

Green Synthesis, Characterization and Bioactivity Study of few Transition Metal Complexes of Schiff base Ligand Derived from 2-Amino-5, 6-Dimethyl Benzimidazole &Benzil

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Abstract

A green synthesis of tetra dentate Schiff base ligand was carried out by treating 2-Amino-5, 6dimethyl benzimidazole with benzil in 2:1 molar proportion under solvent free condition in a scientific microwave oven. High yield was obtained after purification. Few metal complexes of Schiff base were synthesized by using Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Ag(I) metal saltsunder solvent free condition in the same microwave oven. All metal complexes showed diverse color at the end of reaction and melting point of each complex confirmed the formation of the metal complex. An in depth characterization of both Schiff base & its representative metal complexes performed by different analytical & spectral techniques including elemental analysis, TLC, IR spectroscopy, ¹HNMR spectroscopy, LCMS, UV spectroscopy and TGA . Antimicrobial activity of Schiff base &it's all metal complexeswere studied against Escherichia Coli, Staphylococcus Aureus & Salmonella Typhi showing significant bioactivity of the metal complexes and Schiff base ligand.

Keywords: Green synthesis, Solvent free,2-Amino-5, 6-dimethyl benzimidazole, benzil.

Introduction

Schiff base ligands have tremendous importance because of their physicochemical properties, simple way of synthesis, interesting bioactivity and increasing industrial applications [1,2]. The importance of Schiff base increases when it is coordinated with metal ions as there is increase is bioactivity compared to Schiff base alone [3-5]. The Schiff base molecule has characteristic



azomethine group (>C=N-) which has ability to form coordinate bond with transition metals resulting in formation of a stable metal complex [6,7]. The current work principally focuses on green synthesis of Schiff base metal complexes under solvent free condition using a scientific micro oven. Most of the researchers are now using this method as it is time saving, lesser costly and very low or zero pollution method [8,9]. This microwave assisted synthesis has various benefits like increased yield, ecofriendly and very low probability of accident [10-12]. The Schiff bases and their metal complexes show a broad range of antimicrobial activities for example anti-inflammatory [13], anti-diabetic, anticancer [14], antitumor [15], antibacterial, herbicidal [16,17] and antifungal [18,19].

Experimental Section

Material methods

All chemicals were purchased from Sigma Alderich, Loba Chem, and Merck. 2-Amino-5, 6dimethyl benzimidazole, benzil were purchased from Sigma Alderich and metal nitrates from Loba Chem and Merck. All chemicals were used as received. Parent Schiff base ligand was synthesized by condensation of 2-Amino-5, 6-dimethyl benzimidazole and benzil in the 2:1 molar ratio in a scientific microwave oven under solvent free condition. After ligand synthesis, metal complexes were prepared by identical method i.e. treating ligand with metal salts in same scientific microwave oven under solvent free condition.

Techniques

All syntheses were performed in a scientific oven, 2450MHzfrequency, 800 W. All melting points were noted on digital melting point apparatus. The electronic absorption spectra were recordedusing UV-VIS spectrophotometer in DMSO solution. The IR spectra recorded on a Schimadzu Dr-8031 instrument. ¹HNMR spectra recorded on Bruker's 400 MHz instrument in DMSO-d6. The mass spectrum recorded by LC-MS spectrophotometer. The thermogravimetric analysis [TGA] carried outwith a heating rate of 10 $^{\circ}$ C min⁻¹ in dynamic nitrogen atmosphere (30 ml/min) using Shimadzu TGA – 50H thermal analyzer. TLC analyses were performed on precoated aluminium plates with silica gel. TLC spots were envisioned in an UV chamber.



Synthesis of novel Schiff base ligand

The novel Schiff base ligand was synthesized by the condensation reaction between 2-Amino-5, 6-dimethyl benzimidazole [1.62 gm, 0.01mol] and benzil [1.1 gm, 0.005mol] under solvent free condition. The reaction mixture was first mixed in a grinder, kept at room temperature for half an hour and then irradiated about 13 minutes periodically at 750 W in microwave oven. The product obtained was then kept at room temperature for some time and then washed with dry ether. The final product was then recrystallized using absolute ethanol to give brownish crystals. The yield obtained was 2.6 gm [96 %] and melting point was found to be 244^{0} C. The progress of the reaction and purity of the product was monitored by using TLC.Solvent mixture used for TLC was n-hexane + ethyl acetate (7:3).



