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ANTIMICROBIAL AND ANTICANCER ACTIVITIES OF NI(II) COMPLEXES DERIVED FROM PYRAZOLE BASED SCHIFF BASES

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Abstract

Many pyrazole based Schiff base metal complexes were reported to show higher activities towards bacterial, parasitic strains and cancer lines. Metal complexes of Ni (II) with various imines have been synthesized successfully in alcoholic medium. The synthesized imines have been characterized qualitatively by ^{1}H NMR and FTIR spectroscopy. While complexes formed are analyzed by FTIR,elemental analysis and TGA-DSC. The antimicrobial activities of the synthesized complexes were screened against the Staphylococcus aurous, Escherichia coliand Candida albicans. In vitro antibacterial screening activity revealed that the complexes IMN2, IMN3 and IMN4 showed moderate activity against tested bacterial strains. While the complex IMN1 showed excellent activity tested bacterial strains. However, the IMN1 complex at 10 mg/ml revealed excellent activity as compared with other complexes. In addition, the anticancer activity of the complex IMN1 to IMN4 was performed on A549 (Lung Cancer cell line by using standard 5-FU). At the different doses (10 µg/ml to 100 µg/ml) of IMN1, IMN2, IMN3 and IMN4, it was observed that the IC50 value of the compound IMN1 and IMN4 showed good activity against A549 (Lung Cancer cell line) ascompared with standard drug 5 FU. So these results suggest that the pyrazole based Schiff bases will be widely used in cancer treatments in future.

Key words: 3-amino pyrazole, imine, Schiff base, antibacterial activity and anticancer.

1. Introduction

Hugo Schiff, a German chemist, reported Schiff base in 1864 first time [1]. The carbonyl group (>C=O) may be a constituent of an aldehyde or a ketone. Because Schiff bases contain an azomethine (>C=N-).It has been found that Schiff bases are being used as chelating ligands in coordination chemistry. The transition metal complexes with Schiff bases have created interest

among researchers. Schiff bases derived from primary amines and carbonyl compounds are an important class of ligands that coordinate to metal ions via azomethine nitrogen. These imines have been studied extensively since last few years. Pyrazole derivatives are potential bioactive molecules in pharmaceutical industry [2]. Keeping in view biological and medicinal properties of pyrazoles, we found it vital to join this moiety with transition metals. These compounds could work effectively against bacteria and cancer cells. The azomethine group has good coordination ability with the transition metal ions. C=N– linkage in these compounds is essential for biological activities. Several azomethines are found to posses remarkable antibacterial, anticancer, antimalerial and antifungal activities (Annapoorni and Krishnan, 2013). The five membered pyrazole moiety has attracted considerable attention as a coordinating ligand for complex formation. The coordinating ability of these compounds has resulted in the synthesis of several complexes with various applications [3-7]. The thermal stability of pyrazole is considerably high. 3-amino pyrazole derivatives possess interesting biological activity.

To the best of our knowledge, we have firstly synthesized various pyrazole based Schiff base metal complexes and characterized by FTIR,elemental analysis and TGA-DSC technique. The synthesized Schiff base metal complexes have been screened for antimicrobial and anticancer activities.

2. Experimental section:

Synthesis of Schiff bases

All chemicals and reagents were analytical grade and purchased from commercial suppliers (Merck and Aldrich). Pyrazole Schiff bases were synthesized by condensation reaction between 3-amino pyrazole and aromatic aldehydes i.e 3-methoxybenzaldehyde, 4-chlorobenzaldehyde, 3-chlorobenzaldehyde, 3, 4 dimethoxybenzaldehyde respectively in molar ratio of 1:1 and at pH 4-5. The crude products were purified by recrystallization from ethanol.

2.1 Synthesis of N-(3-methoxybenzylidene)-1H-pyrazol-3-amine (IM1)

The Schiff base was prepared by reacting 3-methoxybenzaldehyde with 3-aminopyrazole in molar ratio of 1:1 in ethanol. To a solution of 3-amino pyrazole (1 mole) in SDS (Abs.alcohol), 3-methoxybenzaldehyde (1 mole) was added. A reaction mixture was refluxed for 10 h. The completion of reaction was monitored by TLC. The resulting reaction mixture was filtered and washed with SDS and dried. The yellow product was purified from alcohol. The yield obtained is 75 %.

IR - ν ($cm^{-1)}$ = 3333.15 (NH), 2924, 2853 (C-H $_{Aliph}$), 1600.96 (-CH=N-), 1491 (-C=C-)
NMR-δ 3.5 (s, 3H), δ 8.02 (s, 1H), δ 7.18 (s, 1H), δ 6.19-7.11(m, Ar-H),

2.2 Synthesis of N-(4-chlorobenzylidene)-1H-pyrazol-3-amine (IM2)

The Schiff base was prepared by reacting 4-chlorobenzaldehyde with 3-aminopyrazole in molar ratio of 1:1 in ethanol. To a solution of 3-amino pyrazole (1 mole) in SDS (Abs. alcohol), 4-chlorobenzaldehyde (1 mole) was added, and reaction mixture was refluxed for 17 h. The completion of reaction was monitored by TLC .The resulting reaction mixture was filtered and washed with SDS and dried. The yellow product was purified from alcohol. The yield obtained is 89 %.

IR - ν ($cm^{-1)}$ = 3333.46 (NH), 2923 (C-H $_{Aliph}$), 1595.27 (-CH=N-), 1491(-C=C-)
NMR : δ 7.91 (s, 1H), δ 5.44-7.28(d, Ar -H)

2.3 Synthesis of N-(3-chlorobenzylidene)-1H-pyrazol-3-amine (IM3)

Schiff base was prepared by reacting 3- chlorobenzaldehyde with 3-aminopyrazole in molar ratio of 1:1 in ethanol. To a solution of 3-amino pyrazole (1 mole) in SDS (Abs. alcohol), 3-chlorobenzaldehyde (1 mole) was addedand reaction mixture was refluxed for 6 h. The completion of reaction was monitored by TLC. The resulting reaction mixture was filtered and washed with SDS and dried. The yellow product was purified from alcohol. The yield obtained is 89%.

IR: ν ($cm^{-1)}$ = 3334.53 (NH), 2923.73 (C-H $_{Aliph}$), 1631.33 (-CH=N-), 1595 (-C=C-)

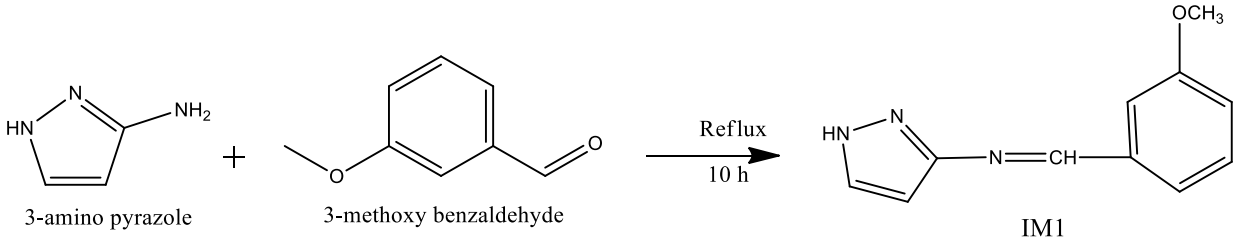
NMR : δ 8.02 (d, 1H), δ 7.71(s, 5H), δ 6.20-7.32 (d, Ar-H)

