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Research Article

Synthesis, characterization and biological screening for antifungal, antimalarial and antitubercular activities of novel bis-imines and their metal complexes

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ABSTRACT

Herein we report the synthesis and characterization of bisimines of ortho-vanillin and iso-vanillin with hydrazine hydrate. Further these bis-imines were used as ligands for the synthesis of metal complexes with transition metals Cu(II), Ni(II), Co(II), Fe(II), Zn(II) Mn(II) and Cd(II). The bis-imines and metal complexes were well characterized and screened for antifungal, antimalarial and antitubercular activities. Bisimines and their transition metal complexes were reported for potential antifungal, antimalarial and antitubercular activities. It is observed that metal on complex formation increased the activity as compared to ligands.

Keywords: Bis-imines, Transition metal complexes, Antifungal, Antimalarial and antitubercular activities

INTRODUCTION

Bisimines are important intermediates for the synthesis of various bioactive compounds used in medicinal, agricultural, pharmaceutical fields and material science (Kumar J, 2017). Schiff bases are very significant due to their stability, chelating properties and biological applications (Abu-Dief AM, 2015). Due to Presence of both nitrogen and oxygen donor atoms in the backbones of these ligands; an attention in this area has been focused on the complexes formed by transition metal ions with Schiff bases. Halogen containing Schiff bases and metal complexes show biological activities like antitumor, antibacterial and antifungal (Arulmurugan S, 2010). They have also been used as bactericidal, fungicidal, anti-tubercular and antiviral agents (Jarrahpour A, 2013).

Metal based drugs were used as medicines for the treatment of diabetes, cancer, anti-inflammatory and cardiovascular disease. Moreover, metal on complex formation increased antimicrobial activity as compared to ligands. Many Schiff base metal complexes with a variety of biological activities have been described in the literature (Da Silva CM, 2011).

Synthesis of new compounds to explore the potential biologically active agents is an area of continued interest. Drug design by molecular manipulation is a productive source of new drugs. Molecular manipulation, combinations of biologically active moieties into one molecule and synthesis of totally new moieties have become popular for the development of bioactive compounds.

Tuberculosis (TB) as a major global health problem and World Health Organization declared tuberculosis is a global health issue (Hameed A, 2017). It is an infectious epidemic caused by diverse species of mycobacteria, collectively termed the *Tubercle bacilli*. Extensive drug resistance developed by the pathogen due to complex structure and characteristics of the mycobacterial cell wall. The emergence of multi-drug resistant (MDR) TB, extensively

drug-resistant (XDR) TB, makes to treat the disease difficult and favors the recurrence of the infection. Moreover, multi-fold infection due to TB enhances the risk of death (Getahun H, 2012). The mycobacteria become resistant since their slow growth phase increases its ability to adapt and acquire new resistance mechanisms. Hence, there is always a demand to discover new anti-TB drugs with enhanced activity against MDR and XDR TB in short time duration.

Malaria is one of the most important tropical parasitic diseases. According to World Health Organization (WHO), around 1 million deaths do occur every year due to Malaria (Lindsay SW, 1998). Human malaria is mainly caused by four species of Plasmodium (*P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*). For the treatment of malarial infections there is a constant need to develop new compounds to the unending problem of the growing resistance to known drugs.

Severe diseases occur due to fungal infections (Levitz SM, 1992). The increasing emergence of fungal resistance to currently available antimycotic agents presents a serious health-problem. Candida albicans, in the oral cavity, lower gastrointestinal tract and female genital tract injection.

Literature survey reveals that several Schiff bases and bisimines possess significant biological activities like anti-inflammatory, insecticidal, analgesic, antioxidant, antiproliferative, antitubercular (Gayakwad DR, 2019; Zaltariov MF, 2019; Kotora P, 2016; Hranjec M, 2011; Hearn MJ, 2009). In continuation of our research interest in the field of synthesis of bioactive heterocyclic compounds (Bhale S, 2019; Kauthale S, 2017) herein we report synthesis and characterization of bis-imines and their metal complexes. All the synthesized compounds were characterized by different spectroscopic methods and screened for antifungal, antimalarial and antitubercular activities.

