

ANTIMICROBIAL STUDY AND SYNTHESIS OF MIXED LIGAND COMPLEX OF Ni(II) ION DERIVED FROM ISATIN SCHIFF BASE AND HISTIDINE

Shobhana Sharma, Poonam Yadav, Seema, Suman Kumari
and Mamta Ranka✉

Department of Chemistry, University of Rajasthan, Jaipur 302004 (Rajasthan) INDIA

✉Corresponding Author: mmt31ran@gmail.com

ABSTRACT

Recently, heterocyclic compounds and amino acids are of great interest to researchers owing to their various biological activities like; antimicrobial, anticancer, antiviral, anti-inflammatory, etc. Isatin is also a vital moiety containing N and O donor heteroatoms, and its Schiff base is used for complexation with metal ions which may alter their activities. The new metal complex of Ni has been synthesized using two ligands, 3-(2-phenylhydrazono) indolin-2-one (PHI) (L_1) and amino acid Histidine (L_2). The newly prepared ligand and the complex are further characterized by determination of molecular weight, element analysis, magnetic moment measurement, UV- visible, 1H NMR, and FTIR spectroscopy. The prepared compounds are examined for antimicrobial evaluation against several strains of bacteria and fungi.

Keywords: Isatin, Histidine, Mixed Ligand Complex, Antibacterial, Antifungal.

RASAYAN J. Chem., Vol. 15, No.2, 2022

INTRODUCTION

Coordination chemistry of Schiff base ligands with biologically potent metal is a versatile field of current research.¹ Schiff bases are shown as a major class of ligands in coordination compound chemistry. Schiff bases are important in the synthesis of several transition metal complexes of biological importance, which provides an important research area due to their facile preparation, high purity, better yield, easy availability, electronic properties, and wide range of applications.² The Schiff-based complexes are poured out extensively in the field of research nowadays.³ Schiff bases and amino acids exhibit diverse importance from the research point of view and have shown remarkable growth during current years due to the flexible properties offered by these complexes in the area of catalysis, industries, pharmaceutical, and biological fields.^{4,5} The Schiff based compounds are addressed because of having azomethine linkage ($-C=N-$) which are derived by the condensation of primary aliphatic/aromatic/heteroaromatic amines with carbonyl compounds (ketones/aldehydes).⁶ Isatin and its derivatives demonstrate high application potential in various fields of medicinal chemistry, such as anticancer, antibiotic, antibacterial, antitumor, antidepressant, anti-inflammatory, antimalarial, antituberculosis drugs, and anti-human immunodeficiency virus and so on.⁷⁻¹³ Isatin belongs to the most versatile and ubiquitous heterocyclic compounds and it shows high application potency, and their metabolites occurrence in the human body and plants, it has stimulated great interest in chemists, pharmacists, and physicians, to study their chemical reactivity.¹⁴ Metal complexes, having Schiff bases, derived from amino acid (histidine/ glycine) play a dominant role in pharmaceutical industries.¹⁵

EXPERIMENTAL

Material and Methods

All used materials were collected from Sigma Aldrich and these were of A.R. Grade. For element analysis of Carbon, Hydrogen, Nitrogen, and Chlorine, the CHNX method was employed. For molecular weight determination, the Rast method was used. Nickel was estimated gravimetrically. For melting point measurement, open capillary method was employed, and melting points were uncorrected. The measurement of magnetic moment and measurement of conductance was done by Gouy's balance model no. HO-ED-EM-08 and Systronic Direct Reading Conductivity Meter, respectively. FTIR spectra and

^1H NMR spectra of the complex were recorded on model SHIMADZU-JAPAN 8400 FTIR spectrophotometer and Hitachi Perkin Elmer spectrometer respectively. The electronic spectrum was recorded employing Perkin Elmer UV lambda 750 UV/Vis spectrophotometer.