2.4 Synthesis of N-(3, 4-dimethoxybenzylidene)-1H-pyrazol-3-amine (IM4)

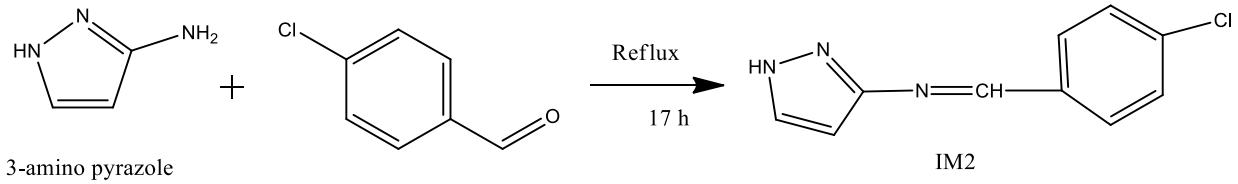
The Schiff base was prepared by reacting 3- chlorobenzaldehyde with 3-aminopyrazole in molar ratio of 1:1 in ethanol. To a solution of 3-amino pyrazole (1 mole) in SDS (Abs. alcohol), 3, 4 dimethoxybenzaldehyde (1 mole) was addedand the reaction mixture was refluxed for 11 h. The completion of reaction was monitored by TLC. The resulting reaction mixture was filtered and washed with SDS and dried. The yellow product was purified from alcohol. The yield obtained is 53 %.

IR - ν ($cm^{-1)}$ = 3274.7 (NH), 2933.12(C-H $_{Aliph}$), 1591.19 (-CH=N-), 1572.60 (-C=C-)

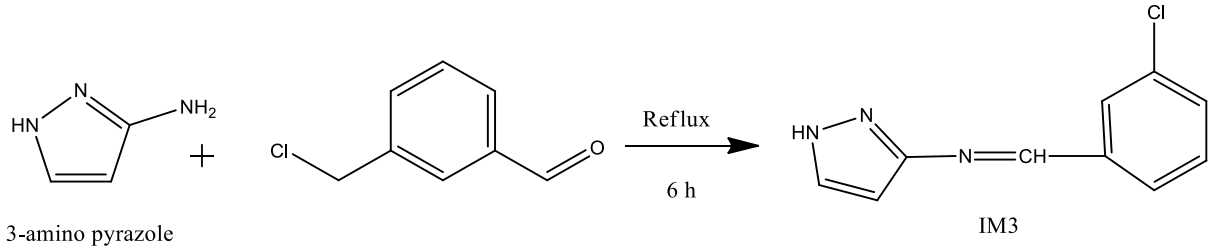
MMR- δ 3.6(s, 3H), 8.6(d, 1H), 7.73(s, 1H), 5.3-7.5 (Ar-H)



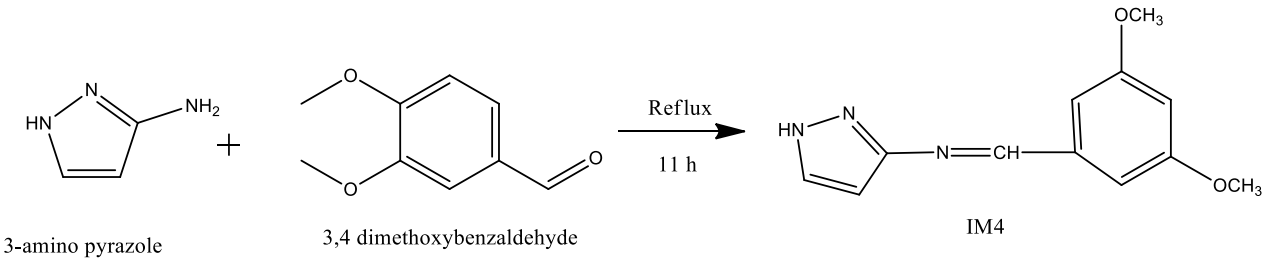
Scheme 1: Synthesis of N-(3-methoxybenzylidene)-1H-pyrazol-3-amine (IM1)



Scheme 2: Synthesis of N-(4-chlorobenzylidene)-1H-pyrazol-3-amine (IM2)



Scheme 3: Synthesis of N-(3-chlorobenzylidene)-1H-pyrazol-3-amine (IM3)



3-amino pyrazole 3,4 dimethoxybenzaldehyde IM4

Scheme 4: Synthesis of N-(3,4-dimethoxybenzylidene)-1H-pyrazol-3-amine (IM4)

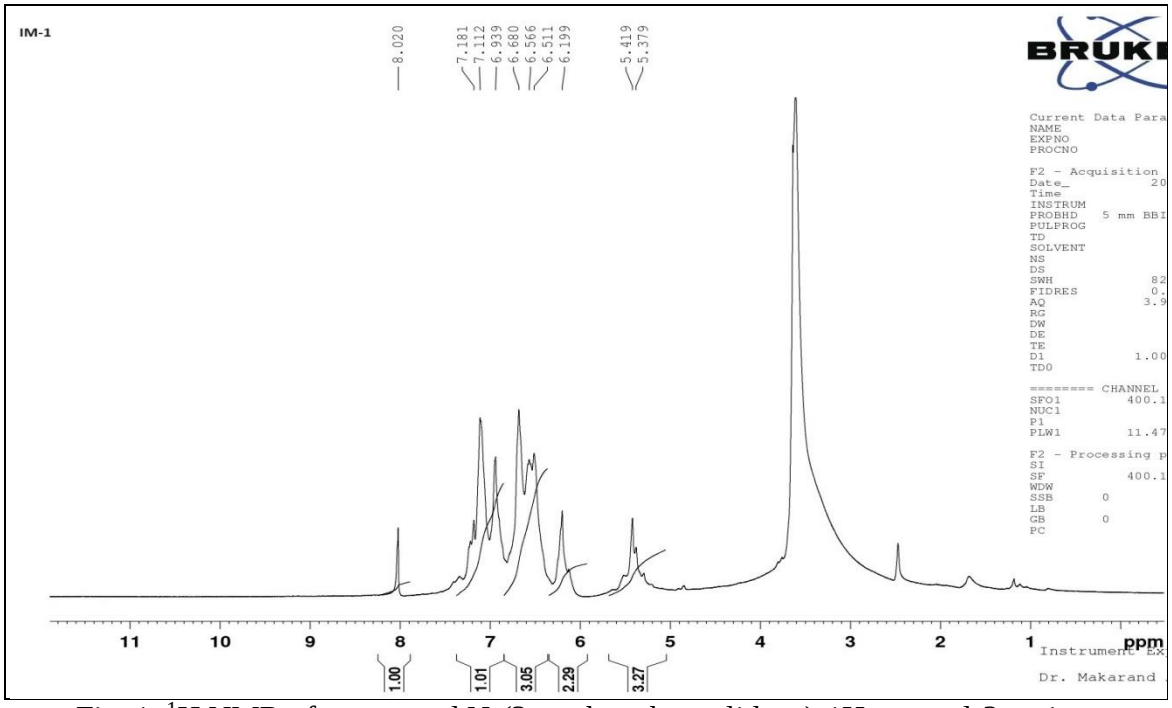


Fig. 1. ^{1}H NMR of compound N-(3-methoxybenzylidene)-1H-pyrazol-3-amine.

