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Synthesis, Characterization and Biological Activity of Transition Metal Complexes of [1-(2-bromo, 5-methoxy benzylidene) hydrazine] Ligand

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Abstract: A series of metal complexes was synthesized from the novel Schiff base [1-(2-bromo-5-methoxybenzylidene)-hydrazine] ligand. The ligand and metal complexes were well characterized by spectroscopic methods. The ligand was found to be monodentate. Stoichiometry of the ligand to metal ions was confirmed to be 4:1. Furthermore, the synthesized metal complexes were screened for antimicrobial, antitubercular, and antimalarial activities, which revealed that Cu (II) and Zn (II) showed excellent antimicrobial activity, Ni (II) was active against *M. Tuberculosis*, and Cd (II) showed excellent antimalarial activity.

Keywords: antimicrobial; anti-tuberculosis; antimalarial; ligand and metal complexes.

1. Introduction

Coordination chemistry is the study of co-ordinate bond between ligands and central metal atom. Schiff's bases are compounds having an imine (C=N) group [1]. Schiff's bases are formed by the condensation of an amine with a carbonyl group. Schiff's base ligands form coordination complexes with metal ions.

Imines constitute an important class of compounds for the synthesis of biologically active entities [2]. Schiff's bases possess remarkable properties due to chelating ability, stability, and antimicrobial activity [3]. They act as ligands for the synthesis of metal complexes. Complexes of Schiff's bases with metals have gained significant attention due to antifungal, antibacterial, antiviral, antimalarial, and antitubercular activity. Metal complexes of Schiff bases containing halogens exhibit good antimicrobial activity [4]. Metal complexes of hydrazones are used as catalysts in different chemical reactions, such as the polymerization of alkenes [5].

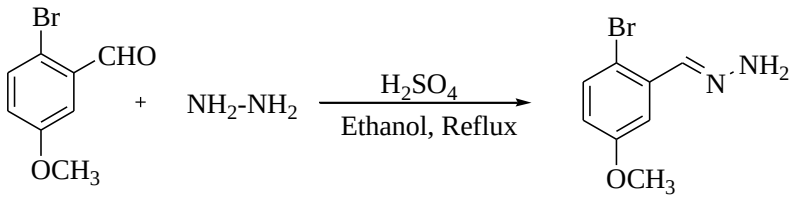
In continuation of our previous work on the metal complexes [6-8]; in the present work, we report the synthesis of some new [1-(2-bromo, 5-methoxy benzylidene) hydrazine] ligand by the condensation of 2-bromo-5-methoxybenzaldehyde and hydrazine hydrate, which was characterized by using different spectroscopic techniques. Transition metal complexes were synthesized from the ligand and furthermore screened for antimicrobial, antitubercular, and antimalarial activities.

2. Materials and Methods

All the purchased chemicals and solvents were analytical grade and used without further purification. Metal salts were used without further purification.

2.1. Synthesis of [1-(2-Bromo-5-methoxybenzylidene)hydrazine] ligand.

2-Bromo-5-methoxybenzaldehyde (1) (1 mmol) was mixed with hydrazine hydrate (2) (7 mmol) in ethanol (3 mL), and 1-2 drops of concentrated sulphuric acid were added to it. The mixture was refluxed for 4 hours at 80° C. The progress of the reaction was monitored by Thin Layer Chromatography by using ethyl acetate:n-hexane (2:8) as the mobile phase. On completion of the reaction, the product was precipitated by cooling the reaction mixture and the addition of ice. The solid product was filtered off, dried, and recrystallized from ethanol, which was confirmed as the pure Schiff base ligand (3) by analysis of its spectroscopic data (Scheme 1).