MATERIALS AND METHODS

For the synthesis, analytical grade chemicals and distilled solvents were used. The IR spectra were obtained on BRUKER and SHIMADZU Model 1600 FTIR Spectrophotometer using KBr disc. Infrared spectra were recorded on a spectrophotometer in wave number region 4000-400 cm^{-1}. NMR spectra were measured on a Jeol ECS 400 spectrometer. Electronic absorption spectra were obtained from a UV-Vis Jasco Spectrophotometer in wavelenth range 200 nm to 800 nm. DMSO solvent is was used for Uv-visible spectra of complexes. The elemental analysis i.e. metal content determined using Atomic Absorption Spectrophotometer (Perkin Make). The X-ray diffractograms of metal complexes were scanned on RIGAKU miniflex-II with radiation (λ=1.5405 Å). Thermogravimetric examination was studied by dynamic or non-isothermal TGA-DTA technique on a SDT-Q600 instrument.

Experimental procedure

Synthesis of Schiff base: Substituted aromatic aldehyde (2 mmol) and hydrazine hydrate (1 mmol) were dissolved in ethanol (5 ml). To it 2-3 drops of glacial acetic acid were added and the mixture was refluxed for appropriate time (2 to 3 h). The progress of reaction was monitored by TLC. After completion of reaction, the content of the flask was poured over crushed ice. The solid obtained was filtered, washed with cold water, dried and recrystallized from ethanol (Figure 1). The synthesized bis-imines were characterized by different spectroscopic methods (Table 1) and screened for antibacterial antifungal, antimalarial and antitubercular activities.

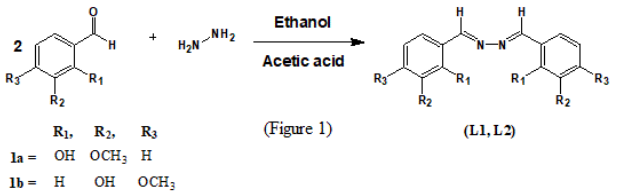


Figure 1. Synthesis of Bis-imine Ligands (L_1 and L_2)

Spectral analysis of ligand: L_1) 6,6'-((1E,1'E)-hydrazine-1,2-diylidenebis(methanylylidene)) bis(2-methoxyphenol):

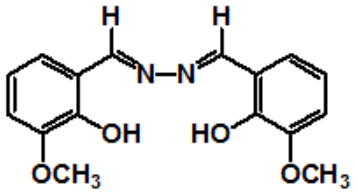
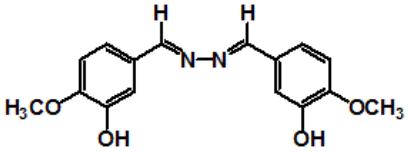
Analytical cal. $C_{16}H_{16}N_2O_4$:C, 63.99; H, 5.37; N, 9.33; found: C, 63.97; H, 5.38; N, 9.34.^{1}H NMR (400 MHz, DMSO-d_6, , ppm): 3.73 (s, 6H, 2 CH_3), 5.76 (s, 1H, -OH), 6.87 (t, 1H, C-5), 6.89 (t, 1H, C-5), 7.42 (d, 1H, C-4), 7.44 (s, 1H, C-4′), 7.45 (d, 1H, C-6), 7.46 (d, 1H, C-6′), 8.84 (s, 1H, -CH=N_{azine}), 8.87 (s, 1H, -CH=N_{azine}). (EI): (m/z) 300.09 [$M^{+\cdot}$].

L_2) 5,5'-((1E,1'E)-hydrazine-1,2-diylidenebis(methanylylidene))bis(2-methoxyphenol):

Analytical cal. $C_{16}H_{16}N_2O_4$: C, 63.99; H, 5.37; N, 9.33; found: C, 63.97; H, 5.38; N, 9.34.^{1}H NMR (400 MHz, DMSO-d_6, , ppm): 3.73 (s, 6H, 2 × CH_3), 5.76 (s, 1H, -OH), 6.87 (d, 1H, C-5), 6.89 (d, 1H, C-5′), 7.42 (s, 1H, C-2), 7.44 (s, 1H, C-2′), 7.45 (d, 1H, C-6), 7.46 (d, 1H, C-6′), 8.84 (s, 1H, -CH=N_{azine}), 8.87 (s, 1H, -CH=N_{azine}). (EI): (m/z) 300.09 [$M^{+\cdot}$].

Table 1. Synthesis of Schiff Bases

Sr. No	Entry	Compound	Time (hr)	M. P. (°C)

1	L_1		2.0	197
2	L_2		2.5	241

Synthesis of metal complex

A mixture of bis-imines (L_1-L_2) and metal nitrates in ethanol was refluxed for appropriate time. The progress of reaction was monitored by TLC. After completion of reaction, the content was treated with liquid ammonia to precipitate the solid. The solid obtained was filtered, washed with cold water, and dried (Figures 2 and 3).