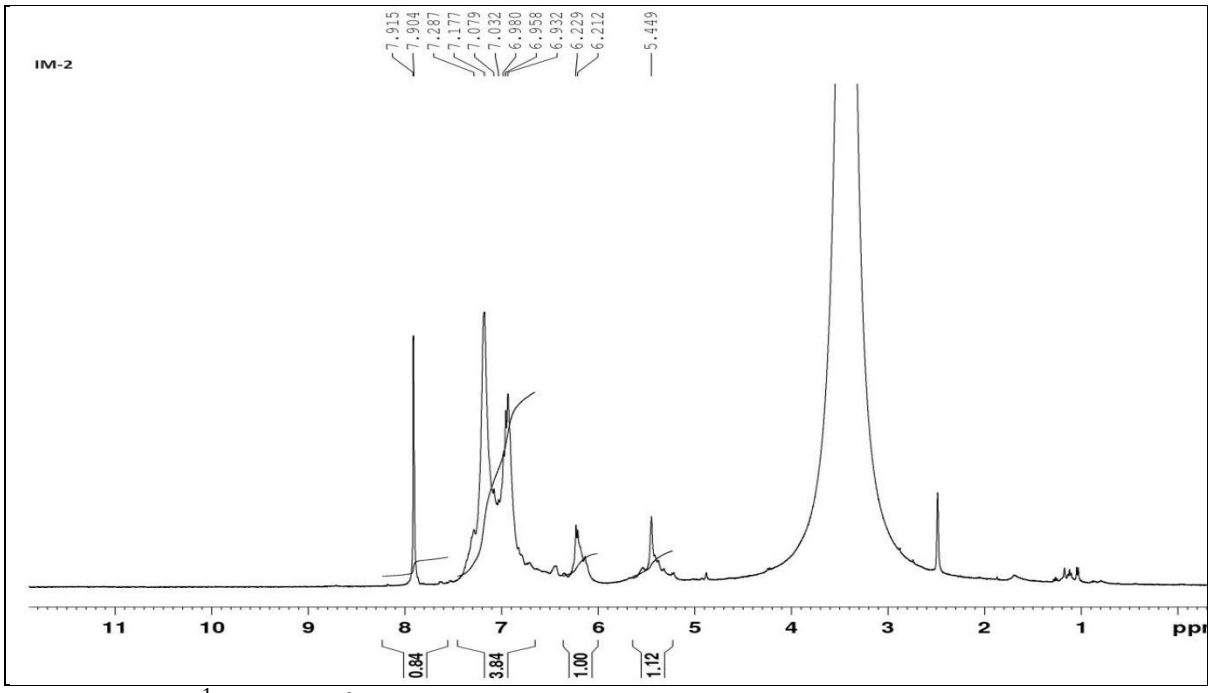


Fig. 2. ^{1}H NMR of compound N-(4-chlorobenzylidene)-1H-pyrazol-3-amine.

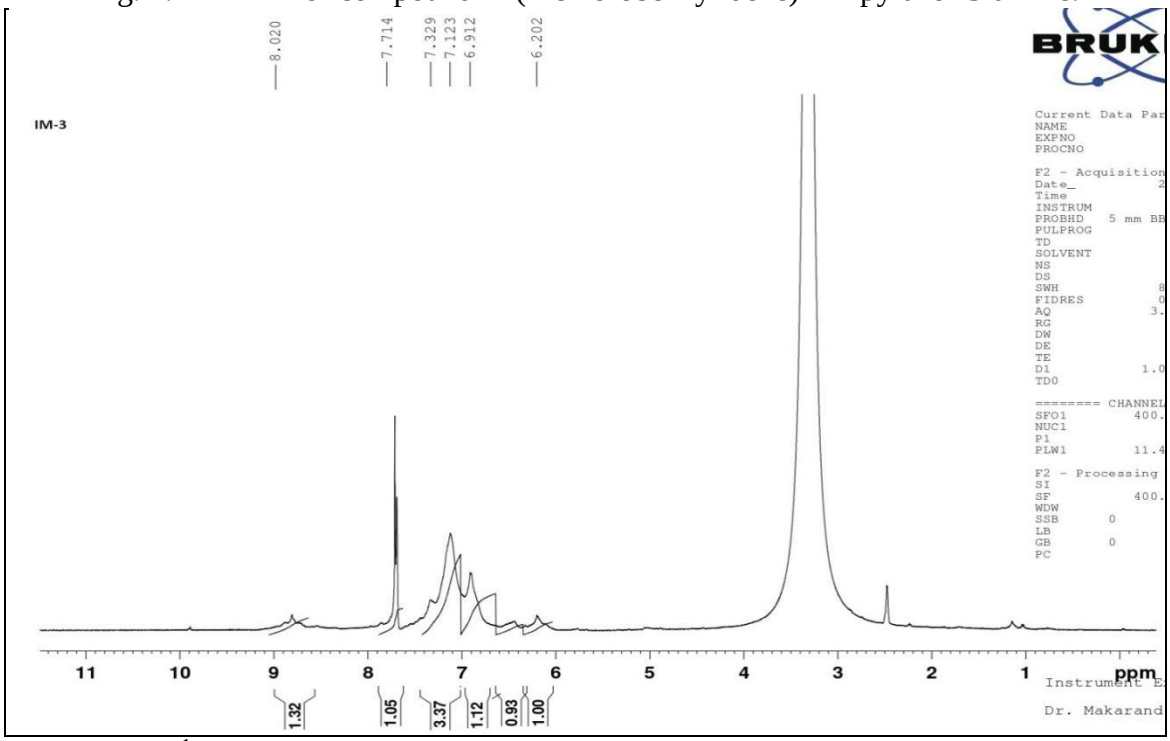


Fig. 3. ^{1}H NMR of compound N-(3-chlorobenzylidene)-1H-pyrazol-3-amine.

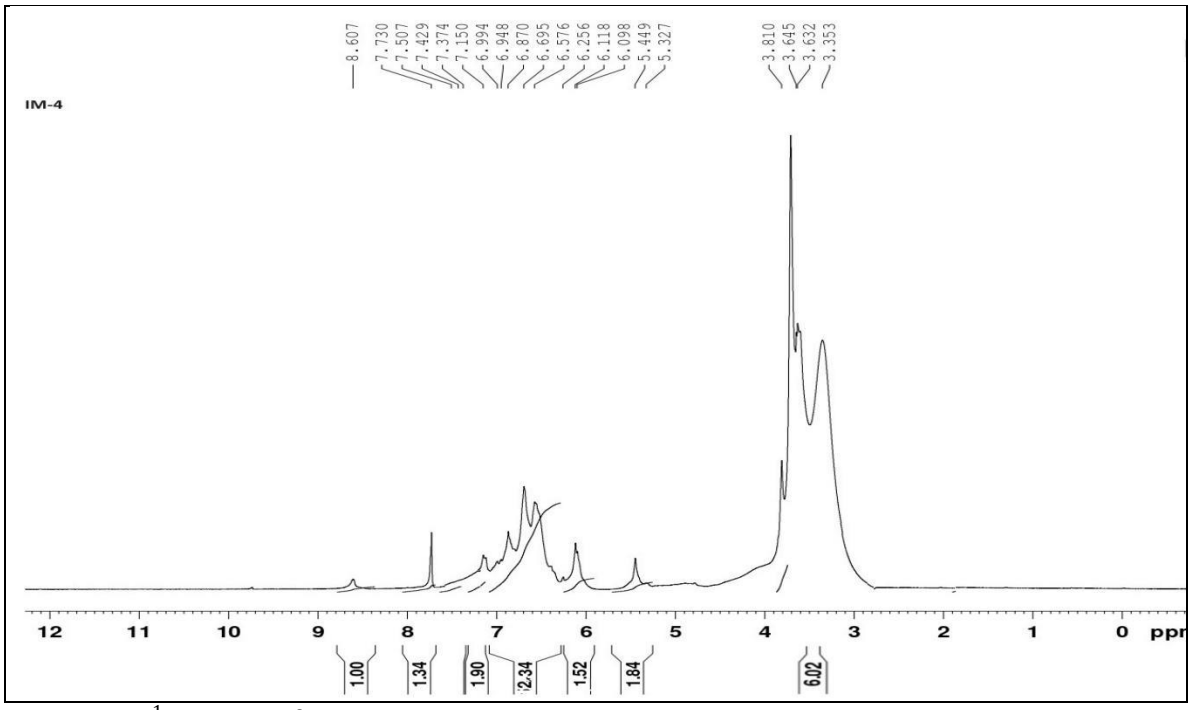


Fig. 4. ^{1}H NMR of compound N-(3,4-dimethoxybenzylidene)-1H-pyrazol-3-amine.

