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Synthesis, Spectroscopic Characterization, molecular docking and In Vitro Antibacterial and antifungal Activity of some Schiff base ligands

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Abstract:

Four Schiff base ligands (L_1–L_4) were synthesized by reacting 1, 3 – Bis(4-aminophenoxy) benzene with 2-hydroxy-benzaldehyde, 5-Chloro-2-hydroxybenzaldehyde, 5-Bromo-2-hydroxybenzaldehyde, 2-hydroxy-5-methylbenzaldehyde. By using ^{1}H NMR, ^{13}C NMR, UV–Visible, FT-IR, HRMS technique synthesized ligand were characterized. These ligands exhibited efficient antibacterial activity against four different human pathogenic bacterial strains such as gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, and gram-positive *Staphylococcus aureus*, *Bacillus substilis* whereas antifungal activity against *Candida albicans* and *Aspergillus niger* fungal strains. The ligand of L_3 act as the most active antibacterial agent and show better antibacterial activity with MIC 135 ± 0.39, 122 ± 0.12, and 130± 0.54 respectively against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis*.The results of the molecular docking analysis for the synthesized compounds indicate that L_1, L_2, L_3 and L_4 exhibit the strong antibacterial activity when compared to standard ampilcillin .

Keywords: Novel Schiff base, Molecular Docking, Antimicrobial and Antifungal Activity.

INTRODUCTION:

Hugo Schiff was the first the chemist who reported synthesis of Schiff base. It is an organic molecule having general formula of $R_1R_2C{=}NR_3$ (R_3 = alkyl or aryl, but not hydrogen) [1-3]. Condensation of Primary amine with an aldehyde or ketone produces Schiff bases. Schiff bases are nitrogen counterparts of aldehydes or ketones where the carbonyl

group has been replaced by an azomethine or imine group [4]. Schiff bases act as ligands because they contain an imine group. [5,6]. There is various imine or azomethine groups in a variety of naturally occurring and synthetic chemicals. It has been proven that the imine group in these chemical compounds is crucial to their biological functions [7-9]. It has been found that few Schiff bases exhibits excellent antifungal, antibacterial, and anticancer properties [10, 11], few Schiff bases and their metal complexes were used in transistors [12], as a catalyst, in pharmaceuticals [13] in defense, pyrotechnic mixes [14]. They have also been used in dyes [15], lasers [16], gas-generating agents [17], nanotechnology [18,19].Remarkable cytotoxic action was shown by some complexes against hepatic cellular carcinoma cells (HepG-2) and colon cancer cells (HCT-116 cell line) [20]. It was discovered few Schiff bases had the ability to intercalatively bind to DNA [21]. Some of the most used organic compounds are schiff bases. They are used as polymer stabilizing agents, catalysts, intermediates in chemical reaction, pigments and dyes [22].

When Schiff base ligand treated with transition metal salts, the resultant heterocyclic moiety forms coordination complex with enhanced physiochemical and pharmacological properties [23-26]. Salicylaldehyde derivatives with one or more halo atoms in the aromatic ring have also been reported showing a number of biological activities, such as antimicrobial and antifungal activities [27].

Multiple antibiotic resistances in bacteria are the root cause of the rise in the death rate due to infectious diseases. The key cause of this particular problem is the absence of effective medical treatments. [28, 29] certainly, there is medical need for the development of new antibacterial medicines with distinct and more effective mechanisms of action [30]. There have been reports that Schiff bases are effective antibacterial agents [8].

The aim of this work is to prepare Schiff base ligands (L_1, L_2, L_3, L_4) condensation reaction of 1, 3 – Bis(4-aminoPhenoxy) benzene with various substituted benzaldehyde respectively and characterized them. In addition, the biological activities were checked to know biological potential of synthesized Schiff base.

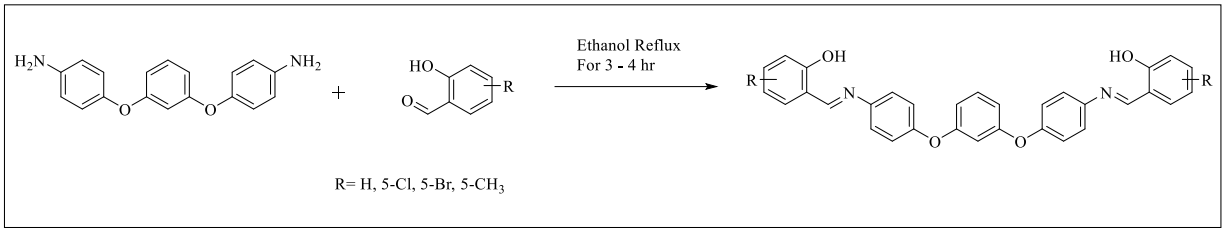
2. EXPERIMENTAL

Materials and Methods

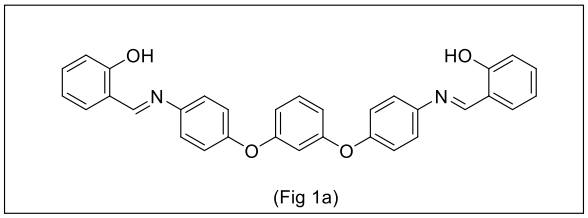
All necessary chemicals and reagents were purchased from Merck chemicals. All commercially available chemicals and solvents were used exactly as they were supplied. All the chemical transformations were monitored by using Thin Layer Chromatography (TLC) on a 2–5 cm percolated E. Merck Silica Gel 60 F254 plates (0.25 mm) and spots were visualized using UV lamp. To find the chemical composition of produced compounds a Perkin-Elmer 1200 FT-IR spectrometer was used. All melting points were determined on a Biotechnic India capillary melting point apparatus and are uncorrected. NMR spectra were recorded with a Bruker AM-400 MHz spectrometer in $CDCl_3$, with $(CH3)_4Si$ is an internal standard for ^{1} H NMR spectra and solvent signals as internal standard for ^{13}C NMR spectra at ambient temperature. Chemical shifts (δ) are reported in ppm. Coupling constants J were reported in Hz. NMR signal splitting patterns were designated as follows: s - singlet, d - doublet, dd -doublet of doublet, dt -doublet of triplet, td - triplet of doublet, t- triplet, q- quartet, p-pentet, bs-broad singlet, m-multiplet, br-broad The electronic absorption of synthesized compounds was examined with a UV-Visible spectrophotometer (Analyticjena, UV-1800). The Schiff base ligands' ^{1}H-NMR data was acquired at 400 MHz with a Bruker Avance. ^{1}H-NMR