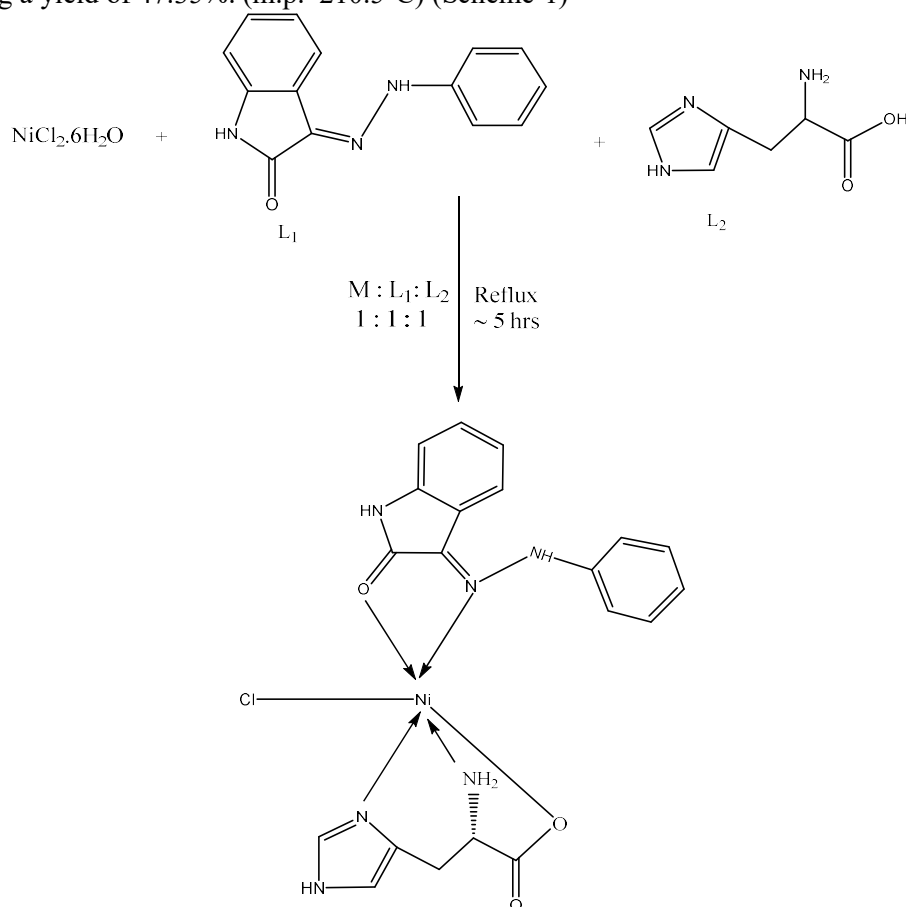
General Procedure

Synthesis of Ligand L_1

PHI [3-(2-phenylhydrazono)indolin-2-one] -Previously reported PHI ligand was used which was synthesized by using the ethanolic solution of Isatin(1.47g, 0.01M) and ethanolic solution of phenylhydrazine hydrochloride(1.44g, 0.01M). 10 ml of each mixed in RB flask and above mixture refluxed for ~ 4 hours by using heating mental with a water condenser. Condensing agent and a small amount of glacial acetic acid were added to the reaction mixture. The reaction was continuously monitored by TLC using a Silica Gel-G plate. Finally, the above reaction mixture was collected on a watch glass, cooled, filtered, washed, recrystallized with ethanol, and dried in a vacuum. The pale yellowish-colored product obtained has a good yield (83.2%).¹ (m.p. 200.5°).

Synthesis of Complex $[\text{Ni } L_1 L_2 \text{ Cl}] \cdot x\text{H}_2\text{O}$

The method, we used for the synthesis of the complex was the Conventional Thermal Method. 10 ml of ethanolic solution of PHI (L_1) (2.37g, 0.01M) and 10 ml of an aqueous solution of Histidine (L_2) (1.55g, 0.01M) were mixed slowly in a round bottom flask, and then a freshly prepared 10 ml of ethanolic solution of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (2.37g, 0.01M) added to this reaction mixture. This mixture was allowed to stir continuously and it was observed that precipitation was not obtained in the round bottom flask. The above content was refluxed for ~ 5 hrs on heating mental. TLC was used to monitor the reaction progress. At the end of the reaction, the product was washed, recrystallized, and dried in a vacuum. We obtained an orangish-yellow product having a yield of 47.35%. (m.p. 210.5°C) (Scheme-1)



Scheme-1: Plausible Synthetic Route of Mixed Ligand Complex

Antimicrobial Study

Agar well diffusion method was used for the investigation of antimicrobial activity (antibacterial and antifungal) of the complex *in vitro*.