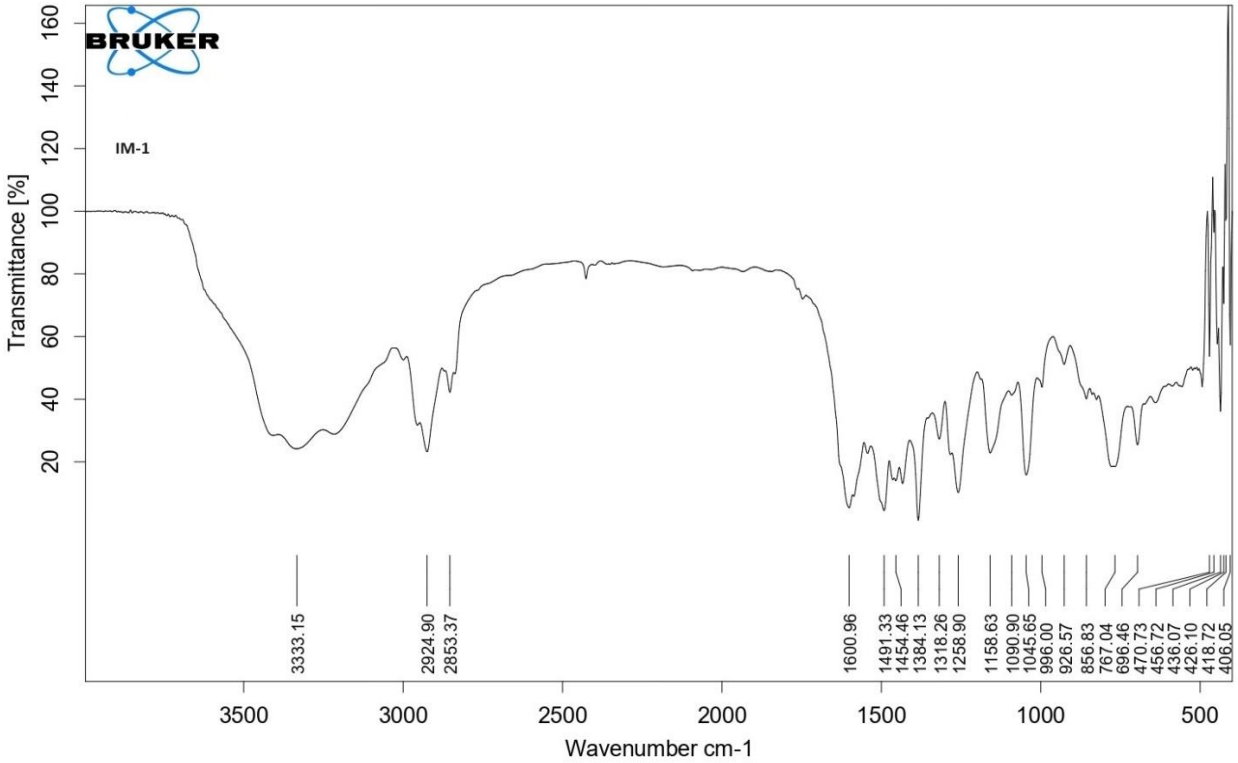


Fig. 5. FT-IR of compound N-(3-methoxybenzylidene)-1H-pyrazol-3-amine.