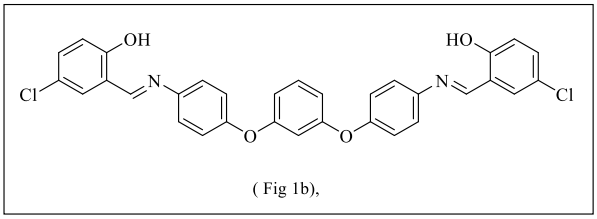
General Procedure for the synthesis of Schiff base ligands (L_1 –L_4)

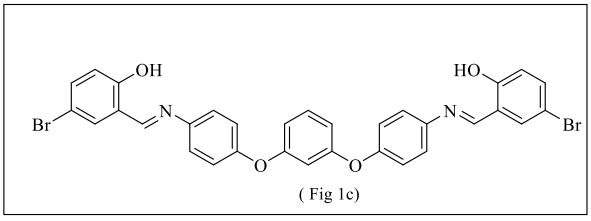
Prepared ligands are tetra dentate consisting of ONNO. 1,3 – Bis(4-aminoPhenoxy) benzene was made to react with 2-hydroxy-benzaldehyde, 5-Chloro-2-hydroxybenzaldehyde, 5-bromo-2-hydroxybenzaldehyde, 2-hydroxy-5-methylbenzaldehyde in 1:2 ratio in ethanol and the reaction mixture is refluxed for 3–4 h to yield Schiff bases L_1, L_2, L_3 & L_4, respectively **(Scheme-I).**

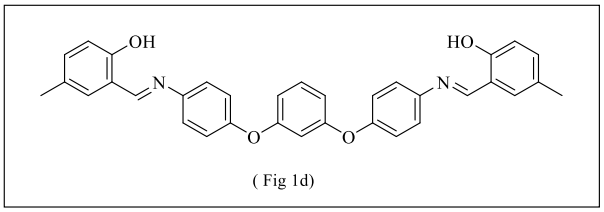


Scheme-I: General Synthetic scheme for Schiff base Ligand (L_1 -L_4)


(Fig 1a)


(Fig 1b),


(Fig 1c)


(Fig 1d)

(Fig. 1).

Spectral data

2,2'-((1E,1'E)-(((1,3-phenylenebis(oxy))bis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene))diphenol (L_1)Fig 1a)

Yield: 81 % %; mp: 106-108°C; FT-IR (KBr, ν/cm^{-1}): 3401 (OH), 3048 (Ar–C–H), 1618 (C=N), 1566 (C=C), 1584, (C-O) 1266,1209,1183,1127; ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm: 13.25 (2H, s, Ar-OH 8.55 (2H, s, Azomethine)7.44 - 7.51 (d, 5 H) 7.33 - 7.35 (s, 1 H) 7.29 - 7.32 (m, 3 H) 7.28 (s, 1 H) 7.11 - 7.12 (t, 3 H) 7.08 - 7.10 (t, 2 H) 6.94 (s, 1 H) 6.92 (s, 1 H) 6.81 (d, *J*=2.25 Hz, 1 H) 6.79 (d, *J*=2.38 Hz, 1 H) 6.74 – 6.75 (t, 1 H); ^{13}C NMR (101 MHz, CHLOROFORM-*d*) δ ppm: 159.94 , 159.73, 158.09, 155.78, 143.14, 135.29, 133.81, 130.35, 122.32, 120.27, 119.59, 118.92, 113.34, 110.21, 109.26, 76.89, 76.68, 76.36; Mass (m/z): 501.18 [M+1].

2,2'-((1E,1'E)-(((1,3-phenylenebis(oxy))bis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene))bis(4-chlorophenol)(L_2)(Fig 1b),

Yield: 79 %; mp:174-176 ◦C; FT-IR (KBr, ν/cm^{-1}): 3418 (OH), 3071 (Ar–C–H), 1619 (C=N), 1506 (C=C), 1378,1358,1260,1240 (C-O); ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 13.20 (2H, s, Ar-OH 8.54 (2H, s, Azomethine) 7.35 (d, *J*=2.50 Hz, 2 H) 7.31 - 7.33 (m, 2 H) 7.29 (m, 3 H) 7.25 (s, 1 H) 7.09 - 7.10 (t, 3 H) 7.07 - 7.08 (t, 2 H) 6.97 (s, 1 H) 6.95 (s, 1 H) 6.79 (d, *J*=2.25 Hz, 1 H) 6.77 (d, *J*=2.38 Hz, 1 H) 6.72 - 6.73 (t, 1 H) ^{13}C NMR (101

MHz, CHLOROFORM-*d*) δ ppm 160.42, 159.61, 158.45, 156.11, 143.54 , 132.85, 131.15, 130.68, 123.72, 122.65, 119.95 ,118.83 , 113.68, 109.58, 77.34, 77.22, 77.02, 76.70. Mass (m/z): 569.10[M+1].

2,2'-((1E,1'E)-(((1,3-phenylenebis(oxy))bis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene))bis(4-bromophenol) (L_3)(Fig 1C), Yield: 80 %; mp:158-160◦C FT-IR (KBr, ν/cm^{-1}): 3423(OH), 2978 (Ar–C–H), 1606 (C=N), 1504 (C=C), 1383,1272,1240,1228 (C-O); ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 13.24 (2H, s, Ar-OH) 8.53 (2H, s, Azomethine) 7.49 -7.50 (d, *J*=2.50 Hz, 2 H) 7.44 – 7.45 (d, *J*=2.50 Hz, 1 H) 7.42 (d, *J*=2.38 Hz, 1 H) 7.28- 7.33 (m, 4 H) 7.25 (s, 1 H) 7.09 - 7.11 (t, 2 H) 7.07 - 7.08 (t, 2 H) 6.93 (s, 1 H) 6.90 (s, 1 H) 6.79 - 6.80 (d, *J*=2.25 Hz, 1 H) 6.77 (d, *J*=2.38 Hz, 1 H) 6.72 - 6.74 (t, 1 H) ; ^{13}C NMR (101 MHz, CHLOROFORM-*d*) δ ppm 161.88, 161.02, 158.58, 155.64, 144.13, 133.06, 132.18, 130.57, 122.54, 119.95, 119.18, 119.08, 117.22, 113.38, 109.32, 77.32, 76.68. 569. Mass (m/z): 658.99[M+1].

