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Synthesis and antifungal potential of some transition metal complexes of tridentate N^2-Benzylidenepyridine-2, 6-Diamine

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Abstract

Cu (II), Co (II) and Mn (II) complexes of Schiff bases of 2,6-diaminopyridine with aryl aldehydes were synthesized and characterized. They were screened for their antifungal potential against various phytopathogenic fungi *viz. Puccinia triticinia, Bipolaris sorokiniana* and *Puccinia striiformis tritici* and the results were compared with the respective ligands and standard fungicide. The complex L_2Cu was found to be most potent against *P. triticinia* (ED_{50} 45 µg/ml) comparable to standard fungicide propiconazole (ED_{50} 40 µg/ml).

Keywords: 2, 6-Diaminopyridine, Schiff Base, Metal complexes, Spore germination inhibition, antifungal activity

1. Introduction

Schiff bases with heterocyclic backbone and their metal complexes are of significant interest owing to their wide spectrum of biological properties. The metal complexes of Schiff bases are considered to mimic the model for the metals embedded in metallo proteins [1, 2] and enzymes[3] and thus, have a great application in the field of bioinorganic chemistry [4, 5]. The bio-potential of the metal complexes are liable to get altered by the design of ligands, choice of metal, bio-potential of metals, bio-potential of ligands [6], and their relative ratios.

2, 6-Diaminopyridine is an important heterocyclic motif having strong chelating properties because of the ring nitrogen atom, with a localized pair of electrons[7]. Its derivatization as Schiff bases [8] have received overwhelming attention as a biologically active compound [9] and reported with wide range of applications as anti-malarial [10], anti-inflammatory [11], anti-tubercular [12], anti-mitotic [13], anti-bacterial [14], tyrosine kinase inhibiting agents [15], and to remove sulphur from ores [16]. In addition they inhibit the growth of wide range of fungal infestations[17], and inflict multiple mode of action for their fungitoxicity ration inhibitors.

Number of complexes of various metal with 2, 6-diaminopyridines have been reported but there is potential to further explore this work for antifungal assay against various phyto-pathogens. In this view, we synthesized metal complexes of 2, 6-Diaminopyridines with non-poisonous bioactive metals *viz.* copper, cobalt and manganese to explore their *in vitro* antifungal potential against three phytopathogenic fungi *viz. Puccinia triticinia, Bipolaris sorokiniana* and *Puccinia striiformis tritici.*

2. Materials and Methods

All the chemicals and reagent used were of AR or LR grades. Melting points were taken in open capillaries in an electric melting point apparatus and are uncorrected. The ^1H NMR, CHN analysis and LCMS-MS spectroscopic analysis was obtained from sophisticated Analytical Instrument Facility, Panjab University, and Chandigarh and FT-IR spectrum were obtained from Central Instrumentation Laboratories (CIL), Panjab University, Chandigarh. ^1H NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer with DMSO and $CDCl_3$ as solvent and TMS as internal solvent. The chemical shifts were expressed in δ (ppm) values. IR spectra were recorded on Perkin Elmer FT-IR spectrometer in the range 400-4000 cm^{-1} using KBr discs. Mass spectra were recorded in terms of mass to charge ratio (m/z) on Waters LCMS-MS Quattro Micro^{TM} API mass spectrometer. Elemental analysis (CHN) was recorded on Flash 2000-organic elemental analyser. The molar conductance was recorded at Department of Chemistry, Punjab Agricultural University, Ludhiana.

3. Chemistry

Synthesis of Schiff base ligands

Schiff bases of 2,6-diaminopyridine were synthesized by condensation of 2,6-diaminopyridine with different aryl aldehydes *viz.* benzaldehyde, 2-chlorobenzaldehyde, 4-florobenzaldehyde in equimolar ratio using glacial acetic acid as catalyst to get N^2-benzylidenepyridine-2,6-diamine (L_1), N^2-(2-chlorobenzylidene)pyridine-2,6-diamine (L_2), N^2-(4-florobenzylidene)pyridine-2,6-diamine (L_3). A single spot on thin layer chromatographic plate showed the purity of the compound. The resultant solution was cooled to room temperature. The yellowish brown coloured solid obtained was filtered, recrystallized from methanol and air dried.

Synthesis of metal complexes

To the hot ethanolic solution of synthesized ligands (L_1, L_2, L_3) (0.02 mol), an ethanolic solution of metal salt (0.01 mol) (MCl_2 where M= Co, Cu, Mn) in the 2:1 molar ratio was added drop wise, with constant stirring. The change of colour and solidification of product was observed for the reaction mixture. Resulting crude solid formed was filtered and washed with methanol several times until the washing becomes colourless. Solid product obtained was air dried to get their respective metal complexes.