(*E*)-*N*-(2-(5,6-dimethyl-1*H*-benzo[*d*]imidazol-2-ylimino)-1,2diphenylethylidene)-5,6-dimethyl-1*H*-benzo[*d*]imidazol-2-amine (Novel Schiff base Ligand)

Synthesis of transition metal complexes

The transition metal complexes were synthesized under solvent free condition by identical microwave assisted method. At first the metal salt was mixed thoroughly with required amount of novel Schiff base ligand, (*E*)-*N*-(2-(5, 6-dimethyl-1*H*-benzo[*d*]imidazole-2-ylimino)-1, 2-diphenylethylidene)-5, 6-dimethyl-1*H*-benzo[*d*]imidazole-2-amine, in a grinder. Then reactant mixture was then irradiated for few seconds to minutes at 750 W. The final product obtained was washed and recrystallized with hot ethanol and then dried at room temperature. The melting



point of each complex was noted on digital melting point apparatus. The metal salts used for synthesis of metal complexes were $MnCl_2$, $Fe(NO_3)_3.9H_2O$, $Co(NO_3)_2.6H_2O$, $Ni(NO_3)_2.6H_2O$, $Cu(NO_3)_2.3H_2O$, $Zn(NO_3)_2.6H_2O$, $AgNO_3and Cd(NO_3)_2.4H_2O$.

Result and discussion

The main features of this green synthesis of novel Schiff base ligand and its corresponding metal complexes were less reaction time and better yield. The reaction mixture was homogeneously irradiated by rotating reactor tray. The overall procedure was repeated for conformation of desired product formation. The irradiation time for the synthesis of novel Schiff base ligand and its corresponding metal complexes was found to be few minutes to seconds. The yield obtained was nearly and above 90 %. Both the ligand and complexes showed distinct color and exact melting points. Each transition metal complex synthesized in this work was found to be in solid state and stable at room temperature. The solubility study of the metal complexes showed that these are insoluble in common organic solvent but soluble in DMF and DMSO.

Elemental composition analysis

Table 1: The elemental analysis (CHIN) data for new parent Schin base ligand	Table	1:	The	elemental	analysis	(CHN)	data f	for new	parent	Schiff	base ligand	d
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Compound	Empirical	Molecular	% C Found	% H Found	% N Found
	Formula	Weight	(calculated)	(calculated)	(calculated)
Novel Schiff	CIIN	406	69.34	6.98	23.68
base ligand	$C_{32}H_{28}N_6$	490	(77.42)	(5.65)	(16.93)



Sr.No.	Molecular Formula	Color	M.P. (°C)	Time	Yield (%)
1	$C_{32}H_{28}N_6$	Brownish	244	13 minutes	90
2	$[C_{32}H_{28}N_6(H_2O)_2]Fe$	Brown	342	60 seconds	90
3	$[C_{32}H_{28}N_6(H_2O)_2]Mn$	Yellow	136	4 minutes	93
4	$[C_{32}H_{28}N_6(H_2O)_2]Co$	Light brown	350	30 seconds	92
5	$[C_{32}H_{28}N_6(H_2O)_2]Ni$	Light green	330	60 seconds	90
6	$[C_{32}H_{28}N_6(H_2O)_2]Cu$	Green	220	70 seconds	91
7	$[C_{32}H_{28}N_6(H_2O)_2]Zn$	Light yellow	280	60 seconds	85
8	$[C_{32}H_{28}N_6(H_2O)_2]Cd$	Light yellow	270	80 seconds	88
9	$[C_{32}H_{28}N_6(H_2O)_2]Ag$	Yellow	203	60 seconds	90

Table 2: The details of physical properties of the novel ligand and its metal complexes

Infrared spectra analysis

Analysis of novel Schiff base ligand: The IR spectra of novel Schiff base ligand showed distinguishing band at 1660.71 cm⁻¹ due to azomethine, v(C=N) [20], stretching. Also a band at 3452.58 cm⁻¹ showed due to NH stretching of benzimidazole ring [20,21]. The novel ligand spectrum showed bands at 1581.63 cm⁻¹ and 1467.83cm⁻¹ due to aromaticv(C=C)stretching [21,22]. These bands confirmed formation of parent ligand.