2,2'-((1E,1'E)-(((1,3-phenylenebis(oxy))bis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene))bis(4-methylphenol) (L_4)(Fig 1D), Yield: 78 % mp:138-140◦C FT-IR (KBr, ν/cm^{-1}): 3425(OH), 2925 (Ar–C–H), 1614 (C=N), 1497 (C=C), 1384,1288,1263,1225 (C-O); ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 12.99 (2H, s, Ar-OH) 8.57 (2H, s, Azomethine) 7.31 (s, 1 H) 7.29 (s, 1 H) 7.29 (s, 1 H) 7.28 (s, 1 H) 7.26 (s, 1 H) 7.20 (d, 1 H) 7.18 (s, 3 H) 7.10 - 7.11 (t, 2 H) 7.07 - 7.08 (t, 2 H) 6.94 (d, 1 H) 6.91 - 6.93 (d, 1 H) 6.78 – 6.79 (d, *J*=2.38 Hz, 1 H) 6.76 - 6.77 (d, 1 H) 6.72 - 6.75 (t, 1 H) 2.32 (s, 6 H) ^{13}C NMR (101 MHz, CHLOROFORM-*d*) δ ppm 161.70, 155.35, 144.09, 133.77, 131.95, 130.35, 122.30, 119.74, 116.77, 115.79, 113.14, 109.09, 20.14, 9.63. Mass (m/z): 529.21[M+1].

Antibacterial activity

The antibacterial activity of the compounds was performed by enumerating the viable number of cells upon in the nutrient broth containing various concentrations of compounds. The viable number is represented by colony count method. The test organisms used on which the antibacterial activity was performed were *Escherichia coli* (NCIM2256), *Pseudomonas aeruginosa* (NCIM-2036), *Bacillus subtilis* (NCIM2063) and *Staphylococcus aureus* (NCIM-2901). In this method, the cells of test organisms were grown in nutrient broth till mid log phase and used as an inoculums for performing antimicrobial test. An approximately, 1×10^5 cells/mL test organisms were each inoculated with 0 to 500 ug/mL concentration of different compounds, separately, and each incubated for 16 to 18 h at 37 ◦C. During this incubation, cells tend to grow and multiply in number. However, if the compounds interfere with growth of cells, the numbers of cells decrease. After 16 to 18 h, viable numbers of cells were recorded by spreading an aliquot from the broth inoculated with test organisms and compounds as colony forming units per milliliter (CFU/mL). Minimum inhibitory concentration (MIC) was determined Ampicillin was used as standards for the comparison of antibacterial activity.

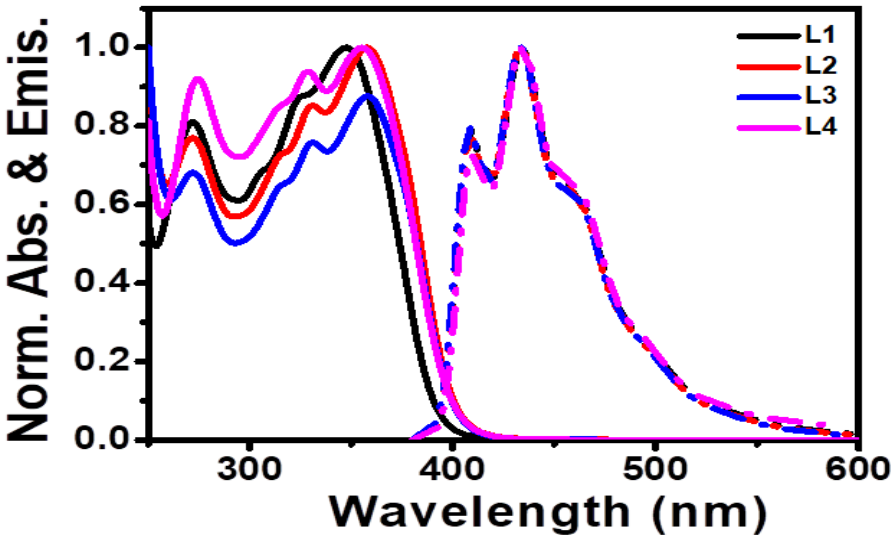
Antifungal Activity

Antifungal activity was determined by dilution method as per CLSI (formerly, NCCLS) guidelines. The synthesized compounds and standard miconazole were dissolved in DMSO solvent. The medium yeast nitrogen base was dissolved in phosphate buffer pH 7 and it was autoclaved at 110 ◦C for 10 min.With each set a growth control without the antifungal agent and solvent control DMSO were included. The fungal strains were freshly subcultured

onto Sabouraud dextrose agar (SDA) and incubated at 25 ˚C for 72 h.The fungal cells were suspended in sterile distilled water and diluted to get 10^3 cells/mL. Ten microliters of standardized suspension was inoculated onto the control plates and the media incorporated with the antifungal agents. The inoculated plates were incubated at 25 ˚C for 48 h. The readings were taken at the end of 48 and 72 h. The MIC was the lowest concentration of drug preventing growth of macroscopically visible colonies on drug containing plates when there was visible growth on the drug free control plates.