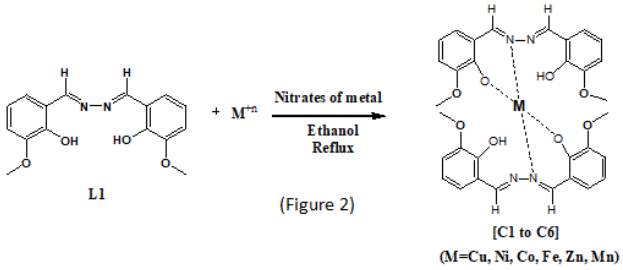


Figure 2. Synthesis of Metal Complexes (C_1-C_7)of Bis-imine Ligands (L_1)

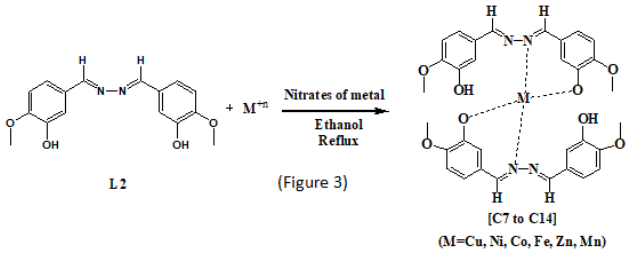
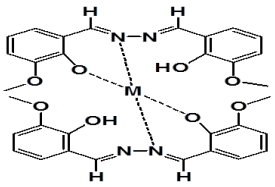
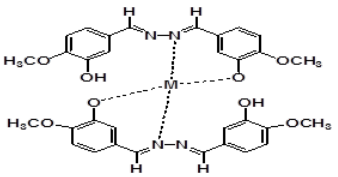


Figure 3. Synthesis of Metal Complexes (C_8-C_{14}) of Bis-imine Ligands (L_2)

All the metal complexes (Table 2) were characterized by different spectroscopic methods and screened for antifungal, antimalarial and antitubercular activities.

Table 2. Synthesis of metal complex

Sr. No	Entry	Compound	Metal ion (M)	Time (hr)	M. P. (°C)
1	C1		Cu	3.5	300
2	C2		Ni	3.7	300
3	C3		Co	3.5	201
4	C4		Fe	3.9	188
5	C5		Zn	3.5	300
6	C6		Mn	3.5	293
7	C7		Cd	4	300
8	C8		Cu	3.4	300
9	C9		Ni	3.8	300
10	C10		Co	4.2	252
11	C11		Fe	3.7	257
12	C12		Zn	3.8	300

13	C13		Mn	3.5	279
14	C14		Cd	4.5	300

IR spectral analysis of complex: Infrared spectroscopic characterization is an important tool used to confirm the formation of metal complexes. IR spectral of transition metal complexes are presented in (Table 3).

Table 3. IR absorption frequencies of metal complexes

Ligand	Complex	v(HC=N)	v(-OH)	v(M-N)	v(M-O)
	Cu	1661	3450	463	569
	Fe	1664	3236	448	517
	Zn	1600	3319	427	512
	Ni	1643	3517	434	556
	Co	1604	3217	462	555
Ortho-Vanillin (L_1)	Mn	1608	3451	471	542
	Cu	1664	3300	428	538
	Fe	1660	3400	411	539
	Zn	1628	3400	440	527
	Ni	1610	3225	465	501
	Co	1626	3215	486	530
Iso-Vanillin (L_2)	Mn	1628	3213	450	523

Infrared spectra were recorded on a spectrophotometer in wave number region 4000-400 cm^{-1}. The spectra bands of metal complexes at 410-486 cm^{-1} were characterized for metal which indicates that the nitrogen atom of ligand was coordinated with metal i.e., (M-N) bands frequency. The appearance of new weak low frequency bands around 500-570 were assigned for the participation of oxygen with metal in the complexes (MO). The strong bands at 3200-3500 cm^{-1} were assigned for vO-H group stretch and 1600-1666 cm^{-1} band assigned for vC=N group. The stretching frequency at 3000-3150 cm^{-1} can be recognized to C-H bond.

UV spectral analysis of complex: DMSO solvent was used for UV-visible spectra of complexes. Maxima in the UV spectra of transition metal complexes are presented in (Table 4).