Analysis of metal complex (L-Fe): The IR spectrum of L-Fe complex showed shift in frequency of azomethine, v(C=N), stretching from 1660.71cm⁻¹ to 1703.14 cm⁻¹ as compared to parent ligand [23-25]. Also band due to imidazole NH stretching shifted from 3452.58cm⁻¹ to 3442.94 cm⁻¹ and aromatic v(C=C) stretching shifted from 1581.63, 1467.83 cm⁻¹ to 1494.83, 1427.32 cm⁻¹. The most characteristic bands of this metal complex are that of M-N and M-O. The M-N band appeared at 578.64 cm⁻¹ and M-O band at 470.63 cm⁻¹. These bands confirmed formation of stable (L-Fe) metal complex [26-27]. The weaker bands observed at 825.53 cm⁻¹ and 715.59 cm⁻¹ are due to OH wagging mode of vibrations suggesting presence of coordinated water (OH₂) molecules in metal complex [28-31]. These last two bands are absent in IR spectrum of novel ligand.



Analysis of metal complex (L-Mn): The IR spectrum of L-Mn complex showed shift in frequency of azomethine, v(C=N), stretching from 1660.71 cm⁻¹ to 1693.50 cm⁻¹ as compared to novel ligand [23-25]. Also band due to imidazole NH stretching shifted from 3452.58 cm⁻¹ to 3302.50 cm⁻¹ and aromatic v(C=C) stretching shifted from 1581.63, 1467.83 cm⁻¹ to 1554.63, 1392.61 cm⁻¹. The most characteristic bands of this metal complex are that of M-N and M-O. The M-N band appeared at 530.42 cm⁻¹ and M-O band at 455.20 cm⁻¹. These bands confirmed formation of stable (L-Mn) metal complex [26-27]. The weaker bands observed at 844.82 cm⁻¹ and 698.23 cm⁻¹ are due to OH wagging mode of vibrations suggesting presence of coordinated water (OH₂) molecules in metal complex [28-31]. These last two bands are absent in IR spectrum of novel ligand.

The IR data of both metal complexes showed the bidentate nature of novel ligand. The IR data of both novel Schiff base ligand and its metal complexes is summarized in Table 3.

Ligand / Complex	υ(C=N)	υ(NH)	υ(C=C)	υ(M-N)	υ(M-O)
	Azomethine	Imidazole	Aromatic		
C. H. N	1660 71	2452 58	1581.63,		
C3211281N6	1000.71	3432.38	1467.83		
$[\mathbf{C} \cup \mathbf{U} \cup \mathbf{N} \cup (\mathbf{U} \cup \mathbf{O})] = \mathbf{E}$	1702 14	3442.04	1494.83,	578 61	470.63
	1703.14	5442.54	1427.32	578.04	470.03
$[C, \mathbf{H}, \mathbf{N}] (\mathbf{H}, \mathbf{O})$ \mathbf{M}	1603 50	2202 12	1554.63,	530 42	455 20
	1095.50	5502.15	1392.61	550.42	455.20

Table 3: Selected Infrared Frequencies (cm⁻¹) of parent ligand and its complexes

¹HNMR Spectral Studies

The peaks observed in ¹HNMR spectra of novel Schiff base ligand clearly show following pattern. The multiple peaks observed at 7.94-7.62 ppm due to H-from aromatic rings. The peak observed at 5.89 ppm (s, 2H) due to H from two imidazole NH group. The peaks observed at 2.5 -2.19 ppm due to H-from methyl groups attached to aromatic rings.



Table 4: Observed ¹HNMR Peaks (ppm) of novel Schiff base ligand

Compound	H- from aromatic group	H-from imidazole	H-from 5,6 dimethyl group
$C_{32}H_{28}N_6$	7.94-7.62 ppm	5.89 ppm	2.50-2.19 ppm

Mass Spectral studies

The mass spectrum study of parent Schiff base ligand showed a peak at m/z 498 (M+2) which corresponds to molecular weight of the parent Schiff base ligand i.e. 496.

