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Synthesis of a chiral schiff base derived metal complexes and its antibacterial activity studies

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ABSTRACT

Six Potential Tridentate Chiral Schiff base ligands and its metal complex were synthesized and characterized, in that the amino alcohol and 2-amino-3,5-dibromobenzaldhehyde were linked by C=N bond, which can be complexes with metal acetate especially copper and nickel acetate, it has Antibacterial activity against several microbes have been tested and found all six compounds exhibits potential antibacterial activity.

Key words : Tridentate chiral Schiff's base ligands, Metal complex and Antibacterial.

INTRODUCTION

A remarkable effort has been devoted by organic chemists over the past 20 years to the Design, synthesis ,characterization and application of diverse chiral Schiff bases reagents are becoming increasingly important in the pharmaceutical, plastic and dye industries generally the chiral compounds has two enantiomers which elicit different properties and responses. Thus the literature survey reveal that some pharmacophores are indeed essential to impact desired therapeutic effect in the molecules. the significant pharmacophores like halogen, Phenolic $-NH_2$,-COOH,-CH=N-,$-C_6H_5$ and chiral centre in the molecules could exhibit broad spectrum activities.

Some of the bioactive chiral compounds already reported ,one of the potential chiral Compound was the hydroxyazole bioisosteres of glutamic acid , also some Compounds like Novel bicyclic acidic amino acids, both the compounds are the biologically important [1-5] based on the nature of compounds ,and recently our group found some chiral compound, which is the Schiff bases of Salicylaldehyde and Amino acid derivatives already reported to possess potential pharmacological activities and biological activities.

some of the bi and tridentate amino alcohol ligands as Schiff bases were already reported [6,7,8].A number of chiral compounds like (+) tartaric acid ,(+)ephedrine,(-)nicotine,(+)cinchonine are very good chiral molecules, and much of the work in this area has been an attempt to mimic the action of enzymes[9]. Already the antibacterial studies on mononuclear complexes of chiral Schiff's base have been reported[10-13].

In the present study synthesize of chiral ligands by treating L-Valinol or L-Phenylalaninol salts with 2-amino-3,5-dibromo benzaldehyde to form schiff base and it has complexed with metal acetate like Copper and Nickel to form the tridentate chiral Schiff base metal complexes. the structures of the synthesized compounds were confirmed by FT-IR,[1]HNMR,[13]CNMR,UV-Vis and Mass spectral analysis.The newly synthesized complexes have been screened for their antibacterial effects against the number of gram positive and gram negative bacteria.

EXPERIMNETAL SECTION

Melting points were determined in open capillary tubes on melting point apparatus (veego, Shankar scientific) and are uncorrected .the ^{1}H and $^{13}CNMR$ spectra was recorded on Bruker NMR 500 MHz using $CDCl_3$ as solvent .Mass spectra was recorded on JEOL GC mate mass spectrometer .The IR Spectra of the synthesized compounds were recorded on FT-IR Spectrophotometer were obtained on a Perkin-Elmer Spectrum One Fourier transform infrared spectrometer (KBr pellet) . Ultraviolet-visible (UV-Vis) spectra were measured with a Lambda 25 spectrophotometer TLC checked the purity of the compounds on pre-coated silica Gel Plates by using methanol: Ethyl acetate (1:9) as a mobile phase and visualized in iodine vapour. Optical rotations of the ligands were recorded using Redolph polarimeter.

Synthesis of 2-Amino-3, 5-dibromo benzaldehyde (A):

2-aminoethylbenzoate (16.5g ,0.10mol) is dissolved in toluene (100ml),cool the mass to 20^0C and added bromine (16.66 g, 0.208mol) in portion wise, then stir the mass at Reflux for 6hr.completion of the reaction monitor by TLC and workup done with addition of water and separate the organic layer degas under vacuum gave ethyl-2-amino-3,5-dibromobenzoate (24.0g),which dissolved in 120ml of THF and chill to 20^0C then lithium aluminium hydride (0.8g) added in three lots and slowly raise the temperature to reflux maintain for 3hr.then quench the reaction with dil.Hcl and addition of 500ml of water gave 20.0g of 2-amino-3,5-dibromophenyl)methanol, which is further oxidized by reflux condition using MnO_2(45.0g) for 8h presence of 1,4-Dioxane as solvent. Afford the pure 2-amino-3,5-dibromo benzaldehyde (16.0g).which melts at 135- 138^0C.(lit : 136-138^0C) . The scheme for synthesis of intermediate and chiral ligands is shown in the following Figure 1 and Figure 2 respectively.