Antifungal assay

The *in vitro* antifungal screening effects of the synthesized compounds were tested against three phytopathogenic fungi of wheat namely; *Puccinia triticinia, bipolaris sorokiniana* and *Puccinia striiformis tritici* by spore germination inhibition technique [18]. Stock solutions (2000 µg/ml) of all test compounds were prepared by dissolving 20 mg of compounds in distilled water (10 ml). Tween 20 (1-2 drops) was used as surfactant. Serial dilutions of 1000, 500, 250, 100, 50 and 25µg/ml were done from stock solution (2000 µg/ml) by adding distilled water as and when required.

Spore suspension was made by adding sterilized distilled water to the fresh cultures of *Bipolaris sorokiniana,* and fresh spores of *P. striiformis tritici* and *P. triticinia* were harvested from the infected leaves. Suspension was filtered through muslin cloth in order to remove mycelia under aseptic conditions. Haemocytometer was used to form standardized spore suspension (1x 10^6 spores ml^{-1}). Small droplets (0.02 ml) of test solution and spore suspension in equal amount were seeded in the cavity of the cavity slides. These slides were placed in Petri plates lined with moist filter paper and were incubated for 24 hrs at 25±1 °C for *B. sorokiniana,* and 24 hrs at 15-17±1 °C for *P. striiformis and P. triticinia*. The numbers of spores germinated were counted and percent spore germination inhibition was calculated by the following formula:

Percent spore germination inhibition (% SGI)

$$\%\ \text{SGI}= \frac{\text{Spore germination in control - Spore germination in treatment}}{\text{Spore germination in control}} \times 100$$

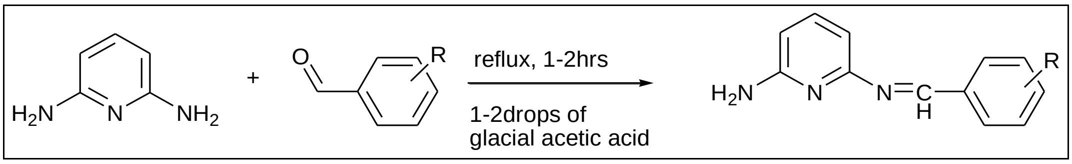
The ED_{50} values for each fungicide were calculated by plotting per cent inhibition in spore germination against different concentrations of different compounds.

4. Results and Discussion

Synthesis and Characterization

The ligands were prepared by the reaction of 2, 6-diaminopyridine with benzaldehyde, 2-chlorobenzaldehyde and 4-florobenzaldehyde in presence of glacial acetic acid as shown in Scheme 1. These synthesized ligands were treated with metal salt of Co, Cu and Mn in the 2:1 molar ratio to get their respective metal complexes. The products were characterized on the basis of elemental analysis, mass spectrometry and different spectroscopic techniques. Physical characterization *viz.* molar conductance, complexometric titrations, colour and melting point were also recorded.

All the analytical data of Schiff bases and their metal complexes is also reported in Table 1 and 2, respectively.



Scheme 1: R= 2-H, 2-Chloro, 4-Floro

Table 1: Analytical data of Schiff bases

S. No.	Ligand	Yield %	Colour	MP (°C)	Molecular formula	MW	Elemental analysis Calculated (expected)		
							C	H	N
1	L_1	80	Yellowish brown	240-245	$C_{12}H_{11}N_3$	197.1	73.07 (72.89)	5.62 (5.45)	21.30 (21.11)
2	L_2	85	Yellow	245-248	$C_{12}H_{10}ClN_3$	231.06	61.89 (62.21)	4.22 (4.35)	18.02 (18.14)
3	L_3	75	Pale yellow	243-246	$C_{12}H_{10}FN_3$	215.09	65.6 (66.97)	4.12 (4.68)	19.23 (19.52)