For the antibacterial study, a dilute solution of the compound was prepared, using 100% DMSO as solvent of amount 1mg/mL. The melted and cooled(48-50°C) Mueller Hinton agar was used as a medium. Bacteria were grown in Mueller Hinton agar medium. To get a solid plate, the molten agar with a standard militant of 1.5×10^8 CFU/mL concentration was spread in Sterile Petri dishes. Then some well-like empty spaces were created in agar-containing plates. The compound, to be examined, (20-80 µg/mL) was poured into these empty spaces of 6 mm diameter. These plates were kept for 12 hrs at 37°C. The zone size of each well is used to measure the antimicrobial spectrum for bacterial species. The diameter defines the activity of the compound against bacterial strain. If the diameter is more, then the effect will also be more against bacterial strain. The inhibitory zone's diameters were created by the test samples, compared with the effect produced by the standard antibiotic, Streptomycin. When pure solvents were used instead of the extract, the properties of every bacterial strain were maintained. The control zones were taken away from the test zones. The resultant diameter was analyzed by using an antibiotic zone reader in mm. To minimize the error the experiment was repeated three or more times and the mean values were measured.

For the antifungal study, the Common Agar Well Diffusion Method was employed. To create an agar plate, selective saprophytic fungi and yeasts were cultured on Sabouraud's dextrose agar, (SDA) in Petri dishes and it was kept at temperature 37°C for 24 hrs at room temperature for two five days respectively. Fungal spores suspensions were created in sterile PBS and their concentration was regulated at 106 cells/ml. The fungal suspension was spread on the surface of the agar medium with the help of a sterile swab. The plates were kept at 25°C for 18-20 min so that they can be dried. Then empty round spaces were prepared in culture media with sterile glass tube and the diameter of the wells was 10 mm and about 7 mm apart. Afterward, 0.1 ml of various concentrations of fresh test compound was introduced to each space. Plates were kept at 37°C for 48 h. Then, the diameter of the inhibitory zone was determined to measure their bioactivities (in mm). The diameter of the inhibitory zone of the compound was compared with standard, Ketoconazole. All experiments were repeated three or more times and mean values were calculated.

RESULTS AND DISCUSSION

All physical properties including elemental analysis, magnetic moment, and conductance of synthesized compounds have been tabulated in Table-1. Spectral data of FTIR and ^1H NMR are tabulated in Table-2 and Table-3. Antimicrobial results are tabulated in Table-4.

On the basis of physicochemical analysis of ligand and complex, we observed that the synthesized compounds are colored with sharp melting point and soluble in ethanol, DMSO, DMF, and CHCl_3 . DMSO is used to measure molar conductance and it was observed at $\sim 12.34 \Omega^{-1}\text{cm}^2\text{mol}^{-1}$ which indicates that the complex $\text{NiL}_1\text{L}_2\text{Cl}$ is non-electrolytic in nature. The magnetic moment (μ_{eff} only) of the complex $\text{NiL}_1\text{L}_2\text{Cl}$ was found at $\sim 2.82\text{BM}$ which shows that the complex is paramagnetic in nature.

Table-1: Physical Properties and Element Analysis of Synthesized Compounds

Compound (Ligands/ Complex) Empirical Formula	Colour/ M.P.	Mol.Wt. Cal. (Found)	% Elemental Analysis Cal. (found)						Mol. Cond. ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$)	μ_{eff} BM Approx .
			C	H	N	O	Cl	M		
PHI (L_1)	Pale yellow/ 200.5°C	237.13 (237.11)	70.90 (70.88)	4.63 (4.64)	17.71 (17.70)	6.74 (6.72)	-	-	-	-
$\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}$										
$\text{NiL}_1\text{L}_2\text{Cl}$	Orangish yellow/ 210.5°C	485.31 (485.30)	49.49 (49.48)	3.91 (3.89)	17.30 (17.29)	9.88 (9.90)	7.30 (7.27)	12.09 (12.10)	12.34	2.82
$\text{NiC}_{20}\text{H}_{19}\text{N}_6\text{O}_3\text{Cl}$										

Recorded FTIR data of the compound provides information about the formation of the bond between metal and ligand as a peak of azomethine ($\text{C}=\text{N}$) at 1612 cm^{-1} observed in the complex which is slightly at a lower

frequency than that of ligands. Some other peaks are also recorded at 618 cm^{-1} and 445 cm^{-1} , which indicates the presence of M-O and M-N bonds in complex respectively.