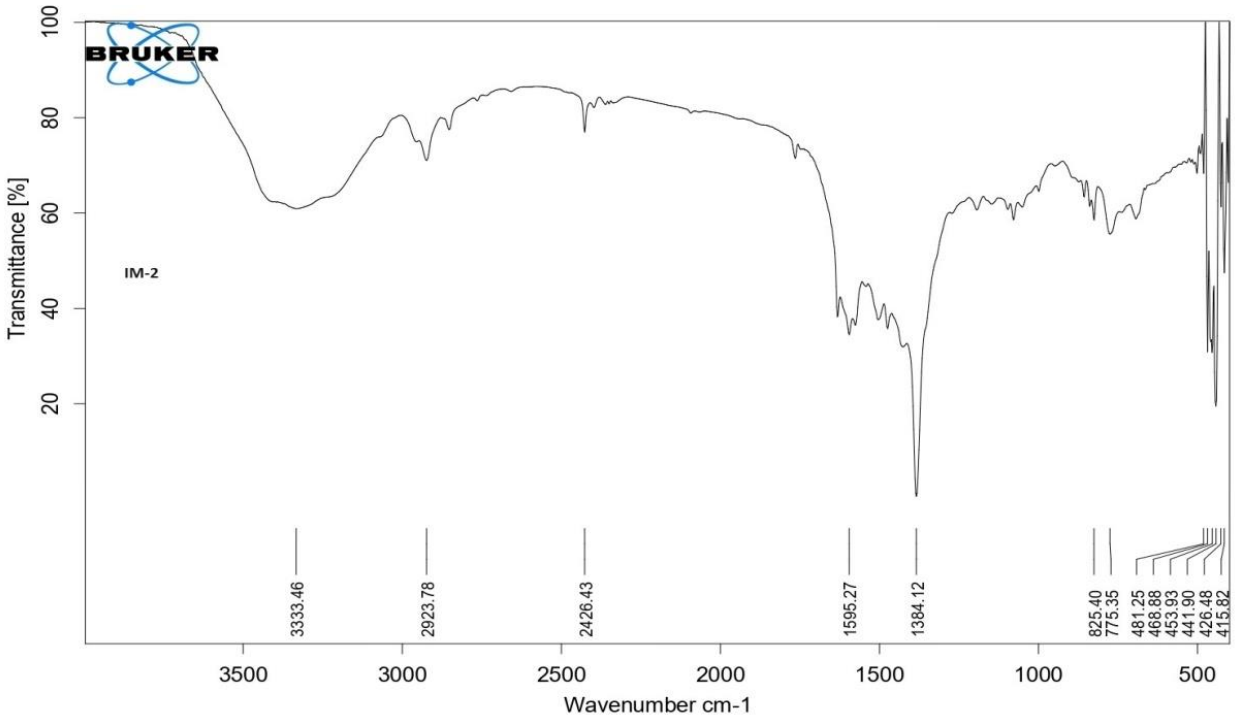


Fig. 6. FT-IR of compound N-(4-chlorobenzylidene)-1H-pyrazol-3-amine

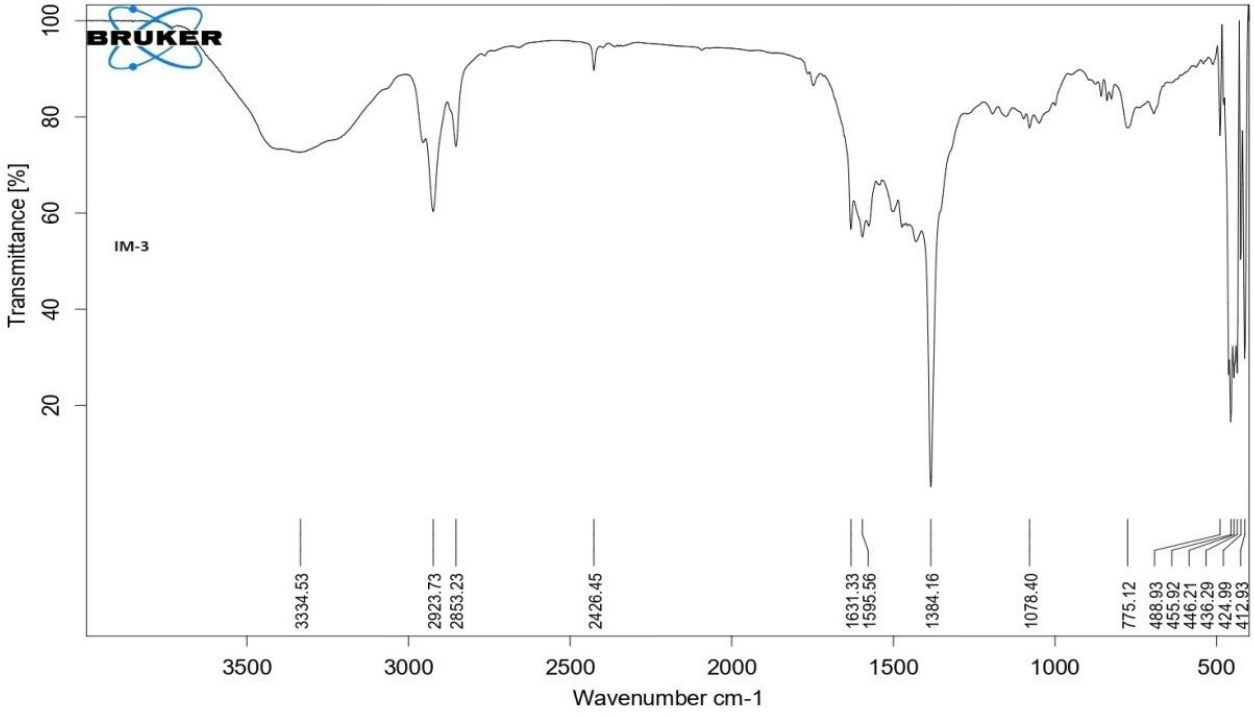


Fig. 7. FT-IR of compound N-(3-chlorobenzylidene)-1H-pyrazol-3-amine

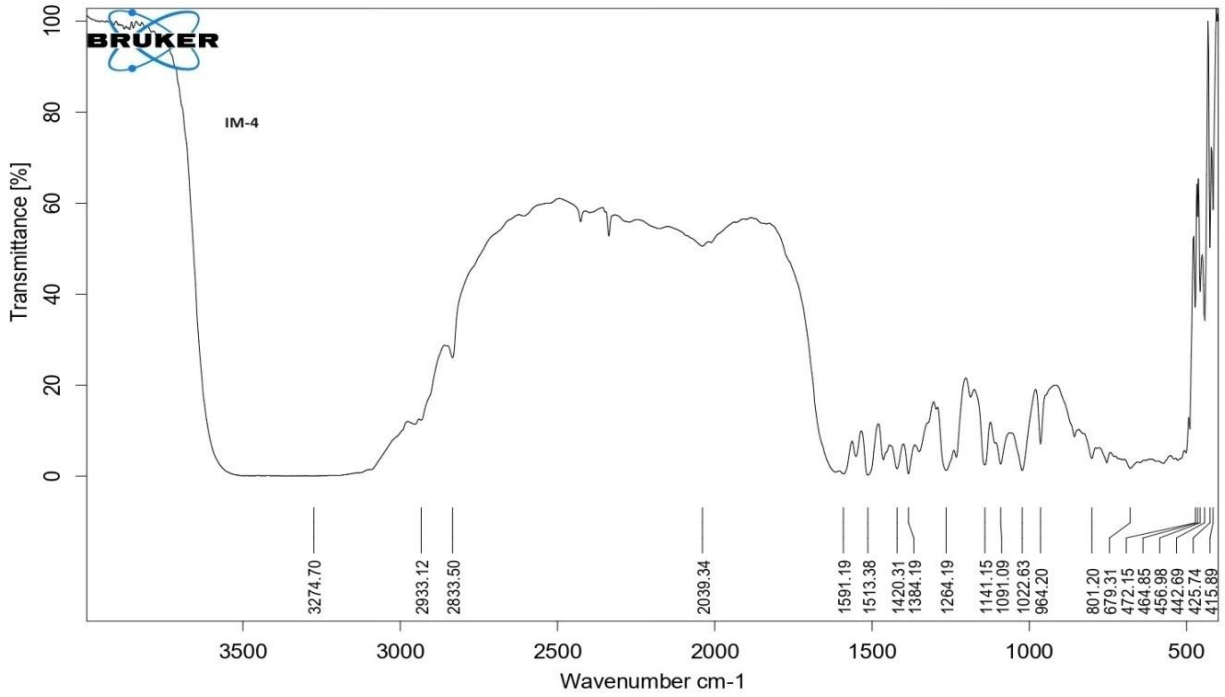


Fig. 8. FT-IR of compound N-(3,4-dimethoxybenzylidene)-1H-pyrazol-3-amine

3. Synthesis of metal complexes

To a suspension of imine in SDS at room temperature, nickel chloride hexahydrate was added slowly. The reaction mixture was then refluxed. The reaction mixture was then filtered off and washed with SDS. The product formed is then dried in oven.

3.1 Preparation of complex (IMN1)

To a solution of imine IM1 (0.200 gm) in ethanol, $NiCl_2.6H_2O$ (0.236 gm) was slowly added with constant stirring and the solution mixture was then refluxed for 8 h. The reaction mixture was filtered off and dried in oven. The product formed was washed with SDS.
Anal.cal. C=45.83, N=29.75=O=18.56, NI=1.91, H = 3.93

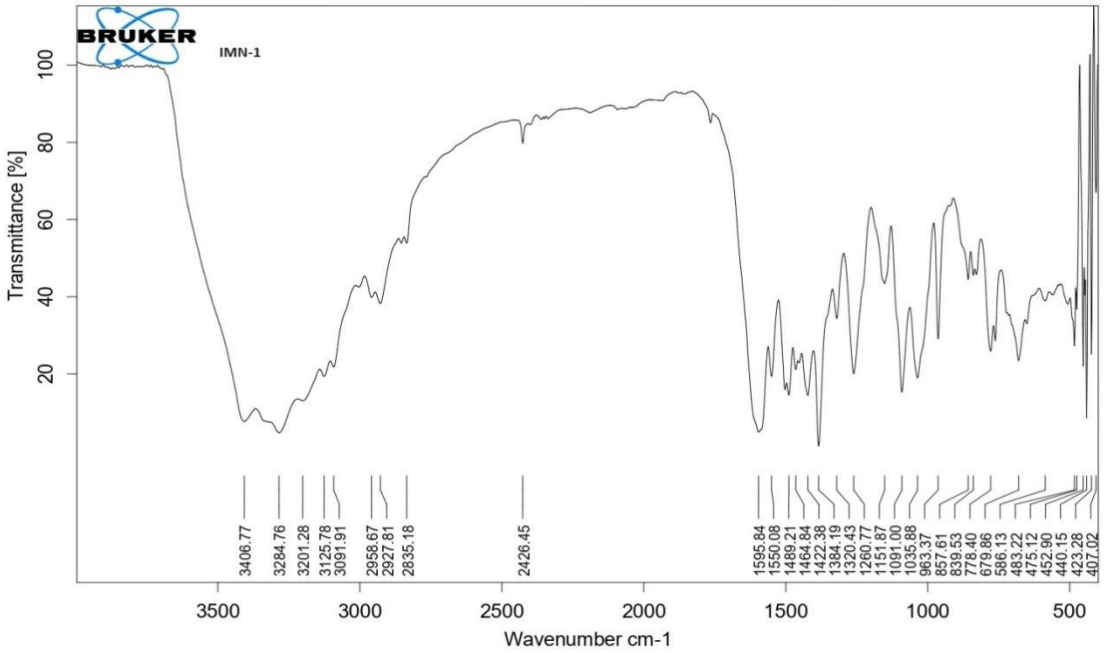


Fig.9. FT-IR of IMN-1

3.2 Preparation of complex (IMN2)

To a solution of imine IM2 (0.200 gm) in ethanol, $NiCl_2.6H_2O$ (0.231 gm) was slowly added with constant stirring and the solution mixture was then refluxed for 11 h. The reaction mixture was filtered off and dried in oven. The product formed was washed with SDS.
Anal.cal.C=61.58, N=31.84, Cl =2.32, NI=0.94, H=3.32