(1) **(2)** **(3)**

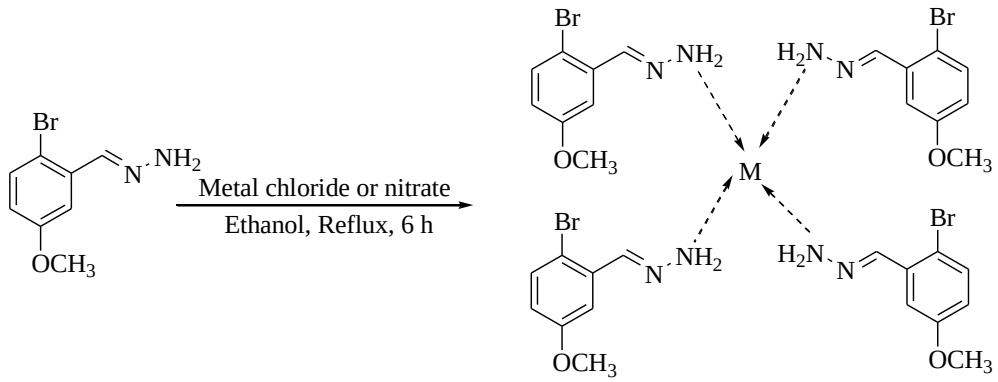
Scheme 1. Synthesis of [1-(2-Bromo, 5-methoxy benzylidene) Hydrazine] Ligand (1SA).

2.2. Spectral data of synthesized ligand.

Yield = 72 %, Yellow solid, M. P. = 162° C; IR (FTIR-ATR, ν cm^{-1}): 3045 (NH), 1620 (Imine C=N).

2.3. General procedure for the synthesis of metal complexes.

The ethanolic solution of metal salts (nitrates or chlorides) was added to the ethanolic solution of the ligand. A slightly basic p^H of the mixture was maintained by adding ammonia, and the contents were refluxed for 5-6 h. The progress of the reaction was monitored by TLC. The resulting product was cooled, filtered off, and dried. Each product was recrystallized from ethanol and confirmed by IR and UV spectroscopy (Scheme 2).



For SA1, 2, 5 : M= Cu(II), Zr(II), Cd(II)

For SA3, 4, 6, 7, 8 : M = Fe(II), Co(II), Sn(II),Ni(II) and Zn(II)

Scheme 2. Synthesis of metal complexes (SA1-8).

The physical and analytical data of the synthesized compounds are mentioned in Table 1.

Table 1. Physical and analytical data of the synthesized compounds.

Sr. No.	Compound	Code	Molecular formula	M.P. (°C)	Color	Elemental analysis (%): Calculated			
						C	H	N	M
1	Ligand (L)	1SA	$C_8H_9B_rN_2O$	162	Yellow	41.94	3.96	12.23	-
2	CuL_4	SA1	$C_{32}H_{36}Br_4N_8O_4Cu$	247	Light Green	39.22	3.67	11.43	6.48
3	ZrL_4	SA2	$C_{32}H_{36}Br_4N_8O_4Zr$	> 300	Yellowish White	38.14	3.57	11.12	9.05
4	FeL_4	SA3	$C_{32}H_{36}Br_4N_8O_4Fe$	270	Light Red	39.53	3.70	11.52	5.74
5	CoL_4	SA4	$C_{32}H_{36}Br_4N_8O_4Co$	230	Light Pink	39.3	3.69	11.48	6.04
6	CdL_4	SA5	$C_{32}H_{36}Br_4N_8O_4Cd$	> 300	Yellowish White	37.36	3.49	10.89	10.92
7	SnL_4	SA6	$C_{32}H_{36}Br_4N_8O_4Sn$	240	Ivory	37.13	3.47	10.82	11.41
8	NiL_4	SA7	$C_{32}H_{36}Br_4N_8O_4Ni$	260	Yellowish White	39.41	3.69	11.49	6.01
9	ZnL_4	SA8	$C_{32}H_{36}Br_4N_8O_4Zn$	236	Yellowish White	39.15	3.66	11.41	6.71

2.4. Infrared spectra.

The band due to the N-H stretch of free ligand was found to be shifted to the lower side in the spectra of all complexes, which indicate the participation of the N-H group in the formation of complexes. Infrared spectral data ν cm^{-1} [9] for C=N, M-N, C-H of metal complexes is presented in **Table 2**.