^1H NMR spectra were carried out in CDCl_3 . Some signals were observed for Ar-H, CON-H, NH_2 , and N-H (Histidine) at 6.95-7.44 (multiplet), 8.94, 3.34, and 11 ppm respectively. In comparison to ligands, the peaks of the complex are slightly shifted towards the lower field.

UV/Vis spectra of the complex were carried out in ethanol in the range of 200-800 nm. We obtained some bands in the range of 250-500 nm because of π - π^* /n- π^* transitions. In comparison with ligand, the UV/Vis bands of complex shifted towards longer wavelength. The bands were shifted towards 370 nm for n- π^* and 294 nm for π - π^* transition, which signs the presence of metal-ligand bonds.

Table-2: FTIR Data of Synthesized Compounds [ν (cm^{-1})]

Compounds	ν (N-H)	ν (C=N)	ν (C=C)	ν (M-N)	ν (M-O)
PHI(L ₁)	3140	1623	1575	-	-
NiL ₁ L ₂ Cl	3131	1612	1552	445	618

Table-3: ^1H NMR Data of Complex (ppm)

Complex	Ar-H	N-H(Histidine)	NH_2	N-H(CONH)
NiL ₁ L ₂ Cl	6.95-7.44 (multiplet)	11.00	3.34	8.94

Spectrum

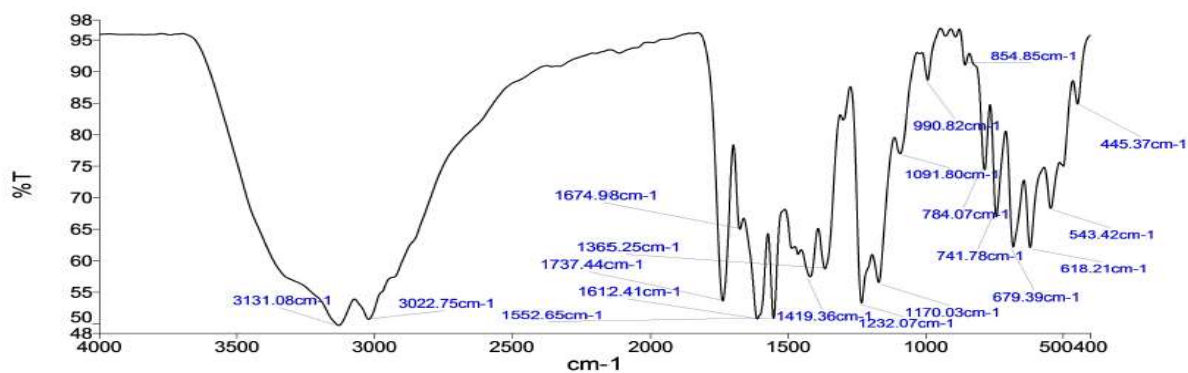


Fig-1: FTIR Spectra of Complex

Antimicrobial studies of the synthesized complex show remarkable activity for bacterial strain *B. subtilis* but less active for *E. coli*. In addition, the complex shows considerable antifungal activity for *T. reesei* but moderate activity seen for *C. albicans*. All results obtained are compared with the standards and concluded. The inhibition effect is exhibited due to the penetrating effect of synthesized compounds.

Table-4: Antimicrobial Activity of Synthesized Compound (Agar Well Diffusion Method)

Test Compounds	Conc.($\mu\text{g/mL}$)	Inhibitory Zone(mm)			
		Antibacterial Activity		Antifungal Activity	
		<i>B. subtilis</i>	<i>E. coli</i>	<i>T. reesei</i>	<i>C. albicans</i>
NiL ₁ L ₂ Cl	80	14	8	17	10
	60	11	6	14	8
	40	2	-	11	-
	20	-	-	-	-
Streptomycin (Standard for Antibacterial)	100	45	44	NT	NT
Ketoconazole (Standard for Antifungal)	100	NT	NT	23	18