Electronic spectra

The electronic spectrum of both metal complexes (L-Fe, L-Mn) recorded in the wavelength region 200 nm to 400 nm in DMSO solution. The electronic spectral data obtained for both the complexes is shown in Table 5.

The electronic spectrum of the Fe (III) complex showed major bands at 48543.69 cm⁻¹(206 nm), 47619.05 cm⁻¹ (210 nm), 44444.44 cm⁻¹ (225 nm) and 35260.93 cm⁻¹ (283.6 nm). The band at 48543.69 cm⁻¹ is due to charge transfer supporting the coordination of the ligand to Fe (III). The last three correspond to ${}^{6}A_{1g}{}^{4}A_{1g}(G) \& {}^{4}E_{g}(G), {}^{6}A_{1g}{}^{4}T_{2g}(G) > {}^{6}A_{1g}{}^{4}T_{1g}(G)$ transitions respectively [32-34]. The electronic transition shown in spectrum suggests octahedral geometry of the complex [32-34]. The major bands also conforms π π^{*} and \Rightarrow π^{*} transitions.

The electronic spectrum of the Mn (II) complex reveals major bands at 49407.12 cm⁻¹ (202.4 nm), 46948.36 cm⁻¹ (213 nm), 43290.04 cm⁻¹ (231 nm) and 35236.08 cm⁻¹(283.8 nm). The band at 49407.12 cm⁻¹ is due to charge transfer supporting the coordination of the ligand to Mn (II). The last three correspond the ${}^{6}A_{1g}{}^{4}T_{1g}(P) \& {}^{4}E_{g}(D), {}^{6}A_{1g}{}^{4}T_{2g}(D), {}^{6}A_{2g}{}^{4}A_{1g}(G)$ transitions respectively [33,35]. The electronic transition shown in spectrum also supports octahedral geometry of the complex [33]. The major bands also conforms $\pi \rightarrow \pi^{*}$ and $n \rightarrow \pi^{*}$ transitions.



Table 5: Electronic spectral data and probable geometries for the metal complexes

Sr.	Complex	UV Vis major bands	Assignment	Proposed	
No.		Absorption maxima		Geometry	
		cm^{-1} (nm)			
		35260.93 (283.6)	$^{6}A_{1g}^{4}\mathbf{F}_{1g}(G)$		
1	[C ₃₂ H ₂₈ N ₆ (H ₂ O) ₂]Fe	44444.44 (225)	${}^{6}A_{1g} {}^{4}\mathbf{F}_{2g}(G)$	Octahedral	
		47619.05 (210)	${}^{6}A_{1g} \stackrel{4}{\Rightarrow}_{1g}(G)$		
		40542 (0 (20))	$A_{1g} \xrightarrow{\bullet} (G)$		
		48543.69 (206)	Charge transfer		
-		35236.08 (283.8)	$^{6}A_{1g} \overset{4}{\rightarrow}_{1g}(G)$		
		43290.04 (231)	$^{6}A_{1g}^{4}\mathcal{P}_{2g}(D)$		
2	[C ₃₂ H ₂₈ N ₆ (H ₂ O) ₂]Mn	46948.36 (213)	${}^{6}A_{1g} \stackrel{4}{}_{1g}(P),$	Octahedral	
			$A_{1g} = B_{g}(D)$		
		49407.12 (202.4)	Charge transfer		



Proposed structure of Metal complexes

[M = Mn(II) / Fe(III) / Co(II) / Ni(II) / Cu(II) / Zn(II) / Cd(II) / Ag(I)]



Thermal analysis of metal complexes

The TGA of both the metal complexes (L-Fe, L-Mn) were carried with in the temperature range from room temperature to 500 0 C. The heating was carried in the nitrogen atmosphere with the linear heating rate of 10 0 C min⁻¹. The thermal data extracted from the thermogram of both the complexes is summarized in Table 6.