Table 4. λ max values in uv spectra of the synthesized metal complexes

Sr. No.	Ligand	Metal in Complex	λ max
1		Cu	206.5
2		Fe	244
3		Zn	315.5
4		Ni	312
5		Co	228.5
6		Mn	309.5
7	$C_{16}H_{16}O_4N_2$ Ortho-Vanillin (L_1)	Cd	231.5
8		Cu	341.5
9	$C_{16}H_{16}O_4N_2$ Iso-Vanillin (L_2)	Fe	334

10		Zn	722.5
11		Ni	229
12		Co	340.5
13		Mn	342.5
14		Cd	278

Biological assay

Antifungal activity: In present study newly synthesized bis-imines and metal complexes were assessed for antifungal activity using broth dilution method. The antifungal activities of the synthesized compounds were investigated against *C. albicans* (MTCC 227), *A. niger* (MTCC 282), *A. clavatus* (MTCC 1323). Measuring the diameter of zone of inhibition in mm, the results were compared with the standard nystatin and greseofulvin. The solution without compound (only DMF) was used as control. The Minimum inhibition concentration for synthesized compound were compared with standard nystatin and greseofulvin as given in (Table 5).

Antimalarial activity: ^{3}H Hypoxanthine uptake is the most commonly used method for assessing antimalarial efficacy of a compound *in vitro*. ^{3}H Hypoxanthine uptake is a standardized model to determine the level of Plasmodium falciparum growth inhibition. Radiolabelled hypoxanthine uptake by parasite is an indicator of its growth and multiplication. Parasites were cultured in the presence of different concentrations of test compounds in media containing reduced concentration of hypoxanthine, Percent reductions were used to plot percentage inhibition of growth as a function of drug concentration. IC50 were determined by linear regression analyses on the linear segments of the dose response curve. IC50 represents the concentration of a drug required for 50% inhibition *in vitro* (Table 6).

Table 5. Antifungal activity of Schiff base and their metal complexes

		Minimum fungicidal concentration µg/ml		
SR. NO.	Code No.	C. albicans MTCC 227	niger MTCC 282	clavatus MTCC 1323
1	L_1	500	1000	1000
2	L_2	250	>1000	>1000
3	C_1	500	500	1000
4	C_2	500	>1000	>1000
5	C_3	250	500	500
6	C_4	500	1000	1000
7	C_5	500	1000	1000
8	C_6	250	500	500
9	C_7	250	1000	500
10	C_8	500	1000	500
11	C_9	500	250	500
12	C_{10}	1000	1000	1000
13	C_{11}	500	>1000	>1000
14	C_{12}	250	500	500
15	C_{13}	500	1000	1000
16	C_{14}	500	1000	1000
Standard	Nystatin	100	100	100

	Greseofulvin	500	100	100

Table 6. Antimalarial activity of Schiff Base and their metal complexes

Minimum inhibition concentration µg/ml		
SR.NO	COMPOUND	MEAN IC50
1	L_1	1.95 µg/ml
2	L_2	0.82 µg/ml
3	C_1	0.44 µg/ml
4	C_2	0.58 µg/ml
5	C_3	1.97 µg/ml
6	C_4	0.28 µg/ml
7	C_5	1.56 µg/ml
8	C_6	0.42 µg/ml
9	C_7	1.10 µg/ml
10	C_8	0.96 µg/ml
11	C_9	1.42 µg/ml
12	C_{10}	1.61 µg/ml
13	C_{11}	1.91 µg/ml
14	C_{12}	0.99 µg/ml
15	C_{13}	0.52 µg/ml
16	C_{14}	1.97 µg/ml
Standard	Chloroquine	IC50-0.020 µg/ml
	Quinine	IC 50-0.268 µg/ml

Antitubercular activities: Test compounds were assessed for antitubercular activity against M. tuberculosis (H37Rv strain) by nitrate reductase assay (NRA). It is based on the ability of Mycobacterium tuberculosis (MTB) to reduce nitrate incorporated in the medium to nitrite, which can be detected by the change in color by adding Griess reagent. NRA was performed on middlebrook 7H11 agar medium incorporated with potassium nitrate: 1000 µg/ml KNO_3 was added in the medium. The media was then divided into two parts; one part containing 0.2 µg/ml of isoniazid (INH), one part without antibiotic was used as the growth control. 0.5 ml of the Griess reagent was added to one drug-free NRA bottle on day 10. If there were any changes in color (light pink), then the corresponding antibiotic-containing bottles were tested for color development. If no color change was observed, then the procedure was repeated at day 14, 21, and finally at day 28. An isolate was considered resistant to a drug if the color in the drug containing bottle was greater than that in the drug-free medium. To check the MIC i.e. the lowest drug concentration to color changes were reported in (Table 7) reveals anti-TB activity of the synthesized compound.