Figure 1: General Scheme for synthesis of Tridentate Schiff base ligands.

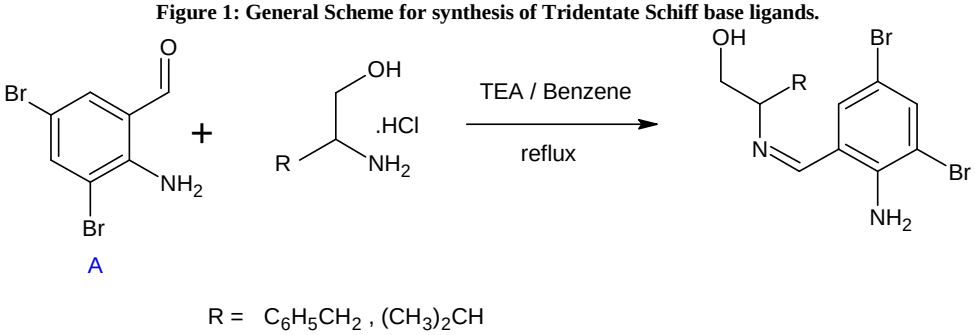
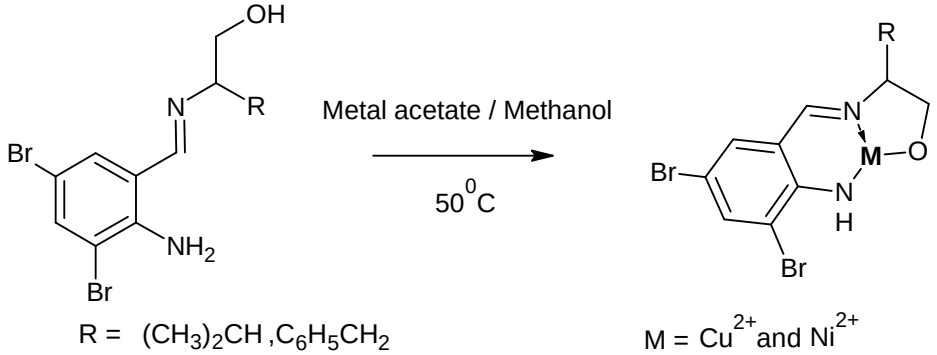


Figure 2: General Scheme for synthesis of metal complexes.



Synthesis of ligand 1 : 2-{[(2-amino-3,5-dibromophenyl)methylidene]amino}-3-methylbutanol (L1)

L-Valinol hydrochloride (5.0g 0.035mol) taken with 100ml of benzene and 2-amino-3,5-dibromo benzaldehyde (9.95g,0.035mol) was added ,to this triethylamine(1.0ml) was added drop wise at 30^0C, slowly raise the temperature to reflux and started collection of water using Dean stark apparatus .It maintained the reflux for 4hrs and cool to room temperature then formed triethylamine hydrochloride was removed by passing the reaction mass to flush column, the organic layer removed under vacuum, which afford 2-{[(2-amino-3,5-dibromo phenyl) methylidene] amino}-3-methyl butanol (L**1**), (11.01g,0.030mol) as white solid, Yield : 83.0%, $^{1}HNMR$ δ 0.92(m, 6H,-CH_3), 1.66(s, 1H, -OH), 1.94(m, 1H, -CH), 3.0(m, 1H, -CH), 3.8(s, 2H, -CH_2), 7.05(b, 2H, -NH_2), 7.3-7.5(2H, ArH), 8.2(s, 1H, N=CH). $^{13}CNMR$ δ18.75, 19.77, 30.21, 64.84, 79.17, 106.3, 110.1, 119.24, 135.15, 135.70, 144.80, 162.820, $[\alpha]^{25}$ = -75.60(C=1 in $CHCl_3$),MS: (m/z) 364.3.