RESULTS AND DISCUSSION

It was found that the synthesized Schiff bases agreed with the expected outcomes. **Table-1** shows chemical and physical data (L_1 to L_4) of all synthesized Schiff bases. Compound values obtained from experiments and those predicted theoretically agree well with the molecular formula. Electronic spectrum of produced Schiff bases ligands (L_1–L_4) have their absorption spectra characterized by two bands in the UV–visible region (Fig. 2). The n → π* transition of the azomethine group accounts for the observed characteristic bright band in the 350–360 nm range, in the lower energy region. It was reported that such ligands shows emission property and showed emission band at 440 nm [31].



(Fig. 2)

Table -1 Analytical and Physical data of Compound studied

Sr. No	Compound	Molecular Formula	Colour	Melting Point
1.	L1	$C_{32}H_{24}N_2O_4$	Light Yellow	106 -108
2.	L2	$C_{32}H_{22}Cl_2N_2O_4$	Pale Yellow	174 – 176
3.	L3	$C_{32}H_{22}Br_2N_2O_4$	Orange	158 - 160
4.	L4	$C_{34}H_{28}N_2O_4$	Orange (Shining with Crystal)	138 - 142

FT-IR spectral studies

The FT-IR spectra of the synthesized Schiff base ligands are as shown in **fig. 3** The FT-IR spectra showed the significant stretching frequencies associated with groups such as enolic –OH, azomethine C=N-, aromatic C= C, -CO-, etc. In the range of 3401–3425 cm^{-1} phenolic–OH stretching vibrations are seen likewise, azomethine stretching, shown in the range of 1594–1618 cm-1. It is a significant functional group [32]. Significant characteristic band in the FT - IR spectra of prepared Schiff base were shown in **Table – 2**

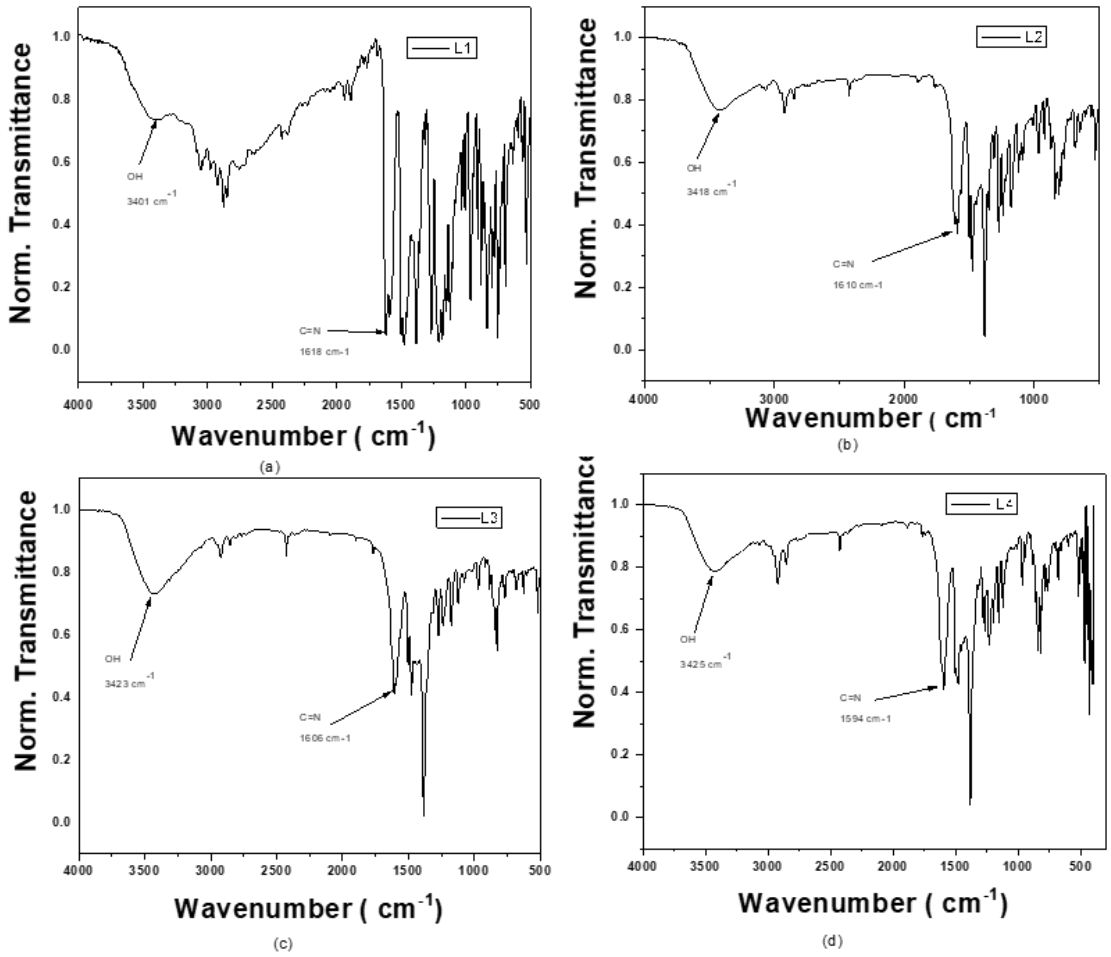


Fig. 3 FT- IR spectra of ligand L_1 (a), L_2 (b), L_3(c), L_4 (d).

TABLE-2

Sr. No	Compound	ν (OH/ H_2O/CH)	ν (C=N)
1.	L1	3401	1618
2.	L2	3418	1610
3.	L3	3423	1606
4.	L4	3425	1594

^{1}H NMR spectral studies

For all synthesized Schiff base ligand, the ^{1}H NMR spectra were recorded in $CDCl_3$ solvent and expressed in parts per million. The synthesized compounds show ^{1}H NMR spectra signals at 6.72–7.50 ppm that are produced by aromatic protons. A singlet that was detected

downfield at 12.99–13.25 ppm in the ^{1}H NMR spectra of the Schiff base ligands and that integrated for two proton is attributed to –OH [33]. Similarly, There is a singlet signal at 8.53–8.57 ppm that is the result of the azomethine proton, which is attached to the carbon nearby the nitrogen atom. [34]. proton ^{1}H NMR of ligand L_1 as shown in Fig. 3 & also ^{13}C NMR spectra of Ligand (L_1) as shown in fig. 4 .