Table 2. Infrared spectral data of metal complexes.

Code no	Compound	ν cm^{-1} (C=N)	ν cm^{-1} (M-N)	ν cm^{-1} (C-H)	ν cm^{-1} (N-H)
SA1	CuL_4	1608	439.77	3070	2962
SA2	ZrL_4	1614	455.20	3072	2954
SA3	FeL_4	1614	451.34	3080	2958
SA4	CoL_4	1610	453.27	3010	2958
SA5	CdL_4	1616	453.27	3080	2958
SA6	SnL_4	1618	457.13	3072	2958
SA7	NiL_4	1616	395.41	3080	2958
SA8	ZnL_4	1614	453.27	3010	2958

2.5. UV spectra of the complex.

UV spectra of the synthesized metal complexes were recorded in DMSO solvent. λ_{max} value in the UV spectra of metal complexes is reported in Table 3.

Table 3. λ_{max} value of the synthesized metal complexes.

Sr. No.	Compound	Sample code	Wavelength (λ_{max})
1	CuL_4	SA1	222.8
2	ZrL_4	SA2	213.60
3	FeL_4	SA3	214.95
4	CoL_4	SA4	216.4
5	CdL_4	SA5	222.86
6	SnL_4	SA6	217.53
7	NiL_4	SA7	212.20
8	ZnL_4	SA8	300.60

2.6. Biological activity.

2.6.1. Antimicrobial study.

The newly synthesized metal complexes were evaluated against four bacteria (*E. coli*- MTCC 443, *P. aeruginosa*- MTCC 1688, *S. aureus*-MTCC 96, *S. pyogenus*- MTCC 442) and three fungal species (*C. albicans*- MTCC 227, *A. niger*- MTCC 282, and *A. clavatus*- MTCC 1323).

The broth dilution method was used for antimicrobial activity [10]. The nutrient medium Mueller Hinton Broth was used to grow and dilute the drug/compound suspension for the microorganisms under test. Solutions of metal complexes were prepared in DMSO solvent. The solution of DMSO (without compound) was used as the control. Each synthesized compound was diluted to get a stock solution of 2000 microgram /mL concentration. For primary screening, 1000 microgram/mL, 500 microgram/mL and 250 microgram/mL concentrations of the synthesized metal complexes were taken. The metal complexes found active in the primary screening were further subjected to all microorganisms for secondary screening. In secondary screening, the metal complexes (found active in primary screening) were diluted to get 200 microgram/mL, 100 microgram/mL, 50 microgram/mL, 25 microgram/mL, 12.5 microgram/mL, and 6.25 microgram/mL concentrations. The control tube, which does not contain the test compound, was sub-cultured with a medium suitable for the growth of the test microbes and placed for incubation at 37 ˚C overnight. The tubes are then incubated overnight. The Minimum Inhibitory Concentration (MIC) of the control test organism is recorded to examine the accuracy of concentrations of test compounds. Before incubation, the extent of growth from the control tube (which served as original inoculums) was compared. The MIC was recorded as the least concentration inhibiting the growth of the organism [11]. The MIC for newly synthesized metal complexes were compared with respective standard drugs for bacteria and fungi, as given in Table 4.

Table 4. Antimicrobial activity of the synthesized metal complexes.

Compound	**Code**	**Antibacterial Activity**				**Antifungal Activity**		
		Minimal Inhibition Concentration				**Minimal Inhibition Concentration**		
		E. Coli	***P. Aeruginosa***	***S. Aureus***	***S. Pyogenus***	***C. Albicans***	***A. Niger***	***A. Clavatus***
		MTCC 443	MTCC 1688	MTCC 96	MTCC 442	MTCC 227	MTCC 282	MTCC 1323
CuL_4	SA1	25	50	125	100	>1000	250	500
ZrL_4	SA2	50	100	25	62.5	1000	1000	1000
FeL_4	SA3	100	500	250	250	500	500	>1000
CoL_4	SA4	50	250	100	500	500	1000	500
CdL_4	SA5	62.5	100	100	250	250	500	1000
SnL_4	SA6	100	62.5	100	62.5	500	1000	500
NiL_4	SA7	100	250	250	100	1000	>1000	>1000
ZnL_4	SA8	250	12.5	100	62.5	250	500	500
Ampicillin	Std*	100	-	250	100	-	-	-
Chloramphenicol	Std*	50	50	50	50	-	-	-
Gentamycin	Std*	0.05	1	0.25	0.5	-	-	-
Ciprofloxacin	Std*	25	25	50	50	-	-	-
Norfloxacin	Std*	10	10	10	10	-	-	-
Nystatin	Std*	-	-	-	-	100	100	100
Greseofulvin	Std*	-	-	-	-	500	100	100