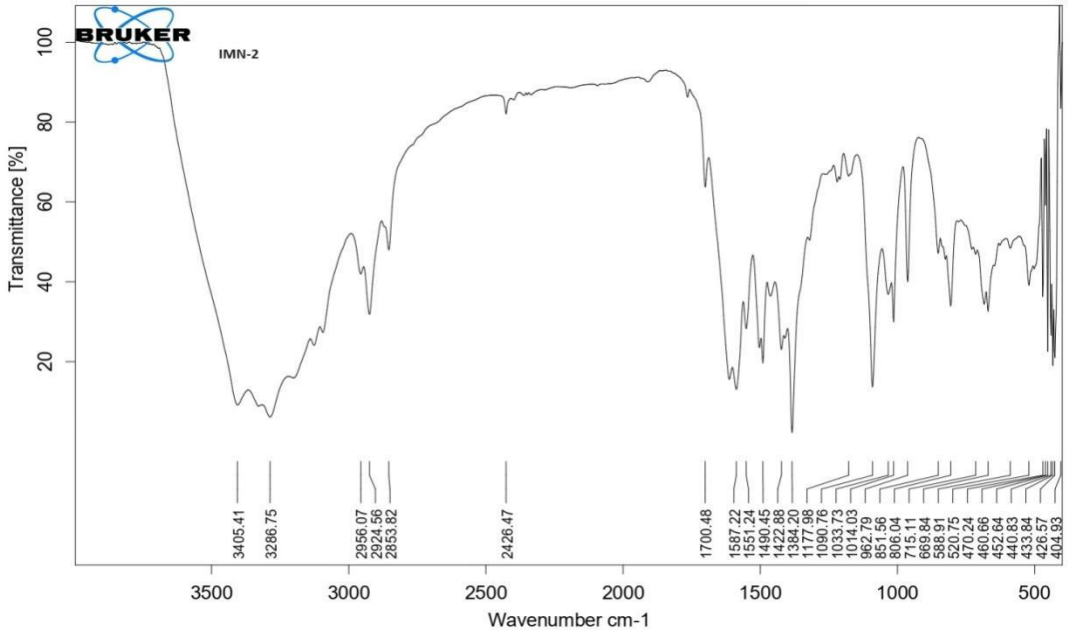


Fig.10. FT-IR of IMN-2

3.3 Preparation of complex (IMN3)

To a solution of imine IM3 (0.200 gm) in ethanol, $NiCl_2.6H_2O$ (0.231gm) was slowly added with constant stirring and the solution mixture was then refluxed for 9 h. The reaction mixture is filtered off and dried in oven. The product formed was washed with SDS.

Anal.cal.C=62.62, N=21.62, Cl =7.6, NI=4.42, H=3.74

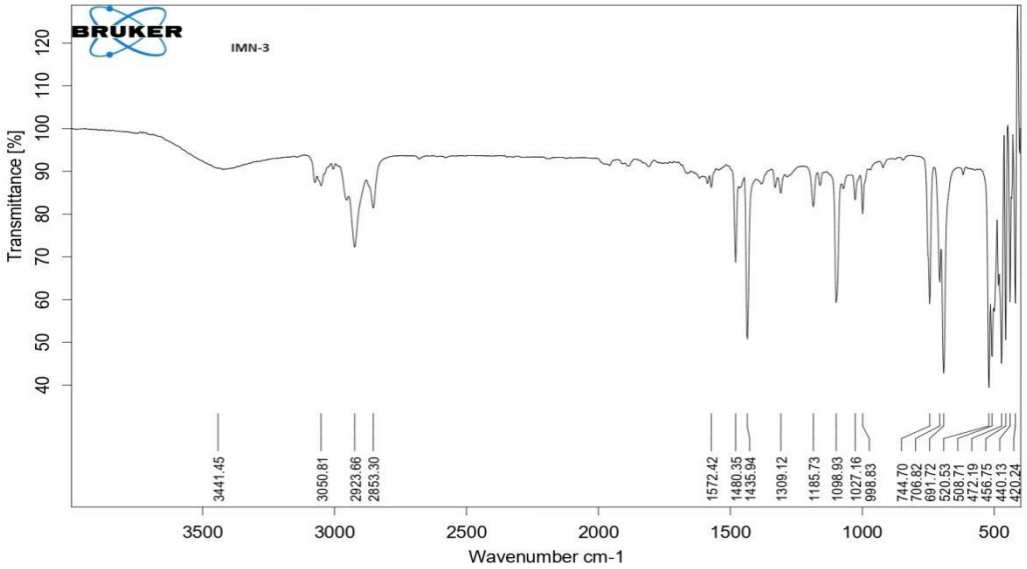


Fig.11. FT-IR of IMN-3

3.4 Preparation of complex (IMN4)

To a solution of imine (0.200 gm) in ethanol, $NiCl_2.6H_2O$ (0.205 gm) was slowly added with constant stirring and the solution mixture was then refluxed for 7 h at room temperature. The reaction mixture was filtered off and dried in oven. The product formed was washed with SDS.