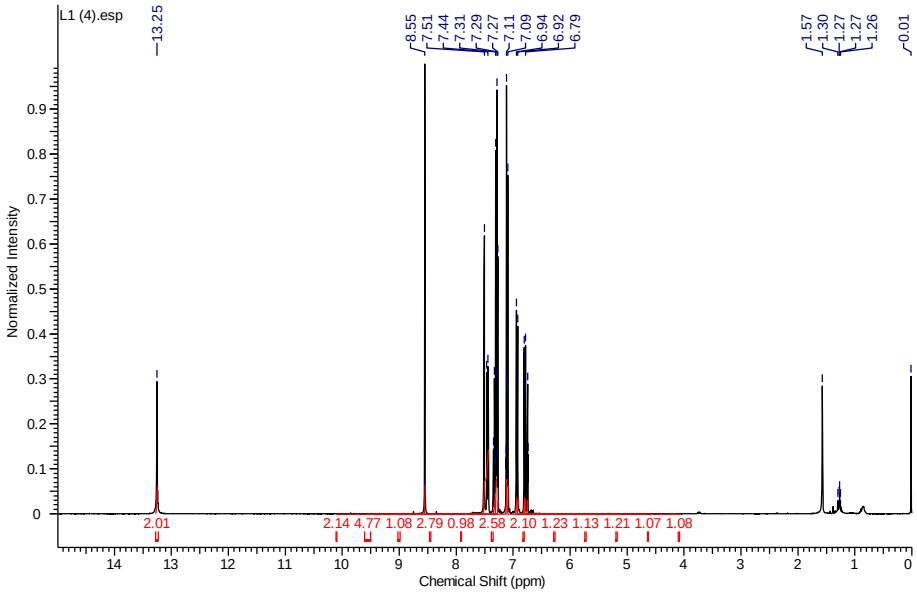


Fig. 3. ^{1}H NMR of ligands L_1

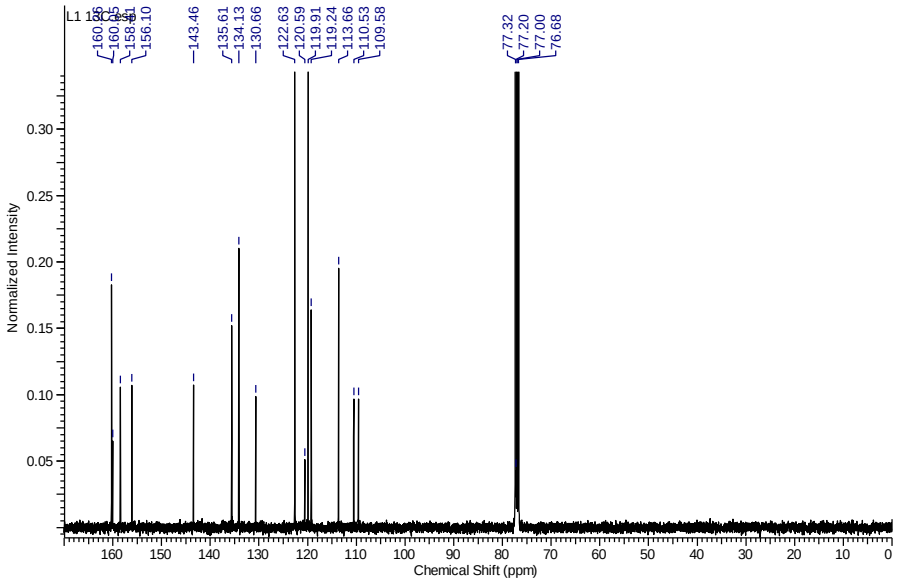


Fig. 4. ^{13}C NMR of ligands L_1

Mass spectral analysis

The mass spectra of all the Schiff base ligands exhibit parent ion peaks, due to their respective molecular ion (M+1), corresponding to the molecular weight and confirming their molecular composition. The proposed molecular formula of these compounds was confirmed by comparing their molecular formula weights with the *m*/*z* values