Synthesis of ligand 2 : 2-{[(2-amino-3,5-dibromophenyl)methylidene]amino}-3-phenylpropanol. (L2)
L-Phenylalaninol hydrobromide(5.0g 0.021mol) taken with 100ml of benzene and 2-amino-3,5-dibromo benzaldehyde (5.85g,0.021mol) was added ,to this triethylamine(1.0ml) was added drop wise at 30^0C, slowly raise the temperature to reflux and started collection of water using Dean stark apparatus .It maintained the reflux for 4hrs and cool to room temperature then formed triethylamine hydrochloride was removed by passing the reaction mass to silica flush column, the organic layer removed under vacuum ,which afford the 2-{[(2-amino-3,5-dibromophenyl)methylidene]amino}-3-phenylpropanol,as crude material which is further purified by CC(ethyl acetate and methanol as eluent) and isolated (7.35g,0.0178mol) as pale yellow powder. Yield : 85.0%, ^{1}HNMR δ 1.2(s, 2H, -NH_2), 1.50(s, 1H, -OH), 2.84-3.07(dd, 2H, -CH_2), 3.52(m, 1H, -CH), 3.84(dd, 2H, -CH_2), 7.1-7.2(s,2H,ArH), 7.5(m, 7H, ArH) and 7.98(s, 1H, C=N),. ^{13}CNMR:δ32.0,39.31,60.10,66.01, 74.61,104.69,107.59, 122.00, 126.43, 128.40, 129.48, 134.52, 135.57,143.72,163.20,203.01,MS:(m/z) 412.11, $[\alpha]_{25}$ = -90.0(C=1 in $CHCl_3$).

Synthesis of ligand 3: [Cu(L1)(OAc)]
A 50ml methanol solution of 4.02g(0.011mol) of the ligand 1 was brought to boiling for 10min and Copper acetate monohydrate was added 2.20g(0.011mol) in a single lot in a boiled ligand solution and 0.1ml of triethylamine was added and continued the reflux for 30min and during which the complex were precipitated and continue stirring for 10min and filtered the material at Room temperature and finally washed with 10ml water followed by methanol and dried gave the 4.0g ,Yield: 76%,the color of the complex was green crystalline powder and Melting point : 182^0C.

Synthesis of ligand 4 : [Cu(L2)(OAc)]
A 35ml methanol solution of 3.50g(0.008mol) of the ligand 2 was brought to boiling for 10min and Copper acetate monohydrate,1.70g(0.0085mol)was added in a single lot in a boiled ligand solution and 0.1ml of triethylamine was added and continued the reflux for 30min and during which the complex were precipitated and continue stirring for 10min and filtered the material at Room temperature and finally washed with 10ml of water followed by methanol and dried gave 3.10g ,which has the yield 70.3%,the color of the complex was greenish brown in color and melting point: decomposed at 196^0C.

Synthesis of ligand 5: [Ni(L1)(OAc)]
A 40ml methanol solution of 4.00g(0.0109 mol) of the ligand 1 was brought to boiling for 10min and Nickel acetate tetra hydrate, 2.75g(0.011mol), was added in a single lot in a boiled ligand solution and 0.1ml of triethylamine was added and continued the reflux for 30min and during which the complex were precipitated and continue stirring for 10min and filtered the material at Room temperature and finally washed with ethylacetate and dried got 4.5g(0.009mol), yield 85.3%,the color of the complex was brown in color and M.pt : decomposed at 202^0C.

Synthesis of ligand 6: [Ni(L2)(OAc)]
A 50ml ethanol solution of 3.50g(0.008 mol) of the ligand 1 was brought to boiling for 10min and Nickel acetate tetra hydrate was added 1.99g(0.008mol) in a single lot in a boiled ligand solution and 0.1ml of triethylamine was added and continued the reflux for 30min and during which the complex were precipitated and continue stirring for 10min and filtered the material at Room temperature and finally washed with ditehylether and dried got 3.15g(0.006mol) ,the yield 74.5%,the color of the complex was dark brown in color and M.pt : decomposed at 220^0C.

Antibacterial activity:
The Antimicrobial activity of the Six novel chiral ligands and its metal complexes were examined against different gram positive (*SA- Staphylococcus aureus ,EB- Enterobacter)* and Gram negative (*PA-Pseudomonas auroginosa ,EC- Escherichia coli , ST-Salmonella typhi*) by using agar diffusion method. Petriplates (100mm) was prepared with 20 ml of sterile nutrient agar (NA) for testing the bacterial assay. The test cultures was swabbed on the top of the solidified media and allowed to dry for minutes. Agar well was cut on the solidified medium using sterile well cutter. Stock solutions of each compound were diluted in DMSO to produce 1000 microgram/ml. The dilutions of the compounds were poured on the agar well (100microliters per well) and left for 10 minutes at room temperature for compound diffusion. Negative control was prepared using DMSO. Ciprofloxacin (5microgram/well) for bacteria were served as positive control. The plates were inoculated with bacteria were incubated at 37^0C for 24 hours. The plates were then incubated at 35 to 37^0C for 24 hours and the zone of inhibition were observed.All the experiments were carried out in horizontal Laminar airflow bench. Zone of inhibition was calculated from triplicate reading.