Anal.cal.C =44.22, N=32.93, O=11.34, Ni=6.49, H=5.0

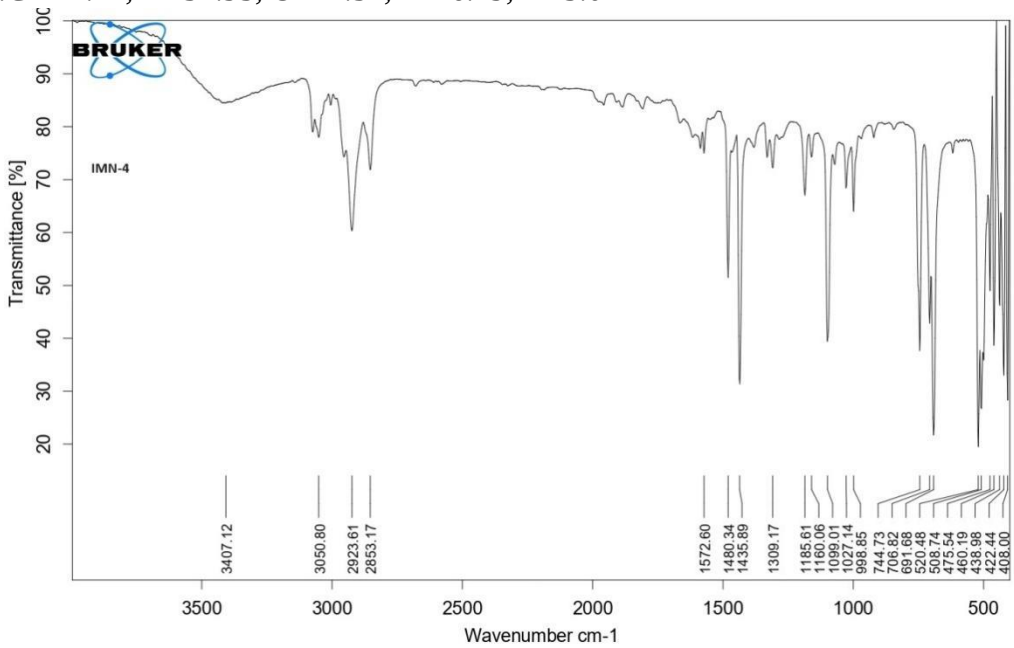
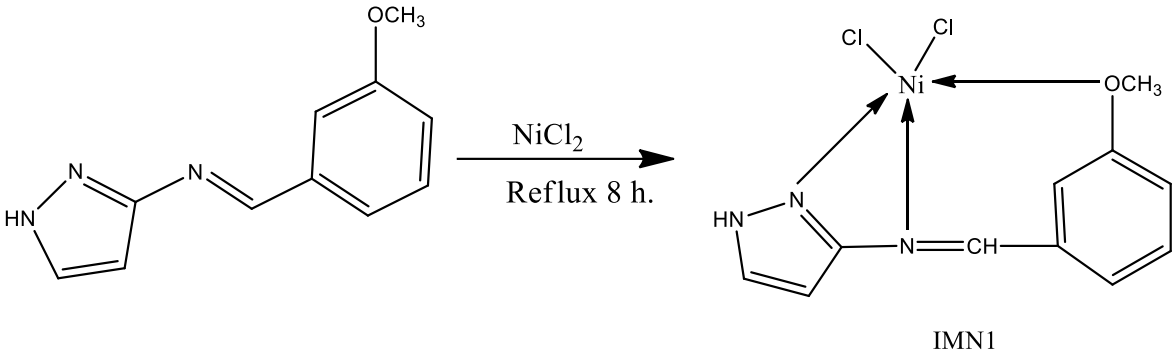
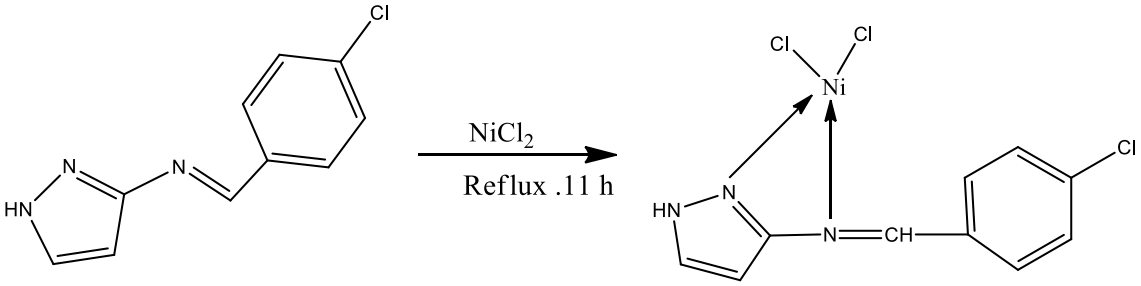


Fig.12. FT-IR of IMN-4



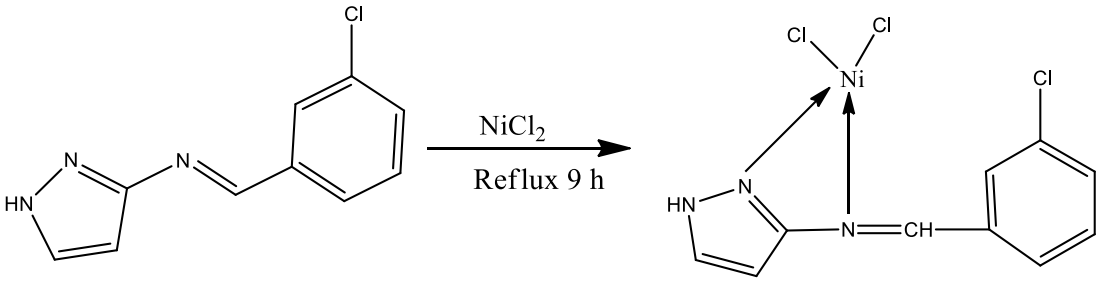
IMN1

Scheme 5: Synthesis of Complex IMN1



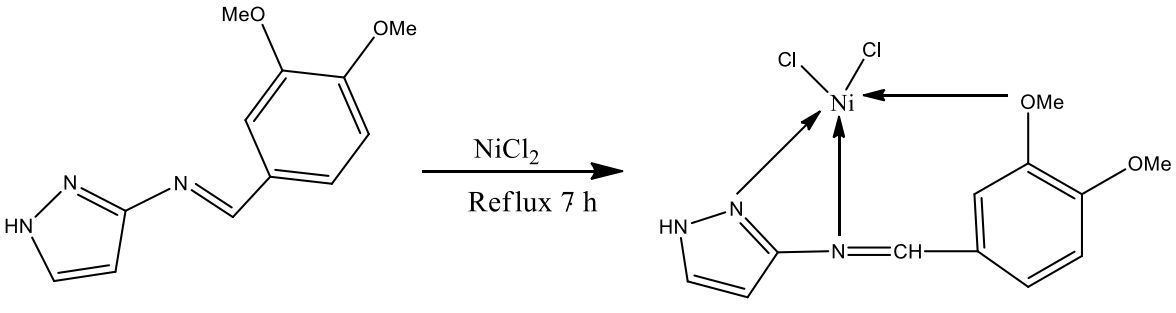
IMN2

Scheme 6: Synthesis of Complex IMN2



IMN3

Scheme 7: Synthesis of Complex IMN3



IMN4

Scheme 8: Synthesis of Complex IMN4

4. Biological Activity

4.1: Antimicrobial Activity-

All the synthesized compounds were tested for their in vitro antimicrobial activities. The microorganisms used for the study consisted of Staphylococcus aurous, Escherichia coliand Candida albicans. The Agar cup plate method was used for testing. The clinically used Streptomycin Fluconazole was employed as a reference material. Biological activities were carried out at Biocyte research & development pvt ltd, Kalanagar, Sangli, Maharashtra – 416416.

Table No. 1: MIC of Compounds with Different pathogenic Bacteria

Sr. No.	Sample	Concentration	Zone of Inhibition (mm) Staphylococcus aureus	Zone of Inhibition (mm) Escherichia coli	Zone of Inhibition (mm) Candida albicans
Standard	Streptomycin Fluconazole	1 mg/ml	39	23	26
1	IMN1	5mg/ml	06	02	04
		10mg/ml	**10**	**04**	**08**
2	IMN2	5mg/ml	04	01	02
		10mg/ml	06	02	05
3	IMN3	5mg/ml	02	04	03
		10mg/ml	04	06	06
4	IMN4	5mg/ml	02	01	02
		10mg/ml	04	02	04

4.2. Anticancer activity

Cells were incubated at a concentration of 1×10^4 cells/ml in culture medium for 24 h at 37°C and 5% CO_2. Cells were seeded at a concentration (70 µl) 104 cells/well in 100 µl culture medium and 100µl sample of IMN1, IMN2, IMN3 and IMN4 in (10, 20, 40, 80, 100 µg/ml) into micro plates respectively (tissue culture grade, and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37 °C and 5% CO_2 in CO_2 incubator (Thermo scientific BB150). After incubation, the medium was completely removed and added 20 µl of MTT reagent (5mg/min PBS). After addition of MTT, cells incubated for 4 h at 30°C in CO_2 incubator. Triplicate samples were analyzed by measuring the absorbance of each sample by a Elisa microplate reader (Benesphera E21) at a wavelength of 570 nm. The activity of complexes against cancer cells (lung cancer cell line) at various concentrations is shown in table No.2.0