Mass spectra of the Schiff base ligand L_1 is depicted in Fig. 5

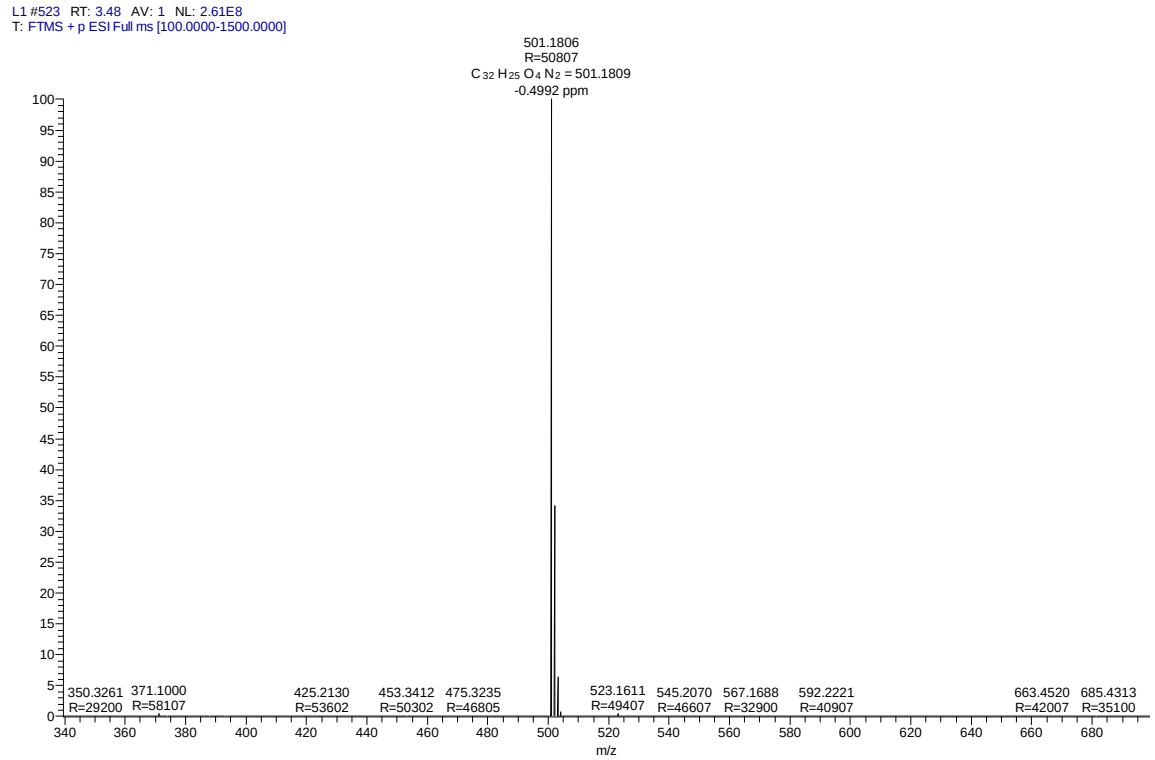


Fig. 5. Mass spectra of ligand L_1

Antimicrobial and antifungal activity:

The in vitro antibacterial and antifungal activities of each produced four ligands (L_1, L_2, L_3 and L_4) have been investigated and the results are collectively shown in Table 3 below. The dilution method [35 - 37] has been employed in this instance for calculating the Minimum Inhibitory Concentration (MIC).

To study the in vitro antibacterial activity of ligands, we have screened all the synthesized ligands against four different human pathogenic bacterial strains namely *gram negative Escherichia coli*, *Pseudomonas aeruginosa* and *gram positive Staphylococcus aureus*, *Bacillus subtilis*. To study the in vitro antifungal activity of ligands, we have screened all the synthesized ligands against two different human pathogenic fungal strains namely *Candida albicans* and *aspergillus Niger*. Here, we have used Ampicillin as standard antibacterial activity and *Miconazole* as standard for an antifungal activity to compare the biological activity results of screened ligands. The bioactivity results showed that most of the synthesized ligands possess better antibacterial activity against the tested bacterial strains as compared to Ampicillin. The ligands of L_3 act as the most active antibacterial agent and show better antibacterial activity with MIC 135 ± 0.39, 122 ± 0.12 and 130 ± 0.54, respectively against all four different tested human pathogenic bacterial strains used in this study. All the synthesized ligands were also observed active against *S. Aureus* with MIC ranging from 287 ± to 166 ± 0.29. Whereas, L_1, L_2, L_3, L_4 ligands show better antibacterial activity against *B. Subtilis* with MIC 296 ± 0.34, 157 ± 0.15,130 ± 0.54, and 166 ± 0.29 respectively. Compound L_3 Contain Br and having high antibacterial activity. It was observed that the antibacterial activity was seen in both gram positive and gram negative bacteria. The results from the current study are compared to previous reports [38, 39]. From the results of biological activity included in Table 3, it was concluded that almost all the synthesized ligands show good antibacterial activity against at least one of the tested strains whereas most

of the synthesized ligands display moderate antifungal activity. Therefore, the synthesized ligands would be acts as better antimicrobial agents.

Molecular Docking:

In order to assess the binding inhibition potentials of the synthesized compounds, we utilized the Autodock Vina program to perform docking simulations, positioning the optimized three dimensional structures of synthesized compounds within the active sites of the Crystal structure of Penicillin-Binding Protein 6 (PBP6) from E. coli in acyl-enzyme complex with ampicillin(PDB ID: 3ITA) [40].

The AutoDockTools 1.5.4 (ADT) was used to prepare the input files for docking [41]. The Quinolone core is extensively used for the design and synthesis of a numerous medicinally active analogs with variety of biological activity [42-43]. All water molecules and Ions were removed from the protein crystallographic structures, polar hydrogens were added and partial atomic charges were assigned by Kollmanunited charges method [44-45]. The pKa values of the residues in the enzyme were calculated to determine if any of them were likely to adopt nonstandard ionization states, using PROPKA 2.0 [46]. The side chains of the lysine, arginine, and histidine residues were protonated, while the carboxylic groups of glutamic acid and aspartic acid were deprotonated. For each ligand, nonpolar hydrogens were merged, Gasteiger charges were assigned, and rotatable bonds were setup. The structures were then saved in the corresponding pdbqt file required by Autodock. A grid box ofm 40×40×40 Å (*x*, *y*, and *z*) was created around the enzymes active pocket with the spacing of1nm in each dimension to evaluate the ligand–protein interactions. The center of the grid box was set to the average coordinates of the crystallography ligand in the pdb structure. Other vina docking parameters were set to default.

For a structural assessment of the antimicrobial potential of the synthesized derivatives, docking studies were carried out with Crystal structure of Penicillin-Binding Protein 6 (PBP6) from E. coli using the Autodock Vina docking tool. The docking results are presented in Table 3

Table-3. In vivo antibacterial, antifungal and Molecular docking of Synthesized analogs in the study

Compound	**MIC Value in µg/mL***						Free Energy of Binding (Kcal/Mol)
	Antibacterial Activity				**Antifungal Actvity**		
	E.coli	***P.aeruginosa***	***S.aureus***	***B.subtilis***	***C.Albicans***	***A.Niger***	
L_1	156± 0.38	138 ± 0.25	287± 0.13	296± 0.34	95±0.89	79±0.11	-6.04
L_2	194± 0.22	124± 0.47	*	157± 0.15	76± 0.05	56±0.72	-5.9906
L_3	135± 0.39	*	122± 0.12	130± 0.54	81±0.38	85±0.23	-6.056
L_4	187±	191± 0.54	188±	166±	101±0.	61±0.9	-6.0146

	0.62		0.16	0.29	34	0	
Ampicillin	100± 0.56	100± 0.08	250± 2.29	250± 0.55			-5.5601
Miconazole	*	*	*	*	25±0.97	25±0.58	*

All of the active compounds efficiently bound to the active site of Crystal structure of Penicillin-Binding Protein 6 (PBP6), forming strong interactions with specific residues within the binding pocket. In the molecular docking study, it was determined that among all the synthesized analogs, L_3 exhibited the highest activity with a binding energy of -6.056 Kcal/mol, followed by L_1 with a binding energy of -6.04 Kcal/mol. Synthesized analogs L_4 and L_2 displayed notable activity with binding energies of -6.0146 Kcal/mol and -5.9906 Kcal/mol, respectively. All compounds demonstrated significant interactions within the active site of Penicillin-Binding Protein 6 (PBP6) when compared with standard.