Minimal Inhibitory Concentration:
The minimal inhibitory concentration (MIC) was determined by both broth dilution assay. For broth dilution assay method, a 24 hour pure culture of *E. coli* was grown in Muller Hinton broth. From the broth, 1000µl of culture was taken and inoculated individually into a set of 5 test tubes that already contained 10ml of fresh Muller Hinton

broth.5 different concentrations of Ligands (1ppm, 10ppm, 100ppm, 1000ppm, 2000ppm) were prepared for testing against *E.coli*. One ml volume of each concentration of Ligands was transferred to the tubes accordingly. The contents in the tubes were mixed thoroughly and incubated overnight at room temperature. The MIC end point is the lowest concentration of plant extracts at which there is no visible growth in the form of zone or turbidity is noticed in the tubes.

The tests were carried for concentrations of 125, 250, 500, 1000µg/ml, DMSO solutions of the three compounds. The inhibition zones caused by the compounds on the different microorganisms were examined.

Minimal Bactericidal concentration:
After MIC determination of the compounds an aliquot of 10µl from the Particular concentration, at which there is no visible growth was observed were inoculated into the fresh Nutrient agar broth. The tubes were kept monitored for culture growth once in one hour for 5 hours by measuring the absorbance at 600 nm using UV-VIS spectrophotometer. Mean while, a 10µl aliquot of compounds from tubes which showed no visible growth in MIC test were seeded into Nutrient agar plates and kept for overnight incubation at room temperature.

The MBC endpoint is defined as the lowest concentration of antimicrobial agent that kills >99.9% of the initial bacterial population where no visible growth of the bacteria was observed on the nutrient agar plates .

RESULTS AND DISCUSSION

Infrared Spectroscopy
The FTIR spectra of the synthesized complexes were recorded within 4000-400 cm^{-1}. Ligand 3 to 6 shows broad lower absorption bands at 3739 - 3879 cm^{-1} which may be assigned for ν(O-H) stretching frequency, lower absorption bands at 3346 - 3600 cm^{-1} which may be assigned for ν(N-H), The strong bands at 1532 to1592 cm^{-1} is attributed to the imines ν(C=N) vibration of the Schiff base, Strong stretching vibrations are observed at 618 and 425 cm-1 may assigned for the ν(Cu-O) and ν(Cu-N) coordination respectively for complex 3 and Strong stretching bands have been observed at 592 and 460 cm^{-1}, has assigned for the ν(Cu-O) and ν(Cu-N) coordination respectively for complex 4 . In the same way Strong stretching vibrations bands at 427 and 614 cm^{-1} may assigned for the ν(Ni-O) and ν(Ni-N) coordination respectively for complex 5 and Strong absorption bands at 429 and 580 cm^{-1} has assigned for the ν(Ni-O) and ν(Ni-N) coordination respectively for complex 6.Thebroad absorption bands at 1415 - 1450 cm-1 for the complex (3 to 6) are attributed to ν(C=O) vibrations. thus the coordination of (C=O) stretching due to the presence of Acetate group in the ligand 3 to 6,which has not present in ligand L1 and L2.same way the strong absorption band of ν(M-O) and ν(M-N) are absent in the ligand 1 and 2.Thus,the formation and coordination of metal complexes with ligand 1 and 2 were confirmed and the absorption values are summarised in the Table 1.

Table 1 : Physical constants and prominent Infra red bands of the Ligands and its metal complex.

			Prominent Infrared bands (cm^{-1})						
Compounds	**Color/ Description**	**Melting point/ decomp. point**	ν_{OH}	ν_{NH}	$\nu_{C=N}$	$\nu_{C=O}$	ν_{C-Br}	ν_{M-N}	ν_{M-O}
Ligand 1 (L1)	White crystalline powder	130^0C	3540	3250	1571	-	681.6	-	-
Ligand 2 (L2)	White powder	144^0C	3740	3394	1523	-	694.1	-	-
[Cu(L1)(OAc)] (L3)	Green crystalline powder	182^0C	3879	3346	1563	1432	680.6	425	618
[Cu(L2)(OAc)] (L4)	Greenish brown powder	196^0C	3815	3600	1565	1428	703.0	460	592
[Ni(L1)(OAc)] (L5)	Brown color powder	202^0C	3739	3539	1592	1450	681.9	614	427
[Ni(L2)(OAc)] (L6)	Dark brown color powder	>220^0C	3802	3593	1520	1415	683.0	580	429