Table No. 2.0 - Effects of compound against A549 (Lung Cancer cell line) by MTT assay

Sr.no	**Sample**	**Concentration (µg/ml)**	**OD(mean)**	**%inhibition**	**IC 50 (µg/ml))**
1	Control		0.322		
2	IMN1	10**µg/ml**	0.227	29.65	17.94
		20**µg/ml**	0.116	64.05	
		40**µg/ml**	0.109	66.22	
		80**µg/ml**	0.099	69.32	
		100**µg/ml**	0.089	72.42	

3	IMN2	10**µg/ml**	0.215	33.37	>100
		20**µg/ml**	0.210	34.91	
		40**µg/ml**	0.205	36.46	
		80**µg/ml**	0.202	37.39	
		100**µg/ml**	0.200	38.02	
4	IMN3	10**µg/ml**	0.232	28.10	>100
		20**µg/ml**	0.228	29.33	
		40**µg/ml**	0.221	31.50	
		80**µg/ml**	0.216	33.05	
		100**µg/ml**	0.214	33.67	
5	IMN4	10**µg/ml**	0.185	42.67	83.97
		20**µg/ml**	0.174	46.07	
		40**µg/ml**	0.169	47.62	
		80**µg/ml**	0.163	49.48	
		100**µg/ml**	0.158	51.03	

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5. Result and discussion

Characterization of the complex

5.1 IR spectral studies

The mode of binding of ligands to the metal ions was elucidated by the IR spectra of the complexes as compared with the spectra of free ligands (Table 3).The formation of ligands is confirmed by the disappearance of stretching vibrations due to aldehyde (–CHO) and primary amine ($–NH_2$) moiety .Instead a strong new band appears at 1620-1600 cm^{-1} corresponding to the azomethine ν (HC=N) group. After complexation, the band due to V(HC=N) shifted to lower frequencies by 15-20 cm^{-1} . This indicates the coordination of azomethine –N to the metal ion. The characteristic IR bands (4000-450cm^{-1}) for the free ligands, when compared with those of its respective complexes, provides meaningful information).The IR bands bands for ν C=N (pyrazole ring) in free ligand spectrum has beenfound to shift to the higher frequency region ,indicating the participation of the tertiary ring nitrogen atom as a potential binding site.

Table 3. Selected Infrared Frequencies (cm^{-1}) for Schiff Bases

Sr. No	IMINE	νcm^{-1} (CH=N) azomethine	Complex	νcm^{-1}(C=N)complex
1	IM1	1600	IMN1	1595
2	IM2	1595	IMN2	1587
3	IM3	1631	IMN3	1572.4

4	IM4	1591	IMN4	1572.60

5.2Elemental analysis

The reported complexes gave satisfactory C, H, N and metal analysis.

5.3 Thermal analysis

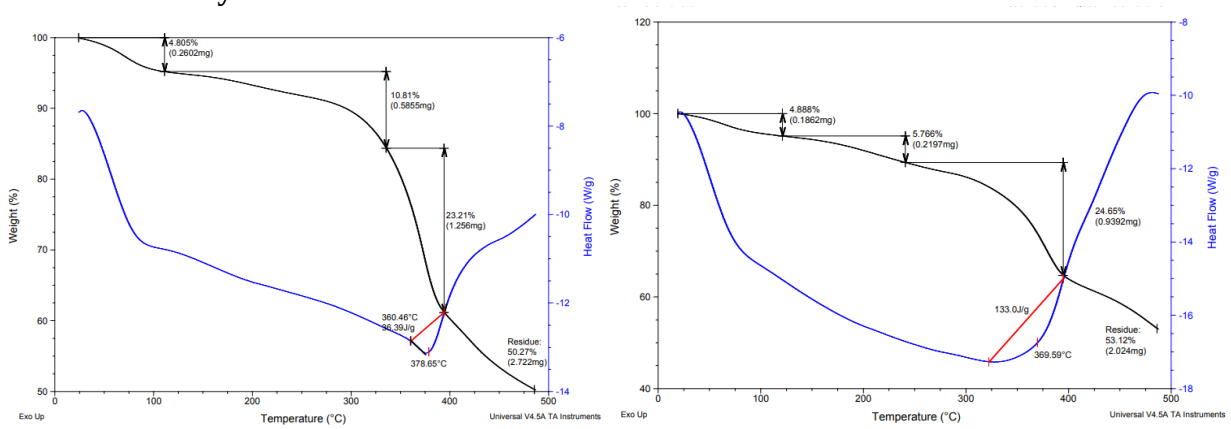

Figure 13: TGA and DSC analysis of IMN 1(left side) and IMN2 (right side) complexes.

The thermal stabilities of IMN1 and IMN2 complexes studied by TGA-DSC are shown in Fig. 13. From the figure, it was observed that weight losses of 50.27% and 53.1 % (char residue) at 500 °C of complexes by three consequent steps. The initial weight losses in first step are 4.805 % and 4.888 % respectively in the range of temperature 50-120 °C due to evaporation of water molecules. However, in the second step, there are different weight losses 10.81% and 5.766 % respectively in the different temperature ranges of 120-340 °C and 120-240 °C observed due to the loss of pyrazole moieties present in the complexes. The weight losses in third step are 23.21% and 24.65 % respectively in the temperature ranges of 340-400 °C and 240-400 ºC due to the desorption of two Ni-bound chlorides as hydrogen chloride. Hence these complexes are stable up to 400 °C and above that dehydrochlorination starts in presence of water vapours [13]. The details of weight loss with temperature decomposition of complexes are shown in table No.4

The DSC analysis of complexes (blue line) shows an endothermic peak at 378.65 ºC and 369.59 ºC respectively, indicating the endothermic reaction caused by the thermal dissociation of Ni-Cl bonds.

Table 4: Thermogravimetric characteristics of IMN1 and IMN2 complexes

Compound	**Decomposition stages**		**% Weight loss**
	Assignment	**Temperature**	
IMN1	1)Water molecules	50-120	4.805
	2)Pyrazole moieties	120-340	10.81
	3) Ni-Cl	340-400	23.21
IMN2	1)Water molecules	50-120	4.888
	2)Pyrazole moieties	120-240	5.766
	3)Ni-Cl	240-400	24.65

6. Conclusion:

New pyrazole Schiff bases were successfully synthesized and characterized by IR, NMR and elemental analysis. Moreover the thermo gravimetric analysis showed that the complexes formed are stable up to 380 ^{o}C. During the study it has been found that samples IMN1 to IMN4 showed moderate inhibiting the growth of bacterial strains Staphylococcus aurous, Escherichia coli as well as fungal strain Candida albicans as compared to standard drug. Among these complexes, complex IMN2 at 10 mg/ml found to be effective against Staphylococcus aureus and Candida albicans. Also the anticancer activity of the compounds IMN1, IMN2, IMN3, IMN4 was performed on the A549 (Lung Cancer cell line by using standard 5-FU). At the different doses (10 µg/ml to 100 µg/ml) IMN1, IMN2, IMN3,IMN4, it was observed that the IC50 value of the compound IMN1 and IMN4 showed good activity against A549 (Lung Cancer cell line) as compared to standard drug 5 FU. Therefore in the future these types of pyrazole based complexes with metals will be widely used in pharmacological study.

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