The L-Fe complex thermogram clearly shows a total weight loss of 73.769 % which may be observed in steps shown in Table 6. At first, the water of crystallization lost in the range of 29.31° C to 150° C and 10° observed weight loss found at 182.64° C. This is followed by loss of methyl and remaining organic moiety resulting in total weight loss of 73.769 % (cal. 78 %) up to 500° C. A stable curve at 500° C indicates formation of stable metal oxide (Fe₂O₃) [36-38].

The L-Mn complex thermogram clearly shows a total weight loss of 31.864 % which may be observed in steps shown in Table 6. At first, the water of crystallization lost in the range of 30 0 C to 160 0 C and 10% weight loss found at 224.49 0 C. This is followed by loss of methyl and remaining organic moiety resulting in total weight loss of 31.864 % at 500 0 C. The lesser weight loss may be due to remaining organic moiety with MnO at 500 0 C [33-35].

[C ₃₂ H ₂₈ N ₆	$(H_2O)_2]Fe$	$[C_{32}H_{28}N_6 (H_2O)_2]Mn$		
Weight loss (%)	Temperature (⁰ C)	Weight loss (%)	Temperature (⁰ C)	
0	29.31	0	30.94	
10	182.64	10	224.49	
20	262.52	20	378.10	
30	285.32	30	493.82	
40	355.77	31.864(Total wt. loss)	500	
50	418.14			
60	459.41			
70	489.86			
73.769(Total wt. loss)	500			

Table 6:	Thermogra	vimetric a	nalytical	data	of metal	complexes
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Bioactivity Study

The antimicrobial activity was measured in terms of % of inhibition *in vitro*. The assay was carried according to the micro assay protocol of Rieckmann and co-workers with fewer modifications. The antimicrobial activity of synthesized novel ligand and its metal complexes were tested against *Escherichia Coli*, *streptococcusAureus* and *salmonellaTyphi* grown at 37 0 C overnight. The minimum inhibition concentration was measured by Micro Broth Dilution method at wavelength 475 nmusing streptomycin as a reference drug. The test samples were prepared by using DMSO solventin the concentration range 4 µg/ml - 100 µg/ml.

The MIC data summarized in Table 7 evidentlyconfirms that Cd (III) complex shows excellent activity against *Escherichia Coli* as compared to novel ligand and rest of the metal complexes. The Mn (II) complex shows excellent activity against *streptococcusAureus* as compared to the novel ligand and rest of the metal complexes. The Ag (I) complex shows excellent activity against *salmonellaTyphi* as compared to the novel ligand and rest of the metal complexes.

Sr.No	Compounds	Minimal Inhibition Concentration (µg/ml)					
•		E. Coli	S. Aureus	S. Typhi			
1	$C_{32}H_{28}N_6$	250	500	250			
2	$[C_{32}H_{28}N_6 (H_2O)_2]Fe$	500	125	500			
3	$[C_{32}H_{28}N_6 (H_2O)_2]Mn$	250	62.5	500			
4	$[C_{32}H_{28}N_6(H_2O)_2]Co$	500	100	125			
5	[C ₃₂ H ₂₈ N ₆ (H ₂ O) ₂]Ni	500	125	250			
6	$[C_{32}H_{28}N_6(H_2O)_2]Cu$	250	125	250			
7	$[C_{32}H_{28}N_6 (H_2O)_2]Zn$	500	250	500			
8	$[C_{32}H_{28}N_6(H_2O)_2]Cd$	100	125	250			
9	$[C_{32}H_{28}N_6 (H_2O)_2]Ag$	500	500	100			

Table 7: Antibacterial activity of ligand and their metal complexes



Conclusion

In the present work, green syntheses of a noveltetra dentate Schiff base ligand, (E)-N-(2-(5, 6-dimethyl-1H-benzo[d]imidazole-2-ylimino)-1, 2-diphenyl ethylidene)-5, 6-dimethyl-1H-benzo[d]imidazole-2-amine and its metal complexes were performed under solvent free condition using microwave method. All compounds prepared were characterized by different analytical and spectral techniques. The principal benefits of this method are lowered reaction time and higher yield. The method reduces risk, hazard and very easy to conduct.

Acknowledgement

The authors are thankful to Shivaji Arts, Commerce and Science College Kannad, Dist. -Aurangabad [MS] India and JES College Jalna, Dist.-Jalna [MS] India for their support.

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