UV-Vis spectra
The UV-Vis absorption spectra of the six compounds L1–L6 in DMSO solution at room temperature are taken. The ligand has absorption bands at around 350 and 345 nm, which originate from π–π* transition of valinol ring, π–π* transition of Phenylalaninol and intraligand π–π* transition of the conjugated backbone, respectively. The maximum absorptions of the metal complexes for Cu(II) are 456 and 437 nm for mononuclear complex of ligand 3and 4,which can be assigned to intraligand π–π* transition of the conjugated backbone and metal-to ligand charge transfer (MLCT) transition. In the absorption spectra of the complexes5 and 6,the characteristic absorption peaks at 292 nm for Ni(II) complex, The spectra of the metal(II) complexes in DMSO solutions are shown that absorption band observed as $n \rightarrow \pi^*$ electronic transition of hydrazone (–NH–N=C–) group involving the whole conjugation and it was summarized in the Table 2.

Table 2 : Ultraviolet Absorption of ligands and its metal complexes.
a) L-Valinol derivative and its metal complexes.

Compounds	UV absorptions in nm	UV absorptions in cm^{-1}
Ligand1 ***(L1)***	350	28571.42
Cu(II)Complex ***(L3)***	456	21929.82
Ni(II)Complex ***(L5)***	292	34246.57

b) L-Phenyl alaninol derivative and its metal complexes.

Compounds	UV absorptions in nm	UV absorptions in cm^{-1}
Ligand2 ***(L2)***	345	28985.50
Cu(II)Complex ***(L4)***	437	22883.30
Ni(II)Complex ***(L6)***	292	34246.57

Antibacterial activity studies

The antibacterial activity of the synthesized ligands and its complexes was done in comparison with Ciproflaxin as standard to reveal the potency of the complexes with five selected strains *E.coli, S. Aureus, P. Auroginosa, and S. Typhi* .The result shows the Ligand 1 and Ligand 2 showed a very good control over the gram positive as well as gram negative bacteria. More over , when the ligands is complexed with metals like nickel and copper , it shows an excellent activity against all the types of bacteria . When compared between the nickel complex and the copper complex, the ligands with the copper complex showed better antibacterial activity. A Minimal Inhibitory Concentration and a Minimal Bactericidal Concentration was also done for all the Ligands .Overall the ligand L3 Posses maximum control over the gram negative as well as the gram positive organisms due to the presence of Copper metal and its coordination and L4 to L6 having moderate. The result of Antibacterial activity, Minimal Inhibitory concentration and Minimal Bactericidal Concentration was summarized in the given Table 3 and Figures 3 and 4.

Figure 3: Minimal Bactericidal concentration

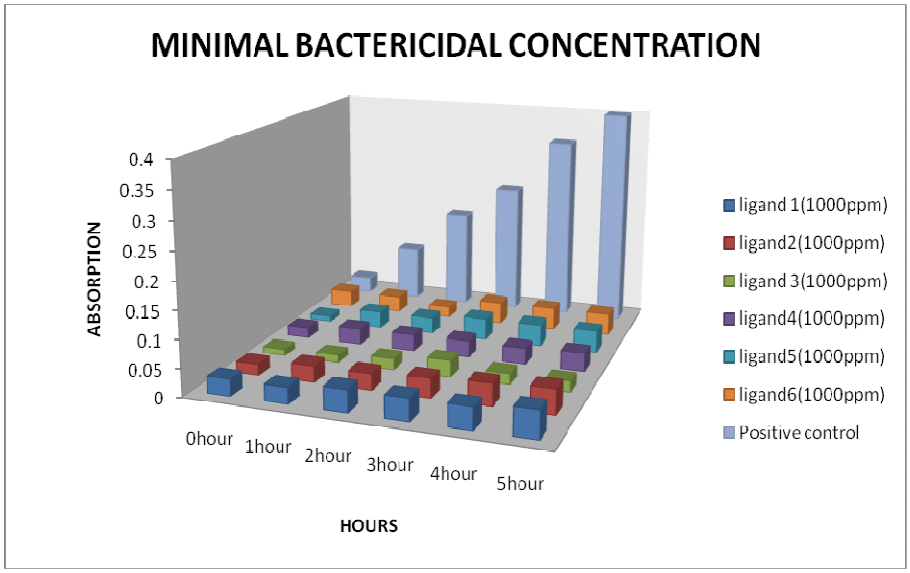


Table 3a:Antibacterial activity of the Ligands against the Gram positive as well as Gram negative bacteria