NT – Not Tested

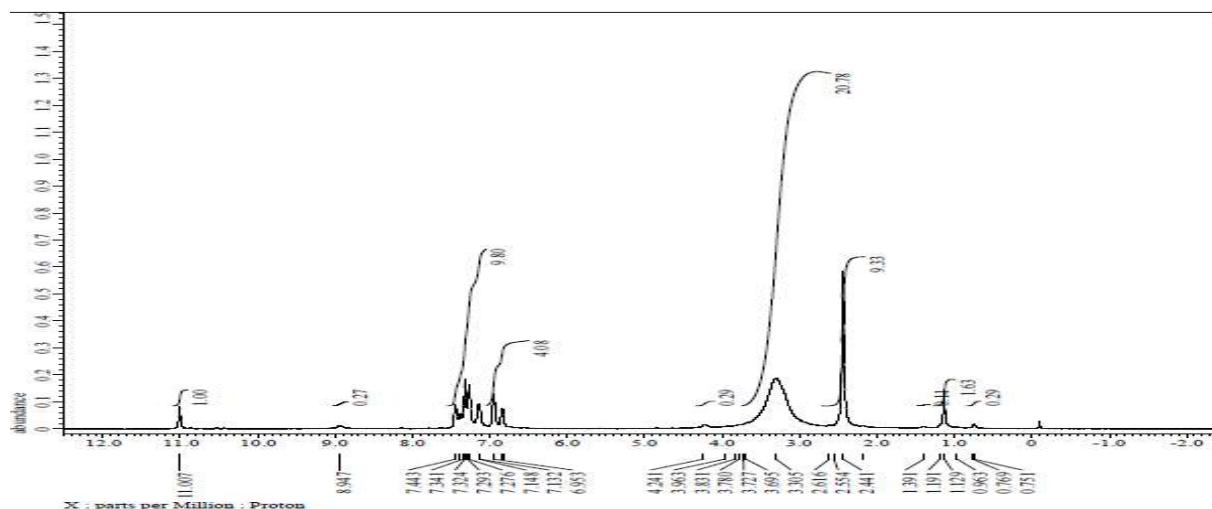
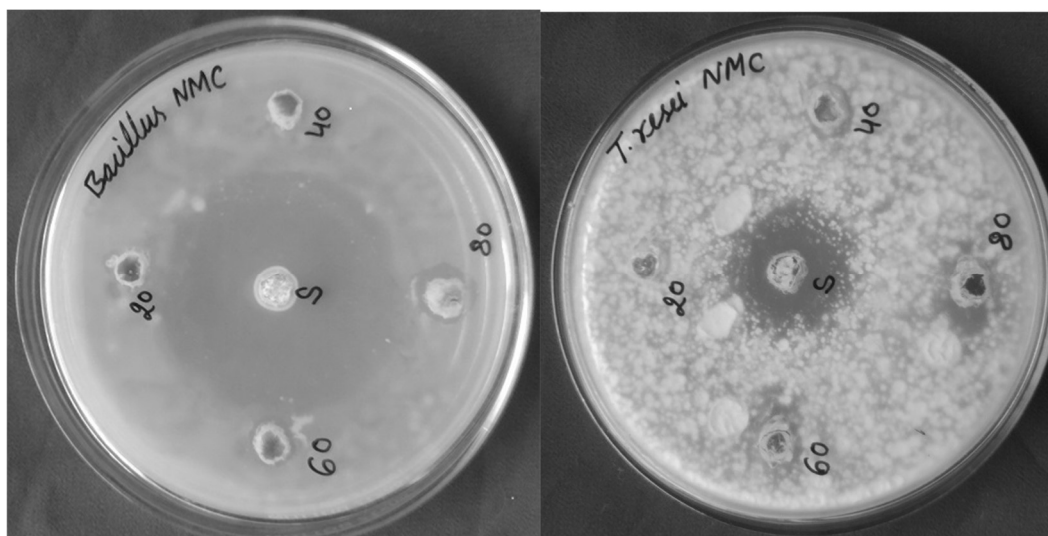
Fig.-2: ^1H NMR Spectra of Complex

Fig.-3: Antimicrobial Study of Complex

CONCLUSION

On basis of all analytical evidence, it is proposed that synthesized complex may have slightly distorted geometry. The synthesized complex is supposed to be non-electrolytic and paramagnetic in nature. No water molecule is coordinated since no FTIR absorption band is seen for this. Moreover, the synthesized complex reveals an inhibitory effect more for the fungal strain compared to bacterial strain, supposed through blocking the active sites of microbes. Further, more derivatives of isatin could be synthesized and novel pharmacophores can be recognized to develop new moieties in drug design.

