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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF ((12E)-4-METHYL-N-((4-OXO-4H-CHROMONE-3-YL) METHYLENE)-1,2,3,-THIADIAZOLE-5-CARBOXYLIC ACID HYDRAZIDE AND THEIR TRANSITION METAL COMPLEXES

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ABSTRACT

A Schiff base (12E)-4-methyl-N-((4-oxo-4H-chromone-3-yl)methylene)-1,2,3,-thiadiazole-5-carboxylic acid hydrazide was obtained by the condensation of 4-oxo-4H-chromene-3-carbaldehyde and 4–Methyl-1,2,3,-thiadiazole-5-carboxylic acid hydrazide and the synthesized schiff base was characterized by various analytical technique such as I.R., ^{1}H-NMR, ^{13}C-NMR and Mass spectrometry. Further it was used for the complexation with different transition metal ions such as Fe (II), Co(II), Ni (II), Cu(II) and Zn (II) by using molar ratio of metal to ligand as 1: 2. The proposed structures of metal complexes have been studied by I.R, Thermogravimetric analysis and ^{1}H-NMR, spectroscopic techniques. The synthesized compounds were evaluated for *in vitro* antimicrobial screening and the results were compared with standard drugs. The antimicrobial result shows the metal complexes exhibit remarkable antibacterial and antifungal activity as compared to parent ligand.

Keywords: NO,ONO donor Schiff base; Co(II), Ni(II), Cu(II) and Zn(II) complexes; Spectroscopic analysis; Antimicrobial activity.

1. INTRODUCTION

The Schiff base and chromones compounds have a wide range of medical applications, where they are used as anti-microbial, fungicidal, antitumor, antioxidant, anti-HIV, anti-inflammatory, psychotropic and insecticidal [1-5]. Many studies on the Schiff bases complexes have shown that these complexes have biological, clinical and pharmacological uses [6, 7]. The chromone moiety execute as the vital constituent in lots of pharmacophores of biological active molecules of synthetic as well as natural origin and many of them have useful medicinal applications [8-11]. 3-Formyl chromone resides in an exclusive position for two reasons, they are carrying a substantial biological activity and they are prominent synthetic intermediates [12, 13]. The notable biological properties, proton affinities, optical properties and the degree of aromaticity of the chromones have paying attention from both theoretical and experimental point of views [14-17]. The Hydrazones are also most extensively used chelating co-ordination ligands in chemistry [18]. They are also important in catalysis and in medicine as antimicrobial, antioxidant and anticancer agents [19]. Metal complexes of hydrazone ligands have been widely studied over earlier periods.

2. EXPERIMENTAL

2.1. Material

All the purchased chemicals were of analytical grade and used without further purification. Solvents were purified and dried according to literature method [20]. Chromone-3-carbaldehyde and 4–Methyl-1,2,3,-thiadiazole-5-carbohydrazide were obtained from Sigma Aldrich. All the metal salts were received from SD fine and E-Merck.

2.2. Physical measurements

Elemental analysis (C, H, N,) was performed using Perkin Elmer CHN analyzer. IR spectra of the ligands and their metal complexes were recorded on Bruker spectrometer within the range of 4000-400 cm^{-1}. Thermal studies of the complexes were carried out on a Perkin Elmer diamond TGA instrument. ^{1}H-NMR and ^{13}C-NMR spectra of the ligands were recorded on

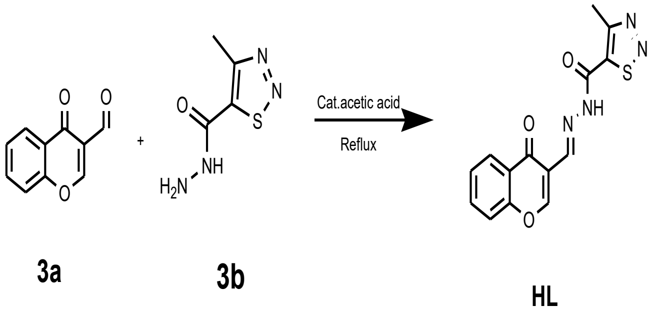
Bruker spectrometer using DMSO-d6 as a solvent and TMS as internal standard. Mass spectra were recorded on water, Qt of micromass (ESI-MS).