Synthesized compound L_3 (-6.056 Kcal/Mol) interact with Non polar aliphatic amino acid residue GLY81 by forming conventional hydrogen bond interaction with hydroxyl group of phenoxy ring with distance of 1.98 Å. Another Non polar aliphatic amino acid residue VAL84 interact with ether bridge Oxygen to from conventional hydrogen bond interaction with distance of 2.02 Å. The Positively charged amino acid ARG194 interact with imine bridge nitrogen atom to form conventional hydrogen bond interaction with 1.88 Å similarly ARG190 interact with hydroxyl oxygen atom to form conventional hydrogen bond interaction with 2.02 Å. The charged amino acid LYS88 and Polar amino acid SER106 interacts with bromine atom of terminal phenyl rings to form carbon hydrogen bond interaction with distance of 2.99 and 3.05 Å respectively. Positive charge amino acid ARG190 interact with hydrogen atom of Imine bridge to form carbon hydrogen bond interaction with distance of 2.50 Å. Hydrophobic amino acid PHE 86 and polar amino acid PRO192 interact with Pi electron cloud of phenyl rings to form Pi-Pi-Stack and alkyl and pi-alkyl interaction with various distance shown in figure 6.

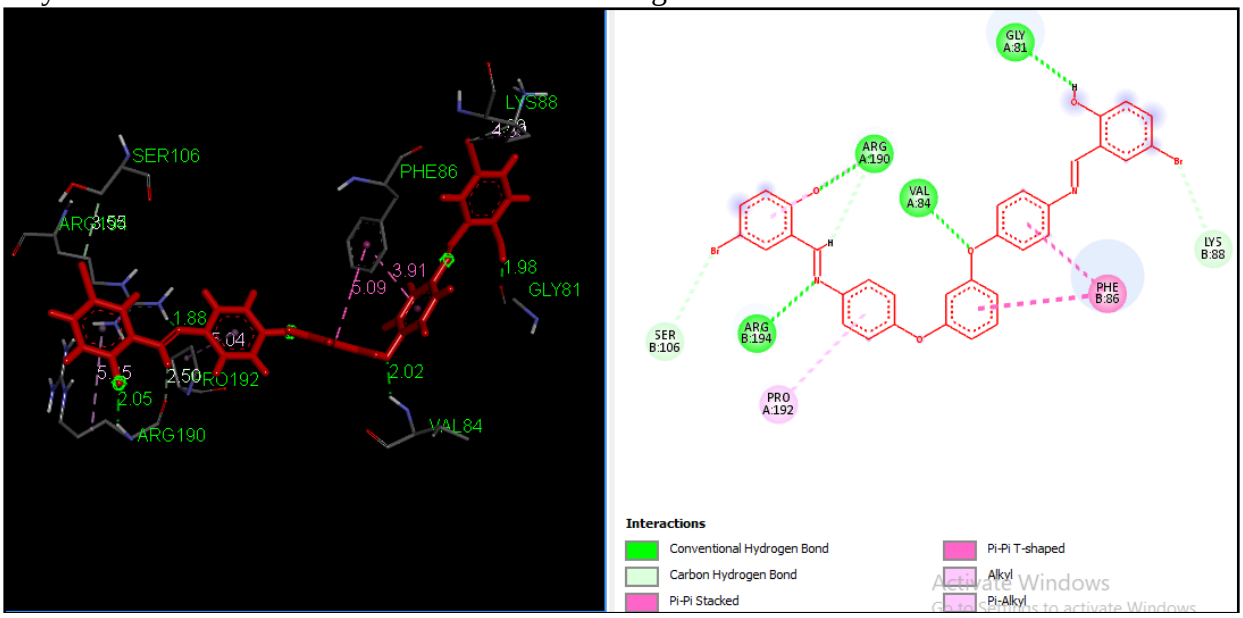


Fig. 6. Binding Pose and molecular interactions of **L_3** in active site of Penicillin-Binding Protein 6 (PBP6).

The synthesized analogue **L_1** (-6.04 Kcal/mol) interact interact with Non polar aliphatic amino acid residue VAL84 by forming conventional hydrogen bond interaction with ether bridge of biphenyl ring with distance of 2.27 Å. Another Non polar aliphatic amino acid residue ILE 104 interact with hydrogen atom of hydroxyl group to from conventional hydrogen bond interaction with distance of 2.15 Å. The Positively charged amino acid ARG194 interact with imine bridge nitrogen atom and oxygen atom of hydroxyl group of terminal ring to form conventional hydrogen bond interaction with 1.96 and 2.23 Å respectively. Negatively charged amino acid GLN105 interact with imine bridge hydrogen atom to form carbon hydrogen bond interaction with distance of 2.84 Å. Polar amino acid SER106 interact ether bridge of biphenyl rings to form carbon hydrogen bond interaction with distance of 3.02 Å. Active site nonpolar, polar and positively charged amino acid residue interact with Pi electron cloud of phenyl rings to form Pi-Pi-Lone and pi-alkyl interaction with various distance shown in figure 7.