Table 2: Analytical data of metal (II) N^2-benzylidenepyridine-2, 6-diamines

Sr. No.	Compounds	Yield	Colour	Solubility	Molar conductance ohm^{-1} cm^2 mol^{-1}	Molecular Mass [M$^+$]
1	L_1 -Co	55%	Light Green	DMSO, DMF	0.07	361.99
2	L_2–Co	45%	White	DMSO, DMF	0.08	395.95
3	L_3-Co	70%	Light Green	DMSO, DMF	0.09	379.98
4	L_1–Cu	70%	Greenish Black	DMSO, DMF	0.06	365.98
5	L_2–Cu	80%	Dark Green	DMSO, DMF	0.07	399.94
6	L_3-Cu	80%	Grey	DMSO, DMF	0.05	383.97
7	L_1–Mn	60%	Pale pink	DMSO,DMF	0.05	357.99
8	L_2–Mn	55%	Pink	DMSO,DMF	0.09	391.95
9	L_3-Mn	65%	Pinkish white	DMSO,DMF	0.06	375.98

The synthesized complexes are intense coloured, thermally stable, non-hygroscopic and insoluble in water but highly soluble in DMSO and DMF. The molar conductance of the complexes has been recorded in 10^{-3}M DMSO at room temperature. The values of molar conductance remain in between 0.04 -0.10 ohm^{-1} cm^2 mol^{-1} suggesting their non-electrolytic nature [19]. Mass spectral data gave the mass in consonance with the expected structures.

Complexometric Titrations: Copper complex solution was titrated with 0.01M disodium EDTA solution using Eriochrome Black T as indicator. End point was noted from red to purplish colour indicated the formation of Copper complex with EDTA. The percentage of copper was found to be 15.2 per-cents very much comparable to theoretical copper per-cent i.e. 15.8%, which was in resonance with the expected structures [20].

^{1}H NMR spectra

The ^{1}H NMR of Schiff base ligands were recorded in DMSO at room temperature. Three types of protons were identified i) resonance exhibits due to =N-H protons around 5.32 ii) characteristic resonance due to azomethine proton in the Schiff base appears around 8.12 ppm iii) the other signals in the region 6.66-7.35 ppm exhibits due to aromatic protons. All these observations support the infrared conclusions [21].

Infrared spectra

The data indicated (Table 3) the involvement of nitrogen of –CH=N- in the bond formation resulting in the increase in stretching frequency of the azomethine group and appearance of a new band in the region 559-683cm^{-1} was in favour of the formation of M-N bond in complexes [22]. Also the increase in stretching frequency of =N-H indicated the involvement of its nitrogen in complexation.

Table 3: Infrared spectroscopic data of the Schiff base ligands and their metal complexes

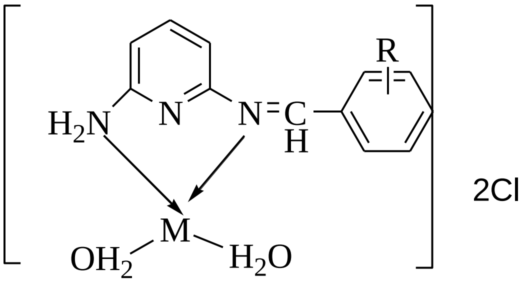
Sr. No.	Compounds	ν_{max} cm^{-1} (-CH=N-)	ν_{max} (M-N)	ν_{max} (=N-H)
1	L_1	1596	-	3372
2	L_1-Co	1645	583	3555
3	L_1-Cu	1615	583	3751
4	L_1-Mn	1601	581	3373
5	L_2	1594	-	3389
6	L_2-Co	1647	586	3553
7	L_2-Cu	1614	514	3446
8	L_2-Mn	1645	617	3390
9	L_3	1597	-	3386
10	L_3-Co	1614	540	3551
11	L_3-Cu	1625	683	3715
12	L_3-Mn	1601	581	3470

Electronic Spectra

The absorption spectra of synthesized Schiff base ligands and their metal complexes were recorded in DMSO. The ligands showed absorption bands between 219-289 nm which were assigned to π-π^* transitions. The other bands for the ligands appeared in the region 300-338 nm which were assigned to n-π^* transitions. In the metal complexes, the π-π^* band and n- π^* bands appeared but with little changes in values of wavelength. The new band in the range 370-412 cm^{-1} supporting the formation of metal ligand bonds [23, 24].