Table 7. Antitubercular activity of schiff base and their metal complex

Sr.No	**Code No**	**MIC µg/ml**
1	L_1	62.5
2	L_2	125
3	C_1	50
4	C_2	100

5	C_3	500
6	C_4	250
7	C_5	250
8	C_6	62.5
9	C_7	12.5
10	C_8	125
11	C_9	12.5
12	C_{10}	500
13	C_{11}	250
14	C_{12}	250
15	C_{13}	500
16	C_{14}	100
Standard	Isoniazid	0.20 µg/ml
		99% inhibition

RESULTS AND DISCUSSION

In present work, bisimines of vanillin and orthovanillin were synthesized with hydrazine hydrate and used as ligands for the synthesis of metal complexes with transition metals. All the synthesized metal complexes were characterized by different spectroscopic methods and screened for antifungal, antimalarial and antitubercular activities.

IR spectral data of metal complexes show a broad band in the region 3200-3500 cm^{-1} corresponding to the stretching frequency of OH, indicating the presence of coordinated water. The spectral bands of metal complexes at 410-486 cm^{-1} were characterized for metal and nitrogen atom of ligand was coordinated i.e., (M-N) bands frequency and 1600-1666 cm^{-1} band assigned for C=N group. The IR stretching frequency at 3000-3150 cm^{-1} can be recognized to C-H bond. Appearance of new weak low frequency bands at 500-566 cm^{-1} were assigned for the participation of oxygen with metal in the complexes (MO). A medium sharp band which appeared at (784 cm^{-1}, 846 cm^{-1}, 887 cm^{-1}) indicates the presence of H_2O in the lattice molecules of the structure of prepared complexes. Broad band which appeared at (3539, 3606, 3483 cm^{-1}) indicates the presence of water molecules out of the coordination sphere. The presence of broad band at 3440 cm^{-1} indicates the coordinated water molecule to central metal atom. Thus, the ligand behaves as bidentate, coordinating through phenolic oxygen and azomethine nitrogen of bis-imine with the metal ion.

DMSO solvent was used for UV-visible spectra of complexes. The peaks were observed in the range of 226.5 to 722.5 nm for all complexes. It was surprising to observe that in L_2 Zn complex showed tremendous increase in λ max value at higher absorption.

The complexes of Cu(II), Fe(II), Zn(II) Ni(II), Co(II), Mn(II) and Cd(II) were formed in metal to ligand ratio of 1:2. Complexes synthesized are crystalline in nature with monoclinic crystal system possessing higher thermal stability.

Most of the complexes showed better antifungal activities. It was observed that metal complexes C_6, C_7, C_{12} and ligand L_2 showed moderate to excellent antifungal activity as compared to standard Nystatin and Greseofulvin against *C. albicans* (MTCC 227) while complex C_9 showed excellent antifungal activity against *A. niger* (MTCC 282).

In antimalarial efficiency study of the synthesized compounds, iron complex C_4 showed excellent antimalarial activity while Cu complexes C_1 and Mn complexes C_6 were active as compared to standard drug chloroquine and quinine. Bisimine ligands L_1 and L_2 exhibited less activity as compared to their metal complexes.

Antitubercular activity of the synthesized compounds was studied against *M. tuberculosis* (H37Rv strain) by nitrate reductase assay NRA methods. Minimum inhibition concentration MIC in µg/ml were measured as compared to standard drug isoniazid. Iron complex of Cd and Ni (C_7, C_9) showed excellent antitubercular activity while Cu and Mn complexes (C_1, C_6) were remarkably active as compared to standard drug isoniazid. Schiff base ligands exhibited less activity as compared to their metal complexes Bisimine ligands L_1 and L_2 exhibited less activity as compared to their metal complexes. due to the formation of metal chelates, which enhanced lipophilic properties over the Schiff base ligands allowing more

efficient ermeation of the metal complex through the thick microbial cell wall leading to better antitubercular activity.

CONCLUSION

In conclusion, we report the synthesis, characterization and biological activity of some novel bis-imines and their metal complexes with transition metals Cu(II), Ni(II), Fe(II), Zn(II), Mn(II). All the synthesized metal complexes were characterized by different spectroscopic methods and screened for antifungal, antimalarial and antitubercular activities. The synthesized compounds C_6, C_7, C_{12} and ligand L_2 showed moderate to excellent antifungal activity. Iron complexes C_4, Cu complexes C_1 and Mn complexes C_6 showed excellent antimalarial activity. Complexes C_1, C_6, C_7, and C_9 showed excellent antitubercular activity as compared to standard drug isoniazid. Bisimine ligands exhibited less activity as compared to their metal complexes.

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