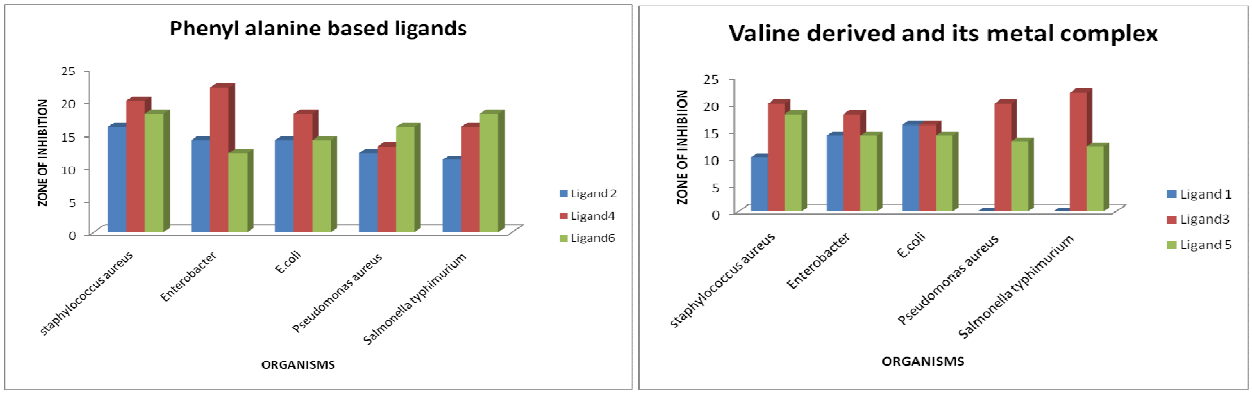
Organism	Ligand 1	Ligand 2	Ligand 3	Ligand 4	Ligand 5	Ligand 6	CIF
	Mean value						
SA	10mm	16mm	20mm	20mm	18mm	18mm	20mm
EB	14mm	14mm	18mm	22mm	14mm	12mm	22mm
EC	16mm	14mm	16mm	18mm	14mm	14mm	18mm
PA	No activity	12mm	20mm	18mm	13mm	16mm	21mm
ST	No activity	11mm	22mm	22mm	12mm	18mm	22mm

SA=Staphylococcus aureus ;EB=Enterobacter ; EC= E.Coli ;PA= Pseudomonas aureginosa ; ST= Salmonella typhimurium ; CIF= Ciprafloxacin

Table 3b:Minimum Inhibitory Concentration, MIC

Sl. no	10ppm	100ppm	500ppm	1000ppm	2000ppm
Ligand 1	Visible bacterial growth	Visible bacterial growth	Slight growth	No growth	No growth
Ligand 2	Visible bacterial growth	Visible bacterial growth	Slight growth	No growth	No growth
Ligand 3	Visible bacterial growth	Visible bacterial growth	Slight growth	No growth	No growth
Ligand 4	Visible bacterial growth	Slight growth	No growth	No growth	No growth
Ligand 5	Visible bacterial growth	Visible bacterial growth	Visible bacterial growth	No growth	No growth
Ligand 6	Visible bacterial growth	Visible bacterial growth	Visible bacterial growth	No growth	No growth

Figure 4: Activity between Ligands and its metal Complexes



CONCLUSION

Thus in the present work we have synthesized L-Valinol and L-Phenylalaninol derived Schiff base chiral ligands and its metal complexes of Cu(II) and Ni(II),were characterized and confirmed by the spectroscopy methods and it confirm that the metal ligand stoichiometry in all these complexes is 1:1. The spectral data show that the ligand act as neutral and tridentate coordinating through nitrogen atom of the azomethine and oxygen atoms of hydroxyl group. Antimicrobial activity of the synthesised complexes was done in comparison with Ciprofloxacin as standard to reveal the potency of the synthesized complexes. In all the five selected strains *E. coli*, *S. Aureus, P. Auriginosa, Enterobacter* and *S. Typhi* showed sensitivity to all complexes at higher concentrations (1000 µg/ml).

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