2.3. Synthesis of the Schiff base ligand

The Schiff base ligand was prepared by condensation of chromone-3-carbaldehyde (**3a**) (1.00 mmole) and 4 -Methyl-1,2,3,-thiadiazole-5-carboxylic acid hydrazide (**3b**) (1.00 mmole) in absolute ethanol (20 ml), 4-5 drops of acetic acid. The mixture was refluxed for 4 hr with continuous stirring. The progress of reaction was monitored by TLC and after completion of the reaction; the mixture was poured on crushed ice and filtered off. The obtained product was recrystallized in absolute ethanol.

2.4. Spectral data of ligand

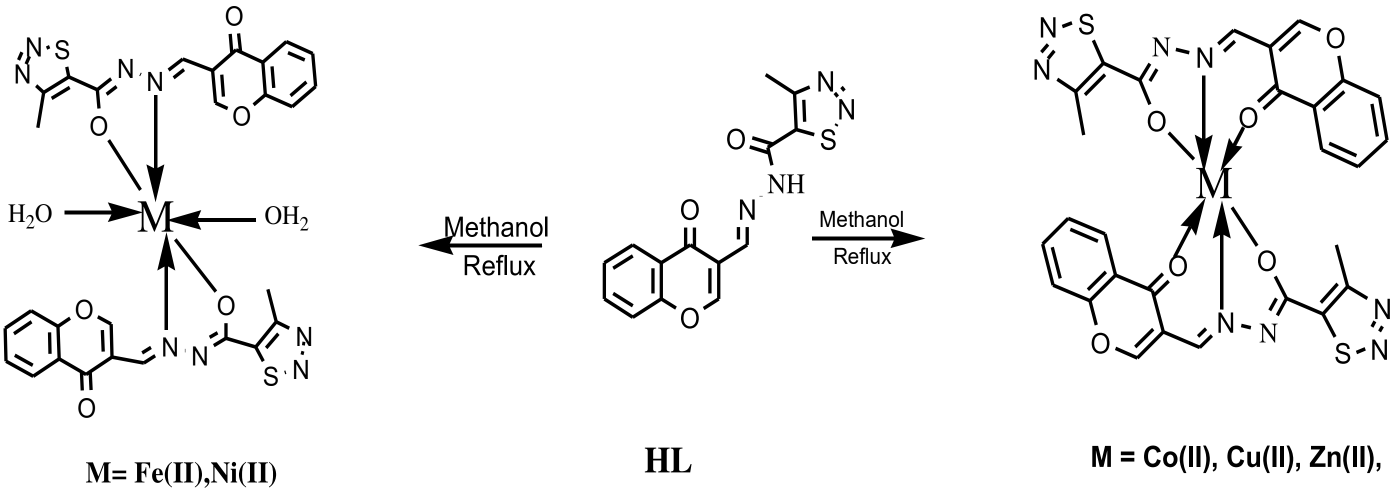
Color: Cream colour , Yield: 74 %, M.P.: 272 °C, Selected FT-IR bands (KBr, cm^{-1}) : 3127.33 ν (NH), 1662 ν(C=O chromone), 1619 ν (C=O hydrazonic), 1590 ν(C=N); ^{1}H NMR (DMSO-d6, δ ppm) 12.49 (S, 1H, NH); 8.07 (S, 1H, HC=N), 7.5-8.3 (m, 5H, Ar-H); 2.97 (S, 3H, $-CH_3$); ^{13}C NMR (DMSO-d6, δ ppm) 193.27 (C=O chromone), 168.20 (C=O hydrazone), 163.70 (-HC=N), 15.32($-CH_3$); Mass spectrum (ESI) $[M+H]^+$ =315.



Scheme: The schematic route for synthesis of Schiff base (**L**)

2.5. Synthesis of metal complexes

The Schiff base ligand HL (0.01 mol) was dissolved in hot methanol solution of corresponding salts (0.005 mol). MX2 [where M=Cu(II), Ni(II), Co(II),Mn(II) and Zn(II), X=nitrate] were mixed together and refluxed with constant stirring for 4-6 h at refluxing temperature. On cooling, colored solids were precipitated out. The products were filtered, washed with cold methanol, dried in air and in desiccator over anhydrous $CaCl_2$ and stored in an airtight sample bottle. All the compounds are colored and are stable to air and moisture.