ACKNOWLEDGMENT

All authors are heartily thankful to the Department of Chemistry, UOR, Jaipur for furnishing research space, chemical, and instrumental requirements. We are very obliged to MRC, MNIT, Jaipur for supplying the facility for spectral studies and Seminal Applied Science Private Ltd, Jaipur for antifungal and antibacterial studies.

REFERENCES

1. S. Kumari, Seema and M. Ranka, *Rasayan Journal of Chemistry*, **14(1)**, 306 (2021), <https://doi.org/10.31788/RJC.2021.1416007>
2. M. S. More, P. G. Joshi, Y. K. Mishra, and P. K. Khanna, *Material Today Chemistry*, **14**, 100 (2019) <https://doi.org/10.1016/j.mtchem.2019.100195>

3. M. Pervaiz, I. Ahmad, M. Yousaf, S. Kirn, A. Munawar, Z. Saeed, A. Adnan, T. Gulzar, T. Kamal, and A. Rashid, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **206**, 642 (2019), <https://doi.org/10.1016/j.saa.2018.05.057>
4. H. Laila, Abdel-Rahman, M. Rafat, El-Khatib, A. E. Nassr, M. Ahmed, Abu-Dief and L. H. Abdel-Rahman, *Journal of Molecular Structure*, **1040(5)**, 9 (2013), <https://doi.org/10.1016/j.molstruc.2013.02.023>
5. K. Siddappa and N. S. Mayana, *Bioinorganic Chemistry and Applications*, **2014(1)**, 483282 (2014), <https://doi.org/10.1155/2014/483282>
6. Y. Satyawana, R. Meena, R. V. Singh and N. Fahmi, *Rasayan Journal of Chemistry*, **12(4)**, 2328 (2019), <https://doi.org/10.31788/RJC.2019.1245459>
7. Suraj B. Ade, M.N. Deshpande, and J.H. Deshmukh, *Rasayan Journal of Chemistry*, **5(1)**, 10 (2012).
8. M. A. Board, M. N. Bhoi, N. P. Prajapati and H. D. Patel, *Synthetic Communications*, **44(7)**, 897 (2014), <https://doi.org/10.1080/00397911.2013.843196>
9. G. Kumari, Nutan, M. Modi, S. K. Gupta, and R. K. Singh, *European Journal of Medicinal Chemistry*, **46(4)**, 1181 (2011), <https://doi.org/10.1016/j.ejmech.2011.01.037>
10. V. V. Vintonyak, K. Warburg, H. Kruse, S. Grimme, K. Hubel, D. Rauth and H. Waldmann, *Angewandte Chemie*, **49**, 5902 (2010).
11. B. K. S. Yeung, B. Zou, M. Rottmann, S. B. Lakshminarayana, S. H. Ang, S. Y. Leong, J. Tan, J. Wong, S. Keller-Maerki, and C. Fischli, *Journal of Medicinal Chemistry*, **53**, 5155(2010), <https://doi.org/10.3998/ark.5550190.p009.385>
12. M. Rottmann, C. McNamara, B. K. S. Yeung, M. C. S. Lee, B. Zhou, B. Russell, P. Seitz, D. M. Plouffe, N. V. Dharia and J. Tan, *Science*, **329**, 1175 (2010).
13. V. Glover, S. K. Bhattacharya, and M. Sandler, *Indian Journal Experimental Biology*, **29**, 1 (1991), <https://doi.org/10.1177/026988119100500304>
14. C.R. Prakash, S. Raja, T. Panneer Selvam, G. Saravanan, V. Karthick and P. Dinesh Kumar, *Rasayan Journal of Chemistry*, **2(4)**, 960 (2009).
15. H. L. Singh and J. Singh, *Bioinorganic Chemistry and Applications*, **2014**, 1 (2014), <https://doi.org/10.1155/2014/716578>

[RJC-6584/2021]