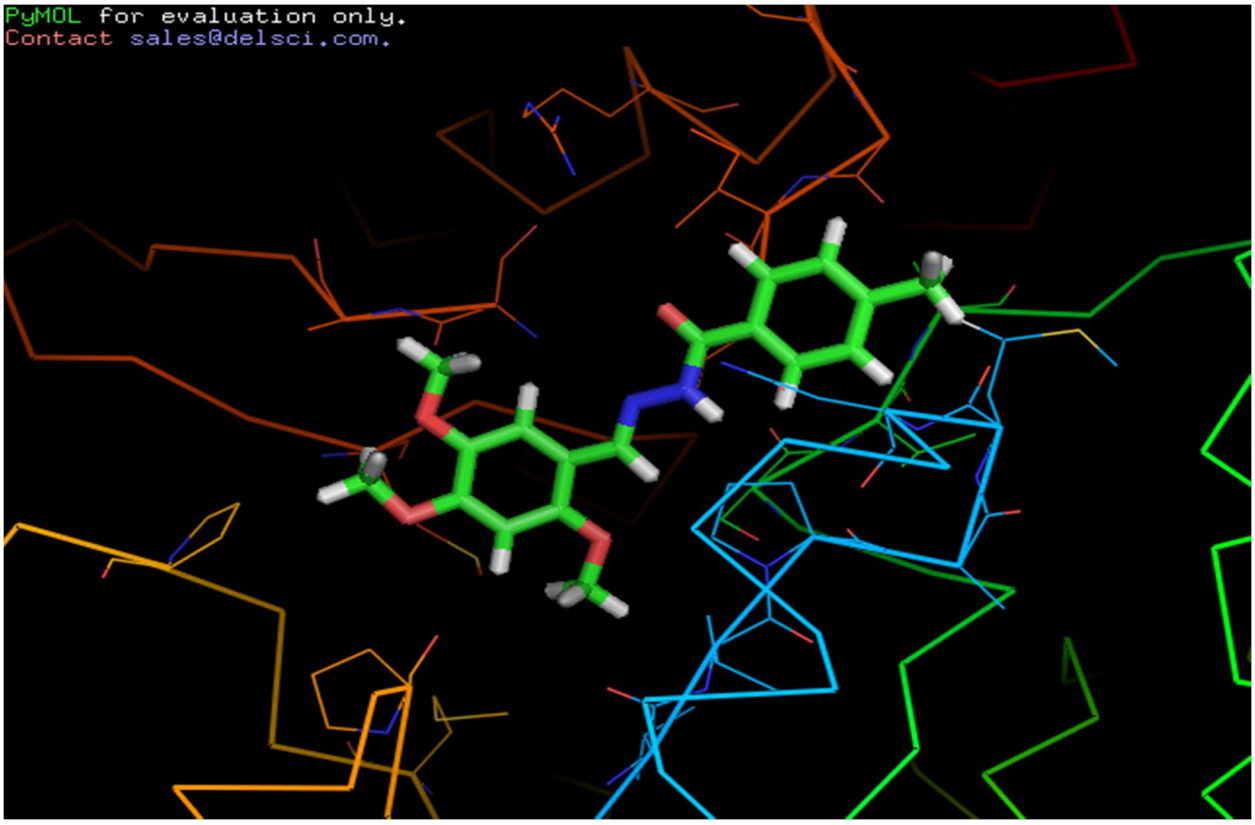


Figure 4: Binding energy analysis of Schiff base derivatives HL with antimicrobial enzymes (GlcN-6-p synthase) using PatchDock.