2.6. Antimicrobial evaluation

The *in vitro* antimicrobial screening of synthesized ligand and metal complexes was performed against four bacteria (*S. Aureus, S. Pyogenes, E. Coli & S. Typhi*) and two fungi (*C. Albicans & T. Rubrum*) by petri-plate containing 30 ml potato dextrose agar and nutrient agar medium, the plates were incubated for 20-24 hr and 24-48 hr for bacteria and fungi stains, respectively. The activities were measured in terms of zone of inhibition in mm. Cefotaxime, Azithromycin and Clotrimazole were used as standard drugs for bacteria and fungi, respectively at 500 ppm concentration of sample as well as drugs. The results of antimicrobial activity of ligand and metal complexes are shown in Table 4.

3. RESULTS AND DISCUSSION

The analytical data and physical properties of the ligand and its metal complexes are listed in Table 1. The Schiff base ligand (HL) is soluble in common organic solvents. The resultant Schiff base complexes are partially soluble in MeOH and CHCl$_3$ but freely soluble in DMF and DMSO. The analytical data indicate that the metal to ligand ratio is 1:2 in all the metal complexes.

Table1: Physical and analytical data of HL and its metal complexes.

Compound	Mol. Formulae (F.W.)	M.P. °C	Colour	Elemental analysis found (calculated.)%			
				% C (cal.)	% H (cal.)	% N (cal.)	% M (cal.)
Ligand (HL)	$C_{14}H_{10}N_4O_3S$ (314.05)	272°C	Crime	52.64 (53.50)	2.92 (3.21)	17.10 (17.82)	-
$[Fe(HL)_2(H_2O)_2]$	$C_{28}H_{22}FeN_8O_8S_2$ (718)	>280°C	Brown	46.22 (46.81)	3.20 (3.09)	15.22 (15.60)	8.32 (7.77)
$[Co(HL)_2]$	$C_{28}H_{18}N_8CoO_6S_2$ (685)	>280°C	wheat	48.21 (49.06)	2.50 (2.65)	15.22 (16.34)	8.08 (8.60)
$[Ni(HL)_2 (H_2O)_2]$	$C_{28}H_{22}NiN_8O_8S_2$ (721)	>280°C	Green	50.49 (46.62)	3.10 (3.07)	15.16 (15.53)	8.04 (8.14)
$[Cu(HL)_2$	$C_{28}H_{18}Co\ N_8O_6S_2$ (690)	>280°C	yellow	48.12 (48.73)	2.55 (2.63)	15.68 (16.24)	8.60 (9.21)
$[Zn(HL)_2$	$C_{28}H_{18}\ Zn\ N_8O_6S_2$ (692)	>280°C	yellow	48.02 (48.60)	2.86 (2.62)	15.81 (16.19)	8.96 (9.45)

3.1. FT-IR spectra

The IR spectra containing relevant vibrational bands of the ligands and their metal complexes are listed in Table 2. The ligands showed a band at 1662 cm^{-1} which is due to ν (C=O) group of the chromone moiety. This band was shifted to lower wave number region 15-40 cm^{-1} in their corresponding metal complexes, indicating the coordination of oxygen atom of carbonyl group of the chromone moiety [21]. The stretching vibration of the azomethine group (C=N) was observed at 1590 cm^{-1} in the ligand. This band was shifted to lower wave number region 20-30 cm^{-1} in their metal complexes, indicating the participation of nitrogen atom of azomethine group in coordination to the metal ion. An appearance of new broad band in the region 3291-3409 cm^{-1} indicates the presence of coordinated water in Fe(II),Ni(II), metal complexes. The coordination of nitrogen and oxygen atoms was supported by the appearance of a non-ligand bands in the range 500-535 cm^{-1} and 400-470 cm^{-1} region due to the ν(M–O) and ν(M–N), respectively [22, 23]. From the above spectral data, it was concluded that schiff base ligand acts as bidentate in Fe(II) and Ni(II) metal complexes due to coordination of two water molecule and tridentate ligands in Cu(II), Co(II), and Zn(II) metal complexes with ONO donor sites.