Std* = Standard

2.6.2. Anti-tubercular activity.

All the synthesized metal complexes were used for an antitubercular activity. *Mycobacterium Tuberculosis* strain (H37Rv) cultures were tested against the synthesized metal complexes. We used MIC to assess the anti-tuberculosis activity. This simple method gives a significant result for the number of antimicrobial agents that are required for inhibiting the growth of specific microbes [12].

The nutrient medium was used to grow microorganisms by the conventional L. J. medium. The size of the inoculum was fixed to 1 mg/mL for test strain. DMSO was used as a solvent to obtain desired concentrations of test compounds. Each synthesized metal complex

was diluted to yield 2000 µg/mL concentration as a stock solution. Serial dilutions were prepared for primary and secondary screening; 500 µg /mL, 250 µg /mL, and 125 µg /mL concentrations of the synthesized metal complexes were taken in primary screening. The synthesized complexes which were found active in primary screening were promoted for secondary screening against *M. Tuberculosis* strain. In secondary screening, the synthesized metal complexes (found active in primary screening) were diluted to yield 100 µg /mL, 50 µg /mL, 25 µg /mL, 12.5 µg /mL, 6.250 µg /mL, 3.125 µg /mL and 1.5625 µg /mL concentrations. MIC is recorded as the maximum dilution showing at least 99 % inhibition. MIC of the synthesized metal complexes was determined and compared with standard Isoniazid and Rifampicin drugs, as given in Table 5, which provides data for antitubercular activity.

Table 5. Antitubercular activity against $H_{37}RV$ stain.

Code	Compound	MIC (µg/mL)
SA1	CuL_4	250
SA2	ZrL_4	500
SA3	FeL_4	125
SA4	CoL_4	100
SA5	CdL_4	250
SA6	SnL_4	100
SA7	NiL_4	62.5
SA8	ZnL_4	125
Standard	Isoniazid	0.20 µg/mL (99% inhibition)
Standard	Rifampicin	40 µg/mL (99% inhibition)

2.6.3. Antimalarial activity.

The synthesized metal complexes were screened for antimalarial activity by using the microassay protocol of Rieckmann K.H. and co-workers with slight modifications. The simple *in-vitro* assay used for the determination of susceptibility of *Plasmodium falciparum* to antimalarial compounds was performed in 96 well microtitre plates. RPMI 1640 medium was used to maintain the cultures of *P. Falciparum* strain. The culture medium was supplemented with 1% D-glucose, 25 mM HEPES, 10% heat-inactivated human serum, and 0.23% sodium bicarbonate.

On treatment with 5% D-sorbitol, the asynchronous parasites of *Plasmodium falciparum* were synchronized to get only the ring stage parasitized cells. Each of the test compounds was diluted by using DMSO to obtain 5 mg/mL concentration as a stock solution, and subsequent dilutions were prepared with a culture medium. 20 µL of the diluted samples were added to the test wells so as to get final concentrations varying between 0.4 µg/mL to 100 µg/mL in duplicate wells containing parasitized cell preparation. In a candle jar, the culture plates were incubated for 36 to 40 h at 37°C. After incubation, lean blood smears from each well were prepared and stained with J. S. Bhattacharya staining. The slides were examined microscopically, and the maturation of ring-stage parasites into trophozoites and schizonts was recorded in the presence of different concentrations of test compounds. The least concentration of test compound, which inhibits the complete maturation into schizonts, was reported as the MIC value. Quinine and Chloroquine were used as standard drugs. The antimalarial activity of the synthesized metal complexes is summarized in Table 6.