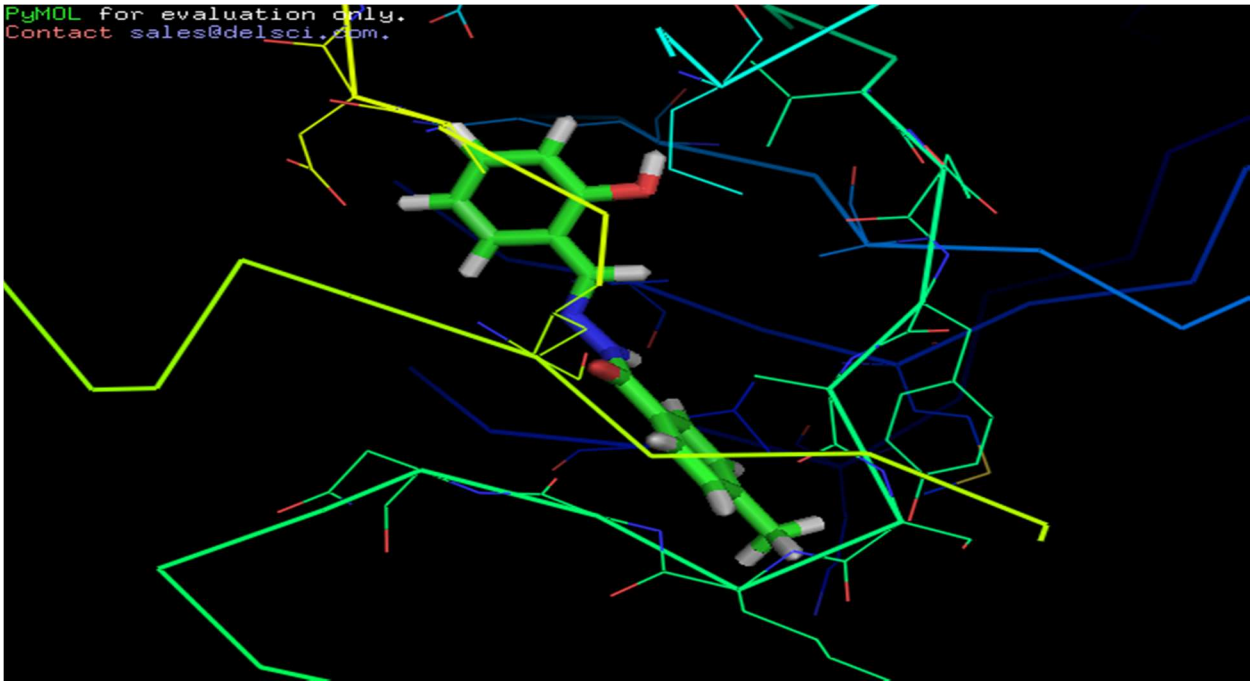


Figure 5: Binding energy analysis of Schiff base derivatives HL with antimicrobial enzymes (EGFR tyrosine kinase) using Patch Dock.

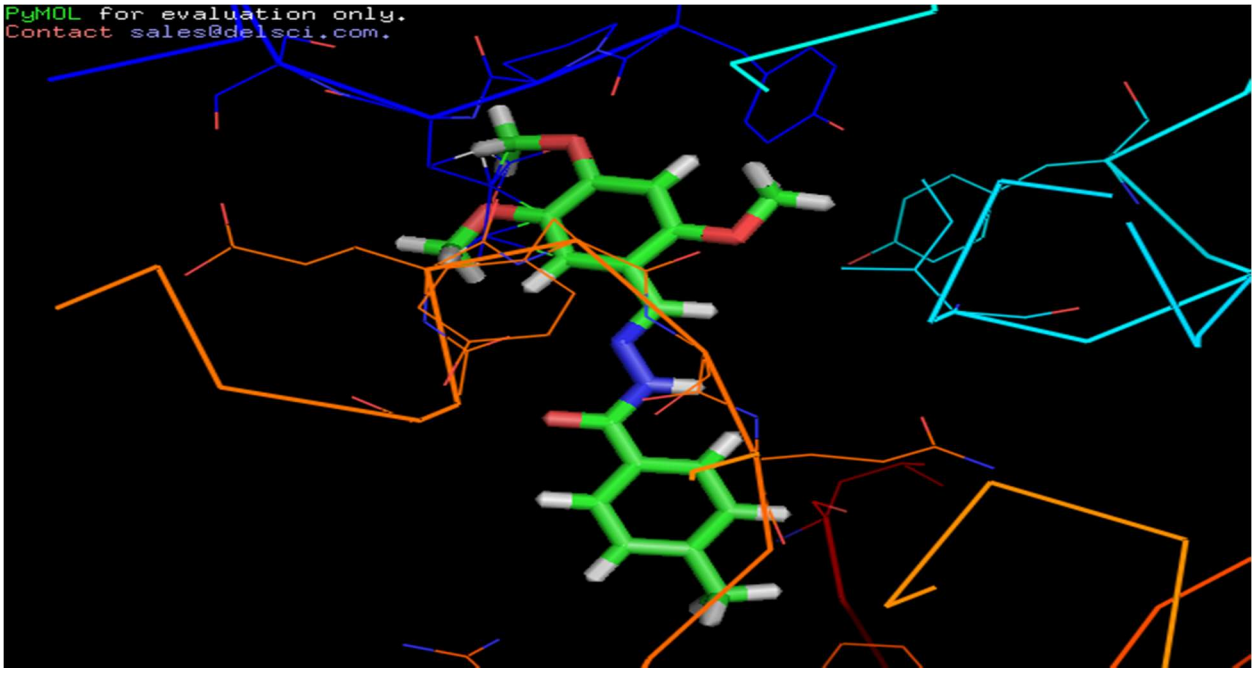


Figure 6: Binding energy analysis of Schiff base derivatives HL with antibacterial enzymes (E.coli) using PatchDock.