Table 2: The selective Infrared frequencies of ligand (HL) and its metal complexes

Compound Name	ν(C=O) Chromone	ν(C=O) ydrozonic	ν(C=N)	ν(M-O)	ν(M-N)
HL	1662	1619	1590	-	
$[Fe(HL)_2 (H_2O)_2]$	1654	1615	1588	528	410
$[Co(HL)_2\ ]$	1606	1588	1558	520	452
$[Ni(HL)_2 (H_2O)_2]$	1654	1627	1580	532	438
$[Cu(HL)_2$	1659	1614	1565	512	470
$[Zn(HL)_2$	1652	1598	1542	548	430

3.2. ^{1}H-NMR spectra and Mass spectrum

The ^{1}H-NMR spectrums of ligand and metal complexes were recorded in DMSO-d6. The spectrum of ligand shows following signals: 12.49 (s, 1H, NH), 8.07 (s, 1H, HC=N), 2.97 (s, 3H, -CH$_3$), 7.5-8.3 (m, 5H, Ar-H). Mass spectra of ligand (ESI) [M+H]$^+$ =315.

3.3. Thermogravimetric analysis

Thermal analysis was used mainly for the confirmation of the water molecule or solvent associated with being in the sphere of coordination or in the outer sphere of the complex [24-26] and the information about its properties, the nature of the products intermediate and final thermal decomposition. From the TGA curves, the weight loss was calculated for the different steps and compared with the theoretically (calculated) weight for the suggested formulas based on the results obtained from the elemental analyzes. Thermal stability of the synthesized metal complexes was done up to 900 °C at a heating rate of 10 °C /min in nitrogen atmosphere. Metal complexes exhibit similar decomposition pattern as evident from their TGA graphs. The TGA graph shoes that decompose of Fe metal complex in three steps within temperature range of 10-700 °C. First step corresponds to the loss of two coordinated water molecules (Found 4.12 %, calcd. 5.01 %) in temperature range of 10-160 °C. The second step corresponds to the loss of $(C_{11}H_6N_2O_2)_2$ (Found 57.12 %, calcd. 59.88 %) in temperature range of 160-400 °C. A peak corresponding to mass loss of (25.54% calcd.27.07 %) at 380-610 °C was due to the loss of $(C_3H_3N_2S)_2$in the third step and as a final product, it leaves FeO as residue. Similar behavior was observed in the TGA curve of metal complexes 3-4. Thermal analysis data of all metal complexes (2-4) collectively given in Table 3.

Table 3: Thermal analysis data of metal complexes

Comp. No.	Molecular formula	Stages	Temp (°C)	Possible evolved species	Residual species	Mass loss	
						Found	Calc.
1	$[Fe(HL)_2\,(H_2O)_2]$ (M.W-718)	1st 2nd 3rd	10-160 °C 160-400 °C 400-600 °C	2 H_2O $(C_{11}H_6N_2O_2)_2$ $(C_3H_3N_2S)_2$	FeO	4.12 57.12 25.54 9.30	5.01 59.88 27.07 9.18
2	$[Co(HL)_2\,]$ (M.W-685)	1st 2nd	10-320 °C 320-650 °C	$(C_{11}H_6N_2O_2)_2$ $(C_3H_3N_2S)_2$	CoO	59.18 27.10 9.33	62.77 28.91 10.76
3	$[Ni(HL)_2\,(H_2O)_2]$ (M.W-721)	1st 2nd 3rd	10-190 °C 190-400 °C 400-700 °C	2 H_2O $(C_{11}H_6N_2O_2)_2$ $(C_3H_3N_2S)_2$	NiO	4.17 59.25 26.10 9.33	4.99 59.63 27.46 10.12

3.4. Antimicrobial activity

The metal complexes exhibit higher inhibition against tested microorganism compared to the free ligand [27, 28]. The value in the respective table indicates that the activity of the Schiff base ligand became more pronounced when coordinated with the metal ions. The presence of azomethine moiety and chelation effect with central metal enhances the antibacterial activities. This enhancement in antