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Molecular docking and Antibacterial activities of Cobalt (II) complexes derived from precursors of Hydrazones

Richa Kothari[1]*, Anurag Agrawal[2] & Sanchita Rai[3]

[1]Department of Chemistry; School of Science; & [2]School of Pharmacy, ITM University, Gwalior-474 005, Madhya Pradesh, India

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The Schiff base ligands in their deprotonated forms have been utilized to synthesize thermodynamically and kinetically stabilized Cobalt(II) complexes. In the complexes, cobalt ion present is in distorted octahedral arrangement and is coordinated by four tridentate ligands in complexes. The synthesized Schiff base ligands coordinate with Cobalt (II) ion through four azomethine nitrogen atoms and two sulfur atoms developing a 6- membered chelate ring. Synthesized Cobalt(II) complexes *via* hexadentate ligands have been characterized thoroughly through various spectroscopic techniques like FT-IR, UV-Vis, ^{1}HNMR, TGA, TEM, SEM, Particle size, Elemental analysis (C, H, N, Co, S) and conductivity measurements. All Cobalt(II) complexes have been evaluated for *in vitro* antimicrobial activity against isolated bacterial strains of *E. coli* (MTCC-1687), *E. faecalis* (MTCC-439), *S. aureus* (MTCC-737) and MR *S. aureus* (Indigenous). All Cobalt complexes show mild to moderate antibacterial activity. The MIC ranged from 50 µg/ mL to 3.125 µg/ mL. All Cobalt(II) complexes displayed *in-vitro* antibacterial activity against both gram-positive and gram-negative bacterial strains. It may be proved that the antibacterial activity of the complexes is related to the cell wall structure of the tested bacteria. *In-vitro* toxicity tests explained the Cobalt complexes were less cytotoxic than the Vancomycin drug on A431 cancer cell lines and the results explain that synthesized Cobalt complexes can act as potent antimicrobial agents and can be considered as a good drug candidate for medicinal chemistry researchers.

Keywords: Antibacterial activity, Cobalt complexes, Disc diffusion method, Schiff base ligands, Spectroscopic characterization

Hydrazone Schiff base ligands with nitrogen donor atoms are a biologically important class of organic compounds that can bind with different transition metal ions with very important biological and non-biological properties and placed a very important role in the last few decades[1,2]. The very interesting point regarding this, these ligands can be easily synthesized by the condensation reaction of aromatic aldehydes and Ketones with a primary aliphatic or primary aromatic amine[3]. The coordination complexes of Schiff base ligands with transition metal ions in biochemistry, medicinal, metallo-organic and inorganic chemistry have great importance because of their wide applications in biological fields like antibacterial, antifungal, antiviral, antioxidant *etc*[4,5]. Transition metal ions display various biological, chemical, optical, electrical and magnetic properties by coordinating with different Schiff base ligands[6,7]. The literature revealed that Schiff base ligands play a very important role by coordinating as Chelating ligands in the main groups and transitions metal coordination chemistry because of their stability in different oxidation states and reduction states[8,9]. The coordination of nitrogen and sulfur donor ligands with transition metal ions gives metal complexes of diverse geometries and because of these unusual geometries of transition metal ions with ligands, these complexes have potential biological activities[10] like anti-cancer, antiviral, anti-malarial, anti-inflammatory, antifungal, antibacterial and antipyretic activity[11-14]. In literature, it has been revealed that metal coordination with Schiff base ligands can tremendously affect the antimicrobial/bioactive nature of the organic ligands; therefore, synthesis of numerous transition metal complexes has been attempts in this biological field[15].

In the past few decades, diverse bacterial infections and their resistance to many antibacterial drugs is a serious growing problem among researchers[16,17]. While in the microbiology field there are numerous classes of antibacterial drugs, which have some considerable developing resistance in most of the pathogenic bacteria like *S. aureus*, MRSA bacteria to existing antibacterial drugs like Vancomycin,

*Correspondence:
E-mail: richakothari70@gmail.com

Tetracyclin[18]. Due to the developing resistance of bacteria to existing antibacterial drugs, it is essential to develop novel chemotherapeutic agents or to increase the bioactivity of the existing antibacterial drugs[19,20]. Literature also reveals that transition metal incorporated antibacterial complexes seems to be a novel research area for developing a new methodology for new antibacterial drugs to prevent and control the growth of broad-spectrum bacterial strains [21,22].

In this paper, we report the template synthesis of hexadentatehydrazone Schiff base nitrogen sulphur donor ligands by the condensation of Schiff base ligands of aromatic aldehydes with primary aromatic amines in the presence of Cobalt(II) Chloride hexahydrate salt. The cobalt (II) complexes were prepared in methanol as a solvent. The synthesized Cobalt(II) complexes were fully characterized by various spectroscopic techniques like UV-Visible, FT-IR, XRD, TEM, SEM, particle size, EDX and screened for the antibacterial activity of ligands and their cobalt (II) complexes against a broad spectrum of gram-positive and gram-negative bacterial strains using Vancomycin antibiotic as a standard drug for comparison of antibacterial activity of compounds.

Materials & Methods

Materials

All chemicals and solvents which were utilized in the Synthesis and biological activities of compounds purchased from Merck [Darmstadt, Germany) and Sigma Aldrich company (St. Louis, MO, USA) and were used without further purification unless otherwise mentioned. UV-Vis absorption spectra were recorded on a Perkin Elmer UV/Vis Lambda 25 using a 1 cm path length cell with dichloromethane solvent. ^{1}H-NMR spectra of ligands were recorded on a BRUKER Advance Neo 500 MHz spectrometer using dimethyl sulphoxide–d_6 as solvent using tetramethylsilane as an internal standard. The Fourier- transform infrared spectroscopy (FT-IR) spectra (KBr pellets) were recorded using a Perkin Elmer FT-IR spectrometer with a wavelength range from 400 to 4000 cm^{-1}. Melting points of compounds were obtained by an electro-thermal melting point apparatus and were not corrected. The elemental analysis was conducted using CHN Analyser, Thermo –Flash EA-1112 Series at the temperature up to 900°C and vanadium pentaoxide (V_2O_5) was used as an oxidizer to prevent inhibition caused by sulphur element. Thin Layer Chromatography (TLC) was performed using n-hexane/EtOAc (1:3) as an eluent. X-ray measurements for the ligands and their Cobalt(II) complexes were performed at room temperature using a Bruker axis D8 using CuK α radiation.

Preparation of schiff base ligands

Schiff Base ligands were prepared by the reaction with carbohydrazones of para-chloroacetophenone, para-nitro acetophenone, para-methoxyacetophenone with 4-methyl aniline and thiosemicarbazide hydrochloride in molar ratio 1:1.

Synthesized of correspondingschiff base ligands as described below

A mixture of carbohydrazone of para-chloro-acetophenone, para-nitro acetophenone, para-methoxyacetophenone and thiosemicarbazide hydrochloride was added to glacial acetic acid as a catalyst to round bottom flask in 50 mL ethanol as solvent. The reaction mixture was refluxed for 4 h at 80-90°C. The progress of the reaction was checked by Thin Layer Chromatography. After completion of the reaction, the resulting mixture was cooled to room temperature. The solvent was evaporated under reduced pressure and the resulting precipitates were obtained. The precipitates were then recrystallized in hot ethanol.

Ligand 1(3{(2E)-2[1-(4-chlorophenyl ethylidene oxopropananide) bis thiosemicarazone) (L_1) ($C_{18}H_{17}O_2N_2Cl$)

Light yellow solid.Yield: 85%, m.pt. (210-214^0), Selected IR data (v, cm^{-1}): 2830,1680,1438, 1360, 1020, ^{1}HNMR (500 MH_2, DMSO-d_6 , δ, ppm): 10.08 (S, 2H, HC= N), 8.06 -7.90 (q, 12.8 Hz, 8H, Ar-H), 7.96 – 7.86 (d, 7.5 Hz, 2H , Ar-H), 7.58-7.65 (d, 7.5 Hz, 2H, Ar-H). UV- Vis (DMSO): λ_{max} (nm) = 280, 360 (Fig. 1).

Ligand 2(3-{(2E)-2[1-(4-nitrophenyl ethylidene oxopropananide) bis thiosemicarazone) (L_2)($C_{18}H_{17}O_4N_3$)

Dark yellow solid,Yield: 84%, m.pt. (208-210^0), Selected IR data(v, cm^{-1}): 3068, 3020, 2978, 2830, 1640, 1120. ^{1}HNMR (500 MHz, DMSO–d_6 , δ, ppm): 10.10 (S, 2H, HC= N), 8.68 -8.62 (m, 2 H, Ar-H), 8.12- 8.10 (m, 5 H, Ar-H), 7.30-7.10 (m, 8H, Ar-H), 3.60 (S, 6H, CH_3). UV- Vis (DMSO): λ_{max} (nm) = 260, 380 (Fig. 2).

Ligand 3 (3-{(2E)-2[1-(4-methoxyphenyl ethylidene oxopropananide) bis thiosemicarazone) (L3) ($C_{19}H_{20}O_3N_2$)

Colourless solid. Yield: 78% m.pt. 210-220°C. Selected IR data (v, cm^{-1}): 3064, 3040, 2995, 2840,

1642, 1160. ^{1}HNMR (500 MHz, DMSO–d_6, δ, ppm): 9.86 (S, 2H, HC=N) , 8.64- 8.60 (m, 2 H, Ar-H), 8.14-8.10 (m, 5 H, Ar-H), 7.20-7.10 (m, 8H, Ar-H), 3.50 (S, 6H, CH_3). UV- vis (DMSO): λ_{max} (nm) = 290, 320 (Fig. 3).

Preparation of Cobalt(II) complexes

All Cobalt(II) complexes were synthesized via a similar procedure. A stoichiometric solution of Schiff base ligand in methanol (4mmol) were mixed with 1 mmol hexahydrate Cobalt chloride and refluxed the resulting mixture for 8 h at 80°C. The coloured precipitate was filtered, dried, washed with ethanol and then dried in a vacuum desiccator.

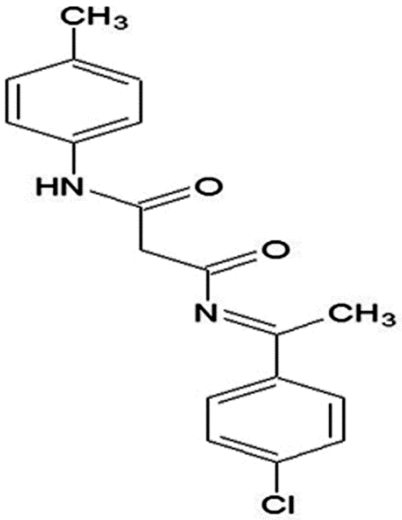

Fig. 1 — Structure of Ligand -1(3{(2*E*)-2[1-(4-chlorophenyl ethylidene oxopropanamide) *bis* thiosemicarazone) (L_1)

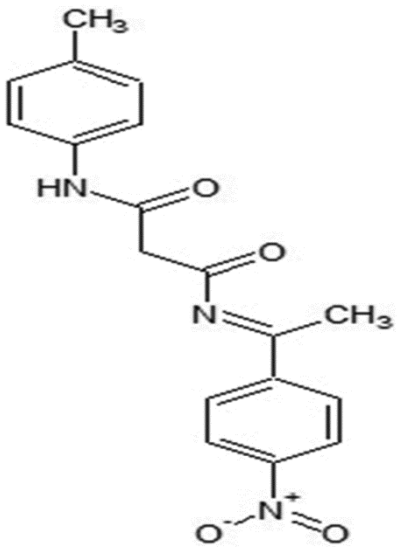

Fig. 2 — Structure of Ligand-2 (3-{(2*E*)-2[1-(4-nitrophenyl ethylidene oxopropananide) *bis* thiosemicarazone) (L_2)

[Co(2-{1-(4-chlorophenylethylideneoxopropananide) bis (thiosemicarbazone) Cl_2 })][CoL_1]

Light green solid, Yield: 89%. Melting point: 250-290°C. Molar conductivity (Ω^{-1} mol^{-1} cm^2): 24. Selected IR data (v, cm^{-1}): 3112, 2982, 1661, 1528, 1420, 1324, 698, 520, 482. UV-vis (DMSO): λ_{max} (nm) = 330, 420, 620, 680 (Fig. 4).

[Co(2-{1-(4-nitrophenylethylideneoxopropananide)bis (thiosemicarbazone) Cl2 })] [CoL2]

Dark green Solid, Yield: 75%, Melting point 320-325°C, Molar conductivity (Ω^{-1} mol^{-1} cm^2): 18.

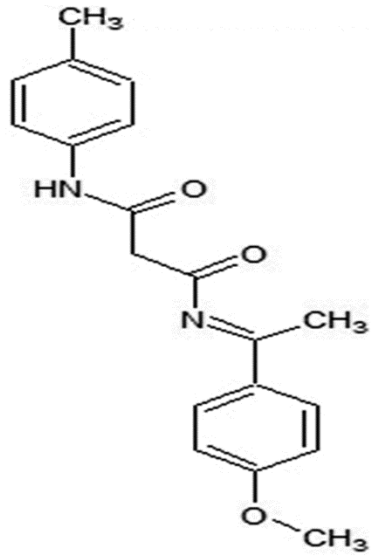

Fig. 3 — Structure of Ligand-3 (3-{(2E)-2[1-(4-methoxyphenyl ethylidene oxopropananide) bis thiosemicarazone) (L3)

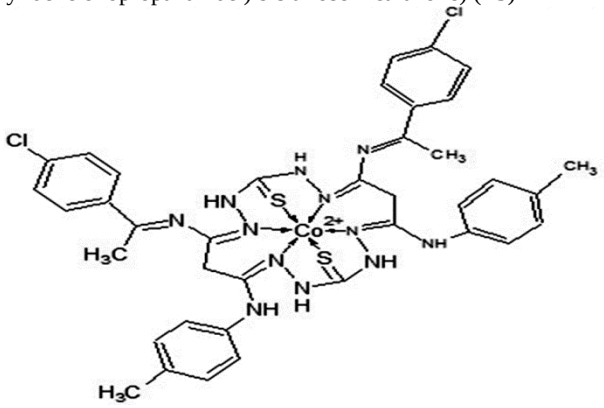

Fig. 4 — Structure of [CoL1]Complex [Co(2-{1-(4-chlorophenylethylideneoxopropananide) bis(thiosemicarbazone) Cl2})] complex

Selected IR data (v, cm^{-1}): 3260, 3116, 1626, 1585, 1360, 668, 520 , 470. UV-vis (DMSO): λ_{max} (nm) = 280, 260, 590, 680, 780 (Fig. 5).

[Co(2-{1-(4-methoxyphenyl ethylideneoxopropananide) bis (thiosemicarbazone) Cl2 })] [CoL3]

Dark Green Solid, Yield: 70% , Melting point 315-312°C, Molar conductivity (Ω^{-1} mol^{-1} cm^2): 15. Selected IR data (v, cm^{-1}): 3240, 3106, 1624, 1560, 1380, 662, 514, 440, UV-vis (DMSO): λ_{max} (nm) = 260, 280, 595, 660, 760 (Fig. 6)

Antibacterial study

Procurement of MTCC cultures of bacteria from PGI Chandigarh which are *E. Coli* (MTCC-1687), *E. faecalis* (MTCC-439) and *S. aureus* (MTCC-737) and indigenous Methicillin-Resistant *S. auresus* isolates was used. Stocks of the experimental compound of concentration of 10 mg/mL were prepared in DMSO passed through 0.22 mm dissociable syringe filter aseptically for sterilization followed by preparation of successive 5 dilutions using sterile distilled water. For antibacterial activity nutrients, agar media plates were prepared for working with bacteria. Bacterial inoculums were taken from the broth of revived cultures using a sterile swab and seeded onto the nutrient agar media followed by punching of 5 wells of 6mm diameter. 10 microliter of different dilution of each compound was poured into every 5 different wells of pre-inoculated culture plates separately with different microbial species. The culture plates were incubated at 37± 1°C for 24 h, respectively. Observations were taken in the form of the zone of inhibition (in mm) after incubation.

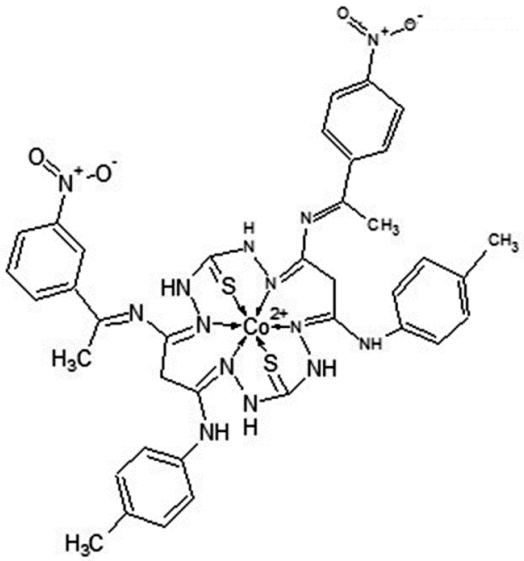

Fig. 5 — Structure of [CoL2][Co(2-{1-(4-nitrophenyle-thylideneoxopropananide)bis (thiosemicarbazone) Cl2 })] complex

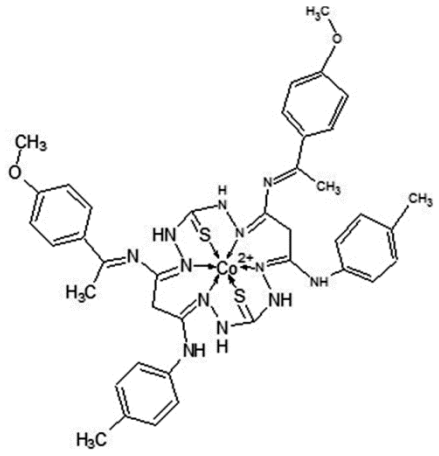

Fig. 6 — Structure of [CoL_3][Co(2-{1-(4-methoxyphenyl ethylideneoxopropananide) *bis* (thiosemicarbazone) Cl_2})] complex

Molecular docking studies of schiff base ligands & their Cobalt (II) complexes

The current research work is based on the antibacterial activity ofSchiff base ligands and their Cobalt (II) complexes evaluated on microorganisms i.e. *S.aureus*, *E.coli*. *etc*. The molecular docking study is carried out on Schiff base ligands and cobalt complexes to identify the antibacterial capabilities through inhibition of thymidylate synthase enzyme. As per our objectives, the three-dimensional crystal structures of the thymidylate synthase enzyme (PDB ID: 4QGG) was achieved from the protein data bank.

The blind molecular docking process was adapted to recognize the binding modes of cobalt complexes and ligands. Before further processing, the retrieved enzyme was cleaned in terms of removal of other ligands and water atoms. Then molecular target *i.e*. thymidylate synthase opened into the AutoDock execution window and hydrogens are added into the enzymes and saved as target .pdbqt. Schiff base ligands and cobalt complexes were drawn through ChemDraw Ultra 8.0 and their energy is minimized using MM2 force field and converted into .pdb format by OpenBable -2.3.2 software[23]. The ligands and their cobalt complexes were also processed into AutoDock execution window and their torsions along with rotatable bonds are assigned and the files are saved as Cobalt complex .pdbqt and ligand.pdbqt, respectively. The nine different conformers were fabricated of ligands and their cobalt complexes and conformers

that showed the lowest binding energy and better interactions with molecular targets were discussed. The docking parameters were defined as coordinates of the centre of binding site with x = 8.949, y = −7.784, z = 2.103 and in case of DNA gyrase, the docking parameters were x = 36.75, y = 42.75, z = 33.00 and binding radius = 1.000 Å and the grid dimension used for all the three (3) proteins are 47.25 × 47.25 × 47.25 Å (grid size) with point separated by 1.000 Å (grid-point spacing).

Results and Discussion

Characterisation of ligands

Three Schiff base ligands(L_1-L_3) were prepared by the condensation reaction of carbohydrazone of para-chloroacetophenone, para-nitroacetophenone, para-methoxyacetophenone with 4-methyl aniline, and thiosemicarbazide hydrochloride in a molar ratio of 1:1. To optimize the reaction condition of starting materials, the reaction of carbohydrazone of p-chloroacetophenone, p-nitroacetophenone,para-methoxyacetophenone and thiosemicarbazide hydrochloride (2 mmol) was carried out in different reaction conditions using different solvents. The highest Yield was obtained up to 85% with a shorter reaction time using ethanol as solvent (Fig. 7).

The ligands were characterized by various spectroscopic techniques like UV-Visible, FT-IR, XRD, TEM, SEM and EDX. The singlet peaks due to the azomethine group were observed in the range of 10.06 - 8.66ppm as a singlet in ^{1}HNMR spectra of Schiff base ligands. It has been observed that azomethine signals of the Schiff base ligands shifted to higher ppm deshielding effect when electron-withdrawing groups(-NO_2,-Cl) was present and signals were moved to a lower ppm (shielding effect) when electron-donating groups like –OCH_3 was present. The signals of the methyl groups (-CH_3) for the ligands are observed in the range of 3.60 - 3.76 ppm. The aromatic protons of the Schiff base ligands are observed in the range of 8.62-6.62 ppm (Fig. 8).

The FT-IR spectra of the ligands showed peaks in the range of 1660-1620 cm^{-1} assigned to ν (C=N). In

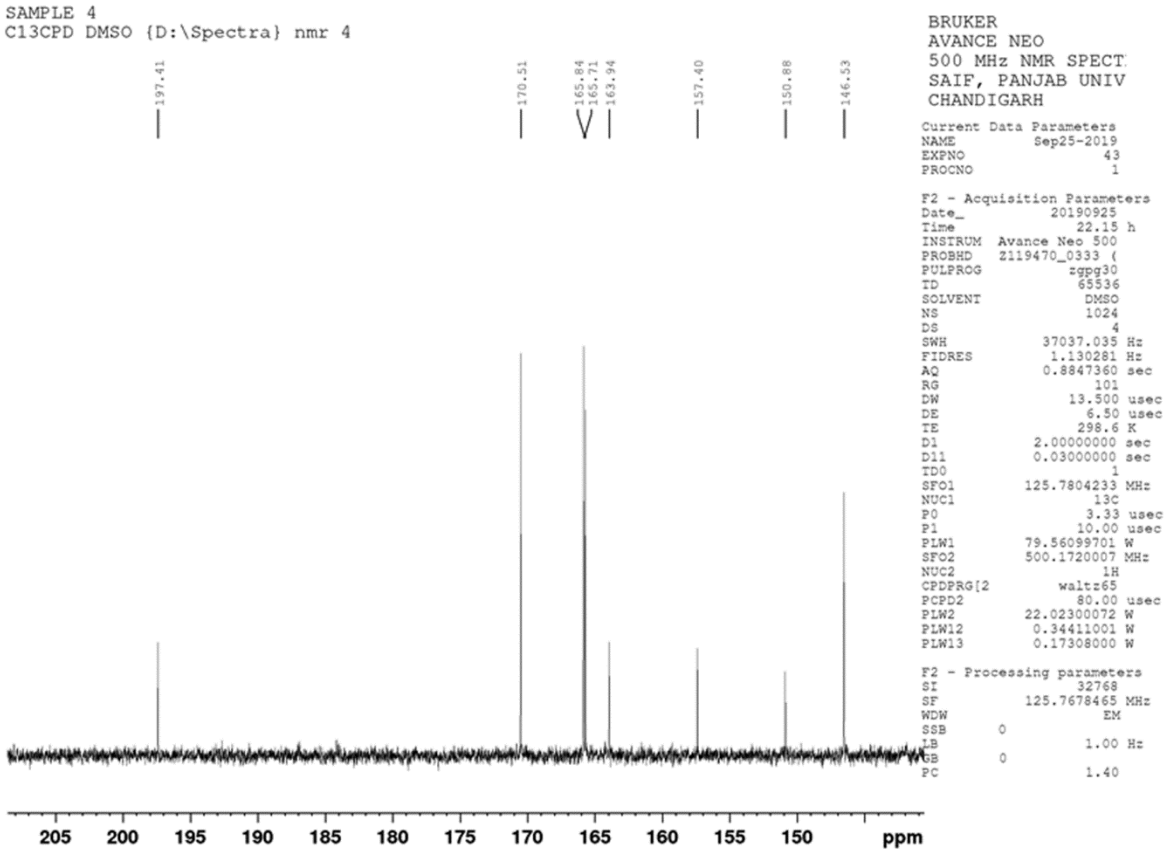

Fig. 7 — ^{1}HNMR Spectra of ligand

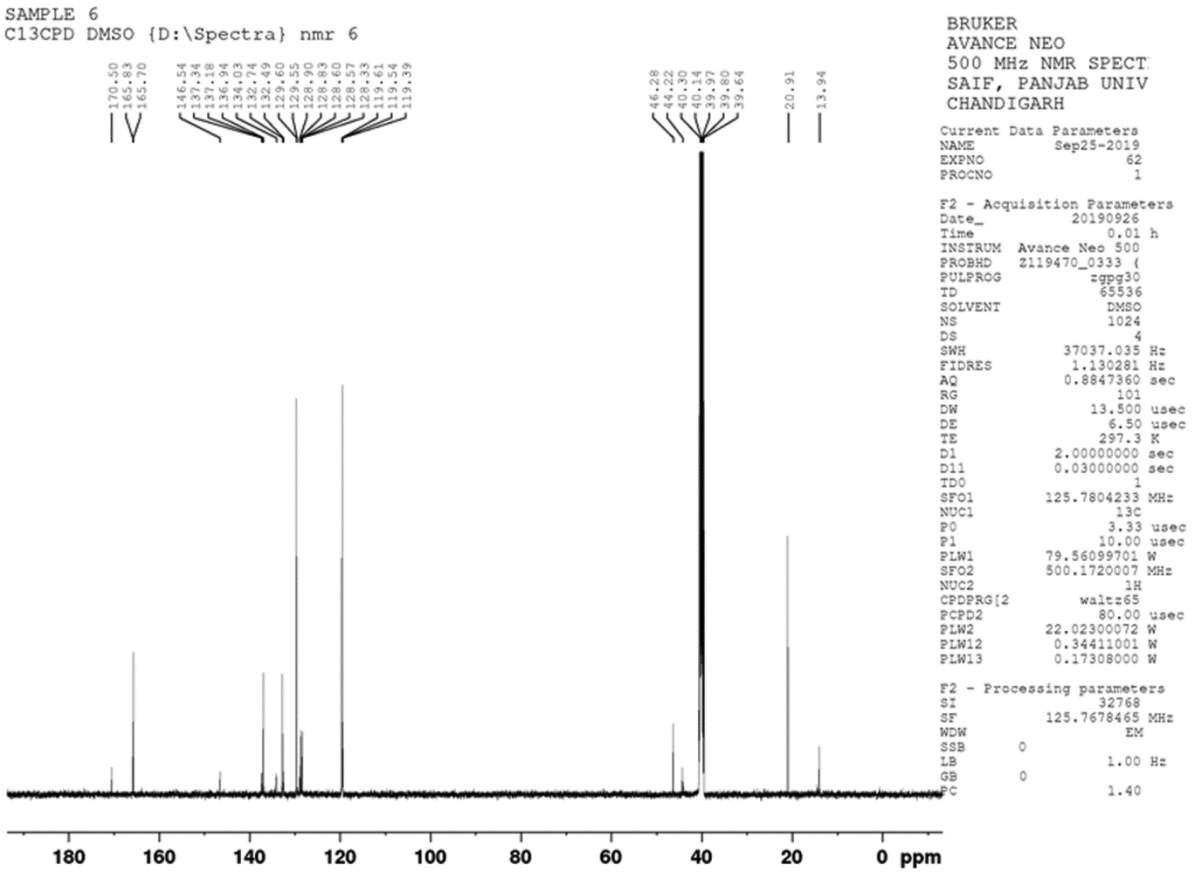

Fig. 8 — ^{1}HNMR Spectra of Coordinated Co(II) Complex

the L_2 Schiff base ligand, the IR peak observed in the range of 1566-1346 cm^{-1} is due to the presence of the nitro group.

The UV-Visible spectra of all the ligands are recorded in dimethyl sulphoxide (DMSO). In the electronic spectra of ligands two peaks appeared which are attributed to n- π* and π - π* transitions respectively. In the UV-vis spectra of the ligands with electron-withdrawing group (-Cl, -NO_2) the π - π* peak shifted to a lower wavelength side (blue shift) as compared to the ligands with electron releasing group like -OCH_3 group. The π- π* transition peak shifted towards the longer wavelength side (redshift) (Table 1).

Characterization of synthesised Cobalt(II) complexes

All synthesized Cobalt complexes are stable and coloured compounds. These complexes were prepared in very good yield (60- 85%). All are insoluble in ethanol, methanol but soluble in common organic solvents like DMSO and DMF. Cobalt complexes were characterized by various spectroscopic techniques like UV-Vis, FT-IR, XRD, TEM, SEM & EDX because the nature of Cobalt is paramagnetic so the ^{1}HNMR technique was not performed. The molar conductance values of the Cobalt (II) complexes in DMSO solvent (10^{-3} M solutions) was calculated at room temperature using the formula-

Table 1 — Electronic spectral of data of Schiff base ligands

Compound	Peak Position (nm)	Assignment
L_1	280	π-π*
	360	n- π*
L_2	260	π- π*
	380	n- π*
L_3	290	π- π*
	320	n- π*

$$\text{Molar conductance}\,(\Lambda\ m) = \frac{\text{Measured conductivity}}{\text{Concentration of the solutions}\ \ (\text{mol/L})}$$

Molar conductance measurements were used to establish the charge of the complexes. The molar conductivity of all Cobalt (II) complexes were lies in the range of (15-24) $\Lambda^{-1}\,mol^{-1}\,cm^{2}$, indicating that all complexes were non-electrolytic in nature.

UV-visible spectra

This is a very useful and reliable technique for the primary identification of formation metal complexes. For this UV- Visible absorption maxima exhibit in the range of 200-800 nm. The results obtained from spectra of cobalt (II) complexes showed approximately 260-780 nm for indicating the formation of cobalt complexes. A broad absorption peak at 590 nm was aroused due to the surface plasmon resonance absorption band along with free electronic vibrations of cobalt (II) complexes in resonance with a light wave (Fig. 9).

FT-IR spectral analysis

This is a very reliable and accurate spectroscopic method to characterize the presence of functionally active sites of the Schiff base ligands (Fig. 10).

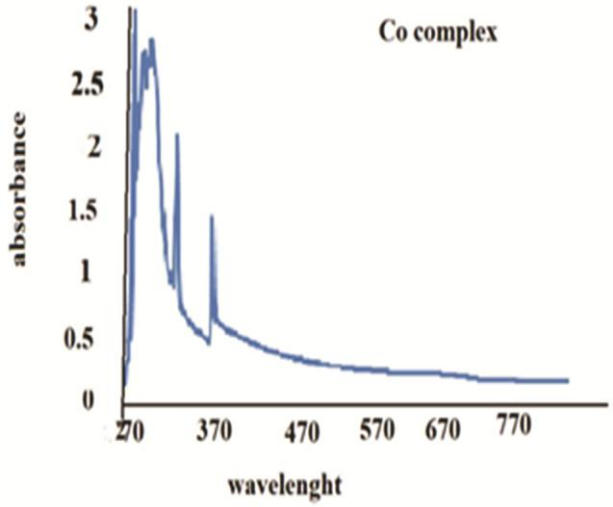

Fig. 9 — UV spectra of Cobalt (II) complex

On the basis of infrared spectra of ligands, it is proved that ligands showed keto-enol isomerism. Keto form of the ligand coordinated with cobalt ion in the divalent state through azomethine nitrogen atoms. Due to the coordination of ligand with cobalt ion, the frequency of infrared spectra of cobalt complexes shifts from 1606 -1608 cm^{-1} (Fig. 11). This result is exactly equivalent to the research work of Tay *et al* (15), in which the infrared frequency of the C=N functional group shifted from 2 cm^{-1} *i.e.* 1621-1619 cm^{-1} after coordination of ligand with cobalt ion. This result was also confirmed by the electronic absorption spectra results where the electronic transition of n- π^* transition of C=N functional group was shifted from 325 nm to 340 nm after coordinating with cobalt (II) ion. Due to the shifting in electronic spectra of cobalt complexes bathochromic shifts were aroused in cobalt complexes due to back bonding of d electrons from cobalt ion to azomethine group of Schiff base ligands and this electronic back bonding decreased the bond energy of the azomethine group of Schiff base ligands.

Powdered XRD

The Powdered XRD technique is a very important crystallographic technique that has been used for the identification of different peaks in the powdered sample of ligand and complex. Figure 12 showed the XRD spectra of the cobalt complex. The XRD diffraction lines are indexed for ligand and its cobalt complex as 101, 102, 103, and 110 phases JCPDS CARD NO (100754 - 441628).

From the full width at half maximum of diffraction peaks of X -rays are employed to calculate the

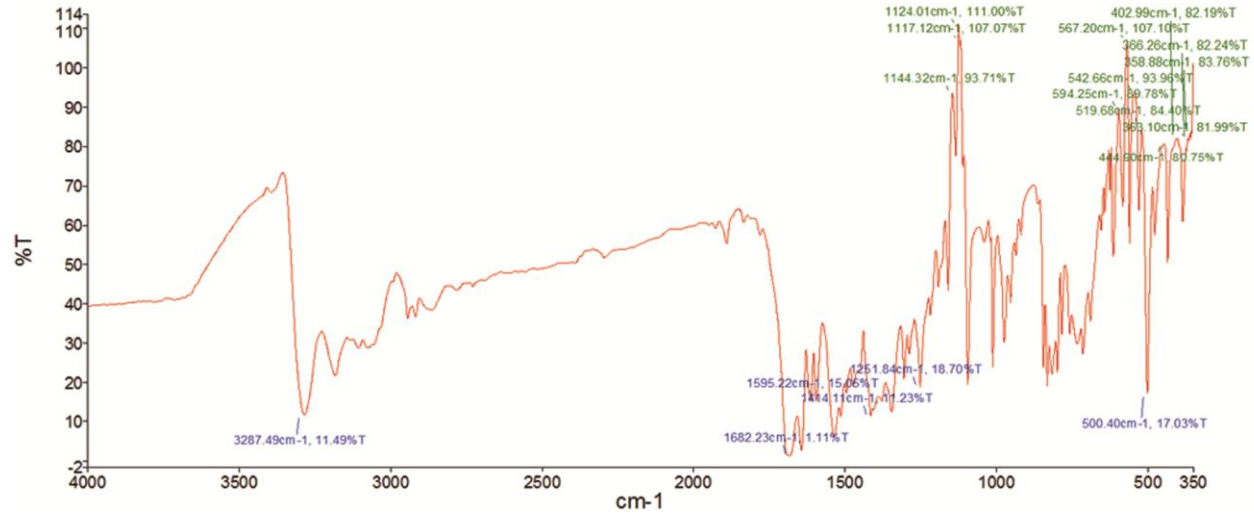

Fig. 10 — FT-IR Spectra of Schiff base ligand

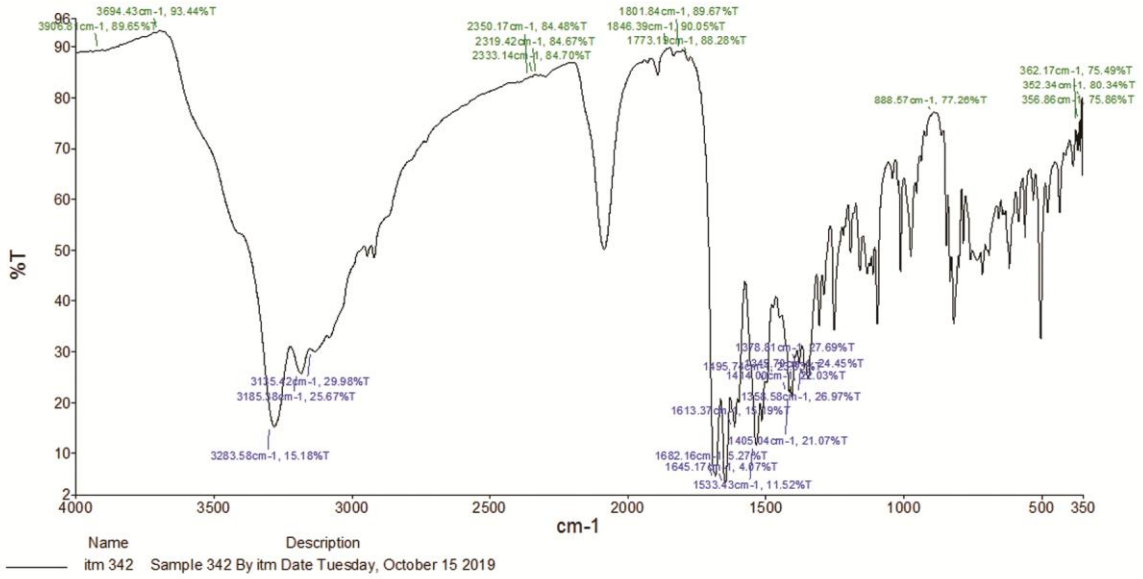

Fig. 11 — FT-IR Spectra of Coordinated Cobalt complexes

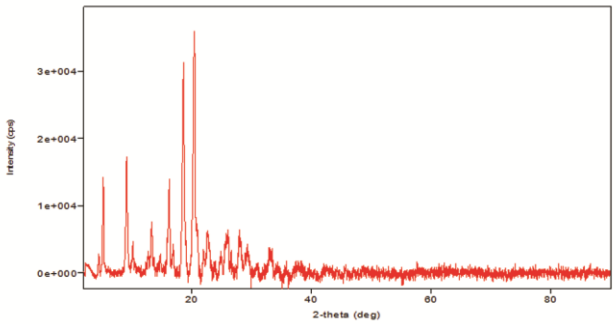

Fig. 12 — XRD Spectra of Cobalt (II) COMPLEX

average crystalline size using Debye- Scherer's equation. *i.e*:

$$D = 0.9 \times \frac{\lambda}{\beta.\cos\theta}$$

where,
D = Crystalline size
λ = wavelength of X- Rays
β= Full width at half maximum of the diffraction peak
θ = Bragg's angle

The particle size of the ligand and its cobalt complexes were below 100 nm-200 micrometres. (Calculated by using Debye Scherer equation) and the width of the x – rays peaks are almost similar to the crystalline size of the particles of the Schiff base ligands and their Cobalt(II) complexes (Fig. 13).

EDX studies confirm the presence of C, N, O, S & Co elements in the complex. The other impurities are also found such as chlorine, calcium elements were identified due to interaction with the solvents during refluxing and recrystallization process (Fig. 14 and Table 2).

TEM images of Cobalt II) complex

The TEM images of the cobalt (II) complexes are shown in (Fig. 15). According to the picture, light coloured cobalt complex is arranged in a cluster form, approximately 100 nm in size, which are expected to the binding with ligand after the generation, and prevent their further growth also. Figure 6 shows the size distribution of cobalt complexes having the arrangement of equispaced particles. Particles of cobalt complexes were not attached but they were separated by equal space which was proved by microscopy visualizing under the high-resolution microscope.

SEM

SEM monographs in (Fig. 16) explain well defined and spherical shape of Cobalt complexes when added to the ethanolic solution of ligand, does not change the shape of Cobalt complex particles but the size of the particle increases with the increase in concentrations. The Cobalt complex particles were arranged into a dense closely packed crystal assembly.

TGA analysis of Cobalt (II) complexes

The TGA figure indicates that the graphs of Cobalt (II)complexes begin to decompose at 240.16°C,

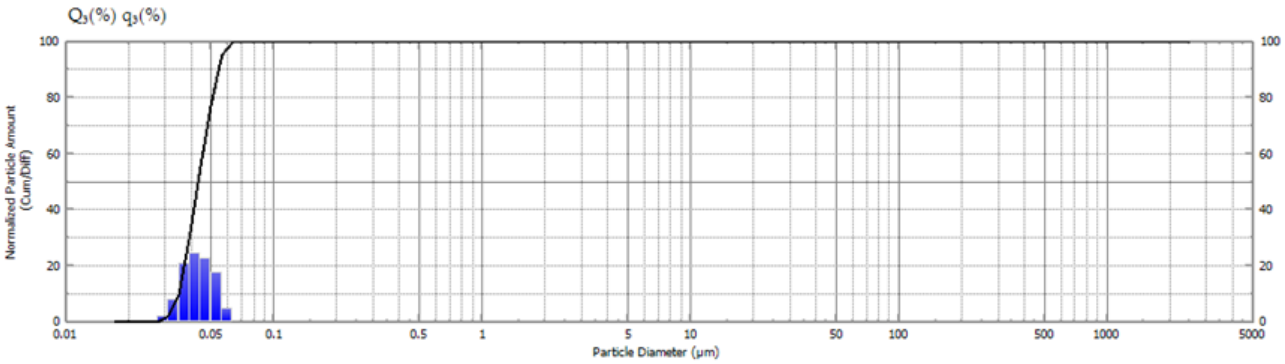

Fig. 13 — Particle size analysis of Cobalt complexes

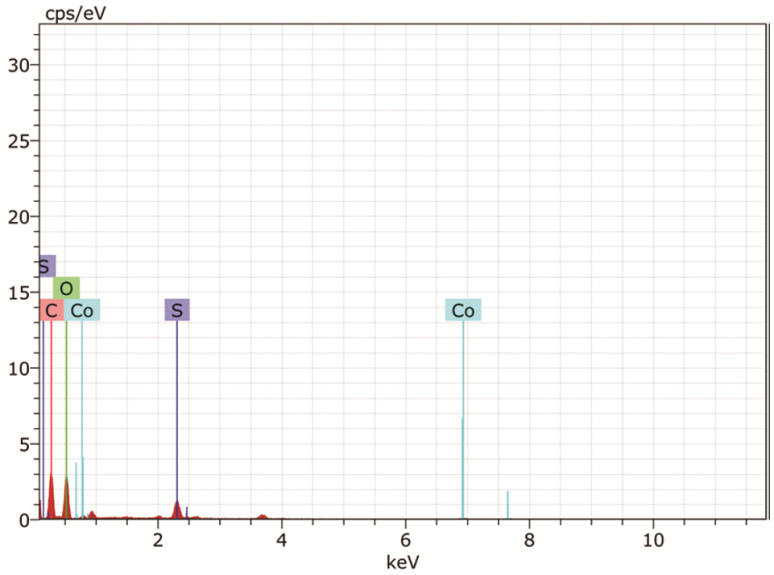

Fig. 14 — EDX Spectra of Cobalt (II) complex

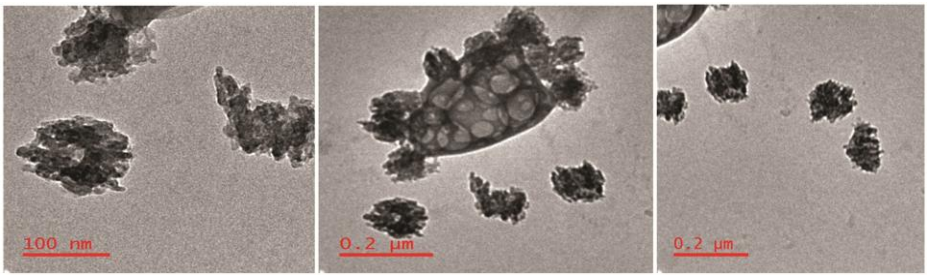

Fig. 15 — TEM Images of Cobalt(II) Complexes

Table 2 — Spectrum test 5168

Element	Series	unn. C [wt.%]	norm.C [wt.%]	Atom.C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	46.99	46.99	56.30	20.79
Oxygen	K-series	45.18	45.18	40.64	20.08
Sulfur	K-series	5.59	5.59	2.510.75	
Cobalt	K-series	2.24	2.24	0.55	0.60
Total:		100.00	100.00	100.00	

251.21°C & 279.30°C, respectively. Comparison of the decomposition temperature of the compounds shows that Cobalt complexes decompose at a higher temperature than their ligands (Fig. 17). The TGA curve for the Cobalt complex displays 2.758 mg weight loss within the temperature range of 240-280°C and exhibit a man loss of 117.26%. The TGA curve of ligands displays 7.26 mg 6.68 mg &

Fig. 16 — SEM Images of Cobalt (II) Complexes

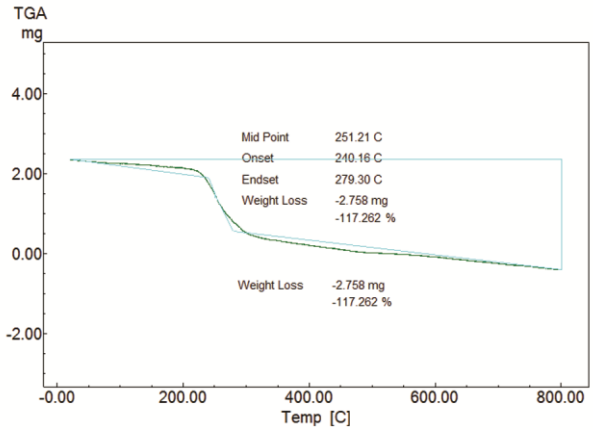

Fig. 17 — TGA Curve of Cobalt (II) Complexes

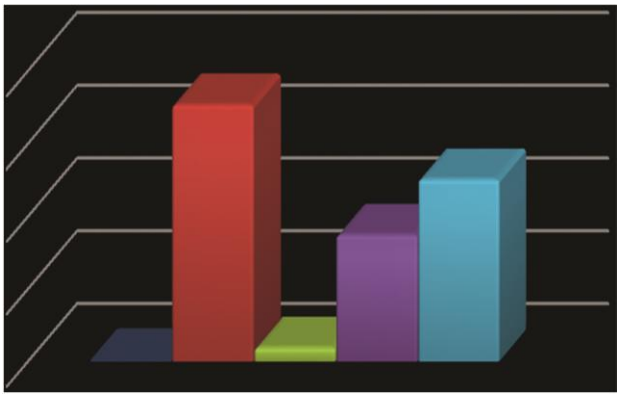

Fig. 18 — Graphical representation for *In vitro* antibacterial activity potential of Schiff base ligands and their Cobalt (II) Complexes in comparison to activity of standard antibiotic Vancomycin

Table 3 — Inhibition of standard drug Vancomycin- HCl against all test microbes

S. No.	Test Microbes	Diameter of Zone of Inhibition(in mm) at different drug concentration				
		50µg/ µL	25µg/ µL	12.5µg/ µL	6.25µg/ µL	3.125µg/ µL
1.	*E. Coli* (MTCC-1687)	11 mM	10 mM	Nil	Nil	Nil
2.	*E. Faecalis* (MTCC-439)	30 mM	28 mM	25 mM	23 mM	20 mM
3.	*S. aureus* (MTCC-737)	27 mM	26 mM	24 mM	22 mM	21 mM
4.	M.R. *S.aureus* (Indigenous)	22 mM	21 mM	19 mM	19 mM	18 mM

Table 4 — Results of antibacterial actibity of carbohydrazone Schiff base ligand (L1-L3) and its Co(II) complex

S. No.	Concentration (µg/µL) in Dichloromethane	*E. Coli* (MTCC-1687)		*S. aureus* (MTCC-737)		*E. Faecalis* (MTCC-439	
		Ligand L1	Co(II) complex	Ligand L2	Co(II) complex	Ligand L3	Co(II) complex
1.	100µg/ µL	10	22	15	28	09	14
2.	50µg/ µL	12	20	12	22	10	15
3.	25µg/ µL	11	21	12	16	14	20
4.	12.5µg/ µL	6.25	14	08	18	10	12
5.	6.25µg/ µL	7.5	17	06	12	nil	nil

7.36 mg weight loss within the temperature range (Table 3).

Antibacterial screening of schiff base ligands and their Cobalt (II) complexes

The *in vitro* antibacterial activity of Schiff base ligands and their Co (II) complexes were evaluated via disc diffusion method and the results of antibacterial activity were summarized in (Table 4 and Fig. 18). The results of ligands and their Cobalt(II) complexes revealed that both are non-toxic in nature to broad-spectrum bacterial species. Results show that synthesized cobalt (II) complexes are more reactive than their ligands(L); even the concentration was increased up to 25µg/µL to 100 µg/µL the possible reason for this could be the absence of hydrocarbon chains in the benzene ring (Fig. 19).

Molecular docking analysis

The molecular docking studies revealed that cobalt complexes and Schiff base ligands both got accessed into the active pockets of molecular targets and interacted with amino acids responsible for target inhibition. Although cobalt complexes interacted with

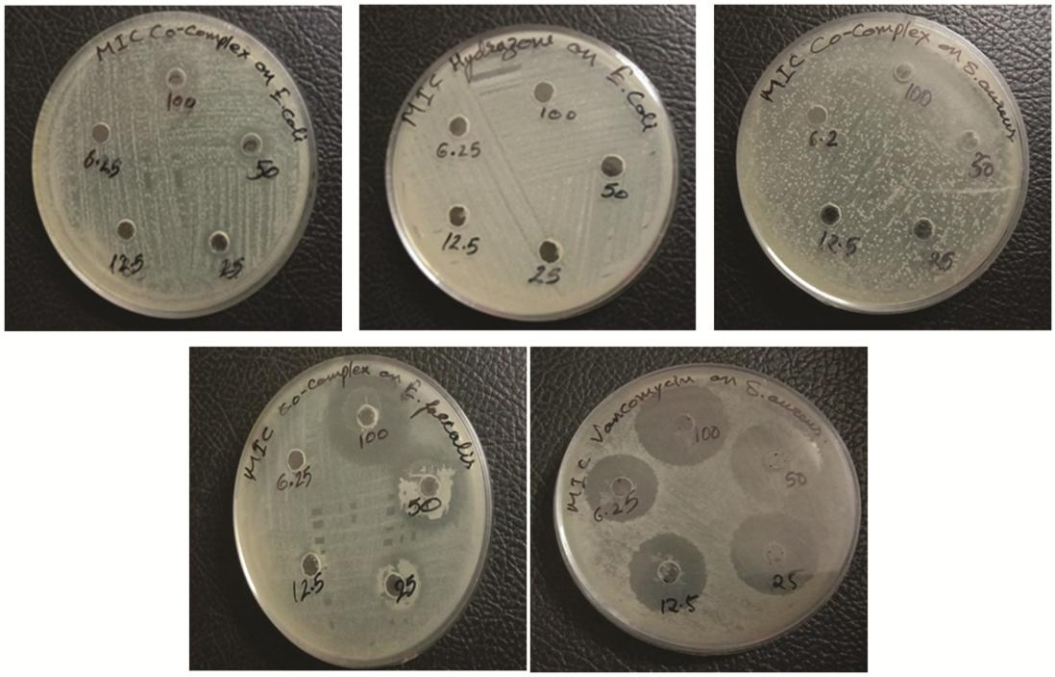

Fig. 19 — *In vitro* Antibacterial Activities of Schiff base ligands and their Cobalt complexes

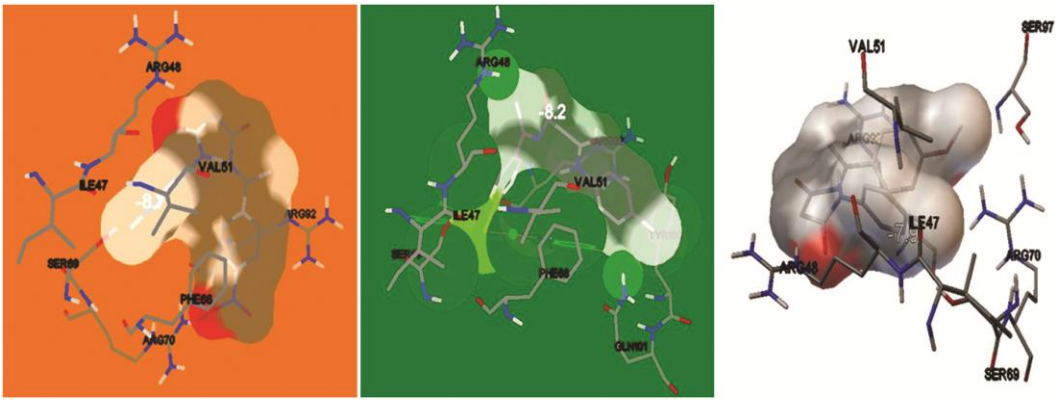

Fig. 20 — Molecular Docking Studies of Schiff Base ligands

Table 5 — Result of docking studies of Schiff Base Ligands and Their Cobalt(II) complexes

Name	Binding Affinities (kcal/mol) with targets	Amino acids involved in the interactions PDB=4QGG	H-bond with Distance
Cobalt complex1	-11.0	Glu11, Arg48, Val51, Leu52, Phe66, Arg70, Arg92, Ser97, Tyr100,	NIL
Cobalt complex2	-9.4	Gln101Arg36, Glu37, Phe66, Arg92, Tyr100, Arg105 Glu11,	NIL
Cobalt complex3	9.1	Glu37, Phe66, Ser69, Arg92, Tyr100, Gln101, Arg105, His160	NIL
Ligand1	-8.2	Ile47, Arg48, Val51, Phe66, Ser69, Arg92, Tyr100, Gln101	NIL
Ligand2	8.7	Ile47, Arg48, Val51, Phe66, Ser69, Arg70, Arg92 Ile47, Arg48,	Arg70
Ligand3	7.9	Val51, Ser69, Arg70, Arg92, Ser97	NIL

amino acid residues more efficiently in terms of binding affinity rather than Schiff base ligands yet cobalt complex could not manage to form the hydrogen bond with residues of the active site in both targets whereas only the second Schiff base ligand could afford the formation of hydrogen bond with the target via Arg70 (Table 5). Results decipher here that significant binding affinity of cobalt complex to thymidylate synthase enzyme confirms that cobalt complexes are more effective against *S. aureus* bacterial strain (Figs 20 & 21).

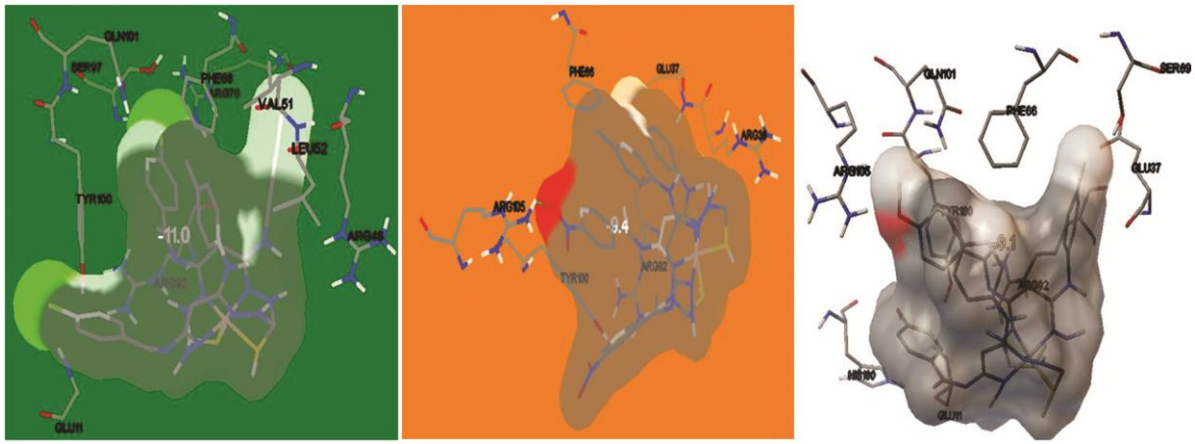

Fig. 21 — Molecular Docking Studies of Cobalt (II) Complexes synthesized from Schiff Base Ligands

Conclusion

In the present study, we reported the synthesis, characterization, molecular docking studies and anti-microbial activity of Cobalt (II) Schiff Base Molecular Adducts. The empirical formula of Schiff base ligands and their cobalt complexes are in agreement with the elemental analysis, FT-IR, NMR, XRD, TEM, SEM, EDX, Particle size and electronic absorption studies. Results of various spectroscopic characterization of ligands and their cobalt complexes revealed that both are stable at room temperature and soluble in DMF & DMSO solvents and ligands were effectively coordinated with cobalt ions via the four azomethinenitrogens atoms and two sulphur atoms of thiosemicarbazone moieties and developed into a coloured hexacoordinate stable Cobalt(II) complexes. The antibacterial activities of ligands and their cobalt complexes revealed that cobalt complexes showed good antibacterial activity against both gram-positive and gram-negative bacterial strains as compared to Schiff base ligands. Molecular docking studies also reveal that cobalt complexes showed excellent binding to the receptor responsible for the antibacterial effect. Results of antibacterial activity of cobalt complexes revealed that Cobalt(II) complexes will be considered as a good candidate for treating antibacterial infections in future for medicinal chemists.

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Conflict of interest

All authors declare no conflict of interest.

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