Table 6. Minimal inhibition concentrations for antimalarial activity.

Code no.	Compound	Mean IC50 values
SA1	CuL_4	1.68 µg/ml
SA2	ZrL_4	1.42 µg/ml
SA3	FeL_4	0.63 µg/ml
SA4	CoL_4	2.06 µg/ml
SA5	CdL_4	0.48 µg/ml
SA6	SnL_4	1.17 µg/ml
SA7	NiL_4	1.03 µg/ml
SA8	ZnL_4	1.07 µg/ml
Standard	Chloroquine	IC50-0.020 µg/ml
	Quinine	IC 50-0.268 µg/ml

3. Results and Discussion

A new ligand **[**1-(2-Bromo-5-methoxybenzylidene) hydrazine] was synthesized from 2-bromo-5-methoxybenzaldehyde and hydrazine hydrate, which was further used as a ligand for the synthesis of metal complexes. Newly synthesized ligand and metal complexes were characterized by different spectroscopic methods. Metal complexes were evaluated for antimicrobial, antitubercular, and antimalarial activities. The IR spectrum of ligand shows broadband in the region 1620 $cm^{-1,}$ corresponding to the stretching frequency for the C=N group. For metal complexes, the spectral band in the region of 395 cm^{-1} to 457 cm^{-1} indicates M-N band frequency i. e. nitrogen of ligand and metal was co-ordinated. The IR band at 3000 cm^{-1} to 3150 cm^{-1} corresponds to the stretching frequency of C-H bonds. The ligand behaves as monodentate, co-ordinating to the metal ion through the nitrogen atom of NH_2 group of the ligand.

In the UV spectra, λ_{max} value for metal complexes ranges from 213 nm to 300 nm. Zn(II), i.e., (ZnL_4) showed λ_{max} value at higher absorption. The metal complexes of Cu(II), Zr(II), Fe(II), Co(II), Cd(II), Sn(II), Ni(II), and Zn(II) were formed in ligand to metal ratio of 4:1.

The results of the antimicrobial activity of the metal complexes showed that Cu(II) and Zn(II) exhibited excellent activity against *P. Aeruginosa* (MTCC 1688), *S. Aureus* (MTCC 96), and *S. Pyogenus* (MTCC 442). Ni(II) and Sn(II) showed activity against *S. Pyogenus* (MTCC 442). Cu(II) and Zr (II) showed remarkable activity against *E. coli* (MTCC 443) as compared with the standard drugs. Cd(II) and Zn(II) showed moderate to excellent activity against *C. albicans* (MTCC 227), *A. Niger* (MTCC 282), and *A. Clavatus* (MTCC 1323). Cu(II) showed good activity against *A. niger* (MTCC 282), Co(II) and Sn(II) were remarkably active against *A. Clavatus* (MTCC 1323) as compared with the standard drugs. The antitubercular activity of the synthesized metal compounds revealed moderate to excellent activity for Ni (II) complex against MTB strain (H37Rv) while Co(II) and Sn(II) were active against MTB as compared to the standard drugs isoniazid and rifampicin. Antimalarial activity of the synthesized compounds was good for the Cd(II), while Fe(II) was active against malaria as compared to the standard drugs chloroquine and quinine.

4. Conclusions

In conclusion, monodentate Schiff base **[**1-(2-Bromo-5-methoxy benzylidene) hydrazine] ligand and its metal complexes were synthesized and characterized by different spectroscopic methods. Antimicrobial activity, antitubercular and antimalarial activity of the synthesized metal complexes indicate that Cu(II) and Zn(II) showed excellent antimicrobial

activity, Ni(II) was remarkably active against MTB while Cd(II) showed excellent activity against the malarial strain as compared to the respective standard drugs.

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Conflicts of Interest

The authors declare no conflict of interest.

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