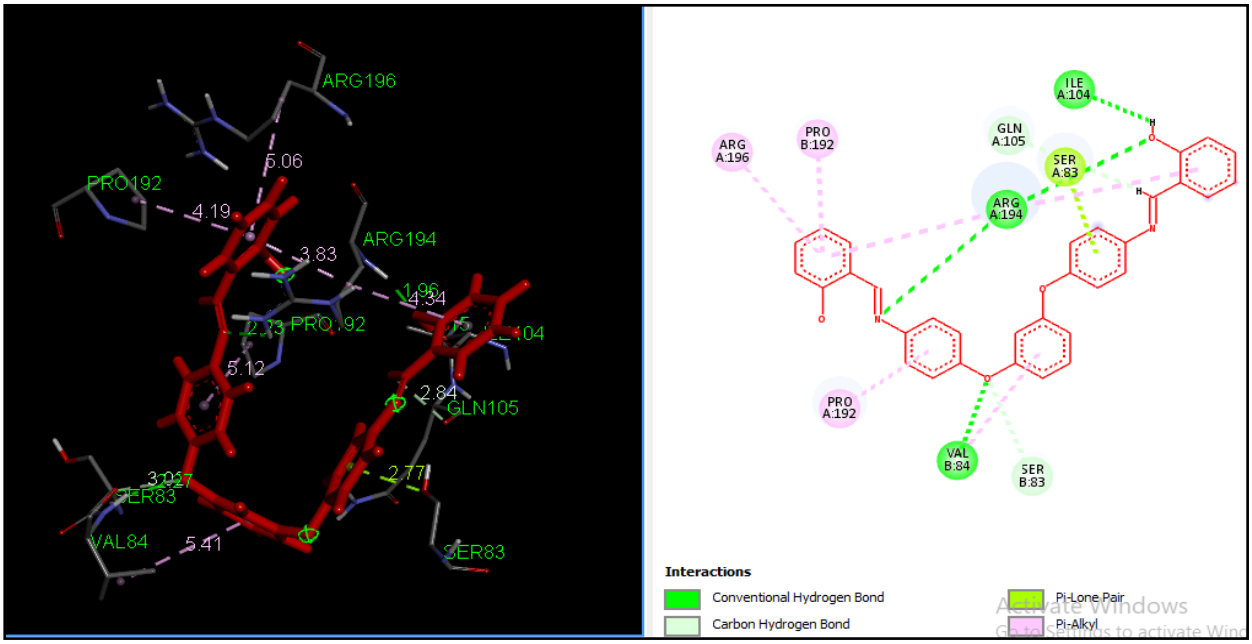


Fig.7. Binding Pose and molecular interactions of **L_1** in active site of Penicillin-Binding Protein 6 (PBP6).

The synthesized analogue **L_4** (-6.0146 Kcal/mol) interact with Non polar aliphatic amino acid residue VAL83 by forming conventional hydrogen bond interaction with ether bridge of biphenyl ring with distance of 2.17 Å. Negative charged amino acid residue GLN105 interact with hydrogen atom of hydroxyl group of phenyl ring to from conventional hydrogen bond interaction with distance of 2.15 Å. The Positively charged amino acid LYS182 interact with hydrogen atom of hydroxyl group of phenyl ring to form conventional hydrogen bond interaction with 1.93 Å. Polar amino acid residue SER83 interact with hydrogen atom of Imine bridge to form carbon hydrogen bond interaction with distance of 2.60 Å. Positively charged amino acid ARG194 interact with Pi electron cloud of phenyl ring to form Pi-Donar hydrogen bond interaction. Active site nonpolar, polar, hydrophobic and positively charged amino acid residues interact with Pi electron cloud of phenyl rings to form Pi-Sigma, Pi-Pi-Stack, alkyl and pi-alkyl interaction with various distance shown in figure 8.

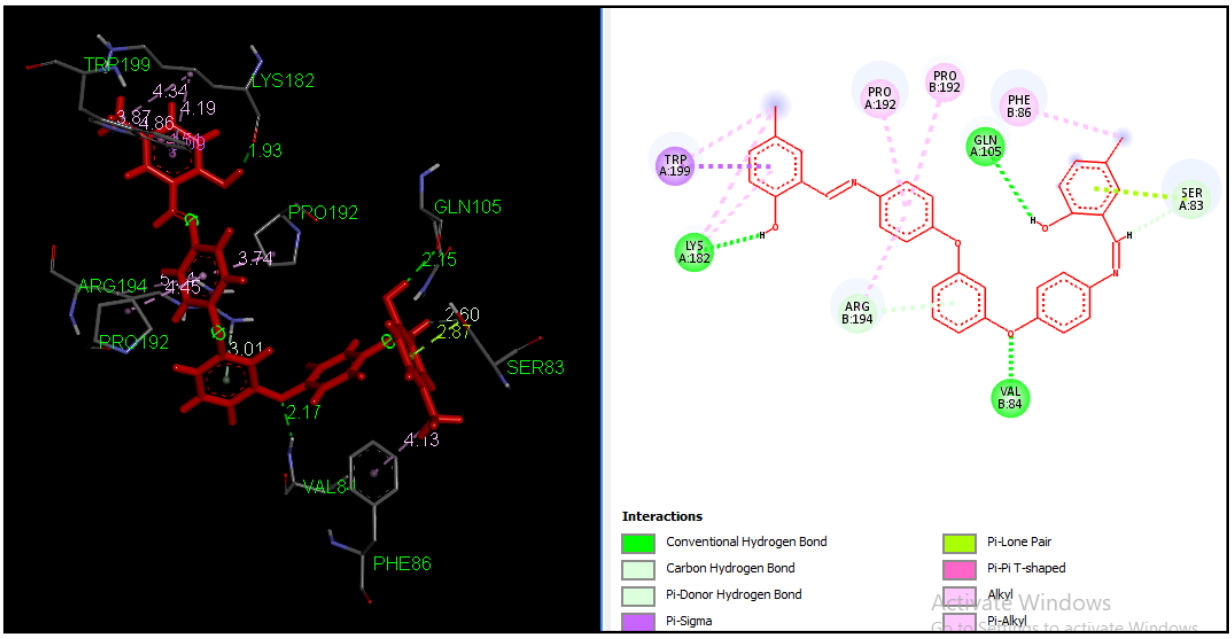


Fig.8.. Binding Pose and molecular interactions of **L_4** in active site of Penicillin-Binding Protein 6 (PBP6).

Conclusion:

In this research, four Schiff bases (L_1-L_4) were successfully synthesized with excellent yield. Spectral analysis confirmed the synthesized chemical structure. The results of the molecular docking study indicate that all the synthesized compounds, L_3, L_1, L_4 and L_2 exhibit the highest level of activity. These compounds show great promise as promising starting points in the quest for new antimicrobial drug candidates.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article

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