Sr. No.	Compounds	λ_{max} (nm)
1	L_1	256 (π-π^*), 365 (n-π^*)
2	L_1-Co	272 (π-π^*), 374 (n-π^*), 420 (C.T)
3	L_1-Cu	245 (π-π^*), 394 (n-π^*), 470 C.T.
4	L_1-Mn	265 (π-π^*), 410 (n-π^*), 435(C.T)
5	L_2	264 (π-π^*), 379 (n-π^*)
6	L_2-Co	264 (π-π^*), 397 (n-π^*),419 (C.T.)
7	L_2-Cu	254 (π-π^*), 371 (n-π^*), 430(C.T.)
8	L_2-Mn	250 (π-π^*), 353 (n-π^*), 435 (C.T.)
9	L_3	255 (π-π^*), 355 (n-π^*)
10	L_3-Co	201 (π-π^*), 297 (n-π^*), 355 (C.T.)
11	L_3-Cu	255 (π-π^*), 371 (n-π^*), 462 (C.T.)
12	L_3-Mn	252 (π-π^*), 286 (n-π^*), 373 (C.T.)

Expected structure of metal complexes



R= 2-H, 2-Cl, 4-F
M = Co, Cu, Mn

Antifungal assay

Schiff base ligands and their metal complexes were screened for their *in vitro* antifungal potential against *Puccinia triticinia, Bipolaris sorokiniana* and *Puccinia striiformis tritici.* It was found that there was direct correlation between concentration and per cent spore germination inhibition. The different compounds inhibited the spore germination variably at different concentrations. Results obtained were compared with the standard fungicide propiconazole (tilt).

Against *Puccinia triticinia,* the ED_{50} values for all the compounds were found to be less than 350 µg/ml. Compounds L_1, L_3, L_3-Cu had showed moderate inhibition of germination of fungal spores with ED_{50} values less than 200 µg/ml. L_2-Cu has the ED_{50} at 45 µg/ml which was very close to the standard. The mechanism for working of Schiff bases may be on the basis of hydrogen bond formation by the azomethine group at the active centres of cellular entities, which cause the interference in normal cellular phenomenon [25]. Metal complexes had more potential fungitoxicity than the Schiff base ligands. Cu complexes of ligands were reported to be more active inhibiting agents [26].

Antifungal activity of compounds against *Puccinia striiformis tritici* was found to show mild toxicity with ED_{50} values of less than 360µg/ml. L_3–Cu were found to possess the best fungitoxicity against the tested fungi with ED_{50} values 49 µg/ml respectively which was comparable to the standard fungicide propiconazole with ED_{50} value 45 µg/ml. L_1-Co and L_2-Cu showed moderate results with ED_{50} values 99 and 80µg/ml. All the Schiff base ligands (L_1, L_2, L_3) had less potential fungitoxicity than their corresponding metal derivatives [27].

Most of the synthesized compounds were found to exhibit mild activity against *Bipolaris sorokiniana* with ED_{50} values less than 500 µg/ml. Complex L_2–Co inflicted best results with ED_{50} value 90µg/ml respectively. Complexes of chlorinated ligands were found to be more effective as compared to others [28]. Chlorinated derivative have influenced the bioactivity of compounds positively; the results were similar to the findings already reported [29].

ED$_{50}$ values have been calculated from the plot of per cent spore germination inhibition against concentrations. Overall Cu complexes were found to inflict best inhibition of germination of spores against the test fungi followed by cobalt complexes. L_2-Cu against *P. triticinia* (ED$_{50}$ 45µg/ml) was comparable to tilt (40 µg/ml), L_2–Co against *B. sorokiniana* was the best against the fungi.

Table 4: ED$_{50}$ values of tested compounds against all three phytopathogens

S. No.	Compounds	*Puccinia triticinia* ED$_{50}$(µg/ml)	*Puccinia striiformis tritici* ED$_{50}$(µg/ml)	*Bipolaris Sorokiniana* ED$_{50}$(µg/ml)
1	L_1	220	360	460
2	L_1-Co	130	99	260
3	L_1-Cu	45	180	110
4	L_1-Mn	96	240	240
5	L_2	90	280	200
6	L_2-Co	75	248	90
7	L_2-Cu	60	80	95
8	L_2-Mn	95	240	110
9	L_3	210	250	220
10	L_3-Co	160	190	200
11	L_3-Cu	85	49	350
12	L_3-Mn	170	160	340
13	Propiconazole (Tilt)	40	35	45

* Standard used Propiconazole (Tilt)

5. Conclusion

Metal complexes of Schiff bases of 2, 6-diaminopyridine and their metal complexes were well synthesized by the suggested methods in excellent yield. Antifungal activity data have shown that the reported complexes have a significant biological activity against *Puccinia triticinia, bipolaris sorokiniana* and *Puccinia striiformis tritici.* L_2-Cu against *P. triticinia*, L_2–Co against *B. sorokiniana* and L_2–Cu against *P. striiformis tritici* were the best against the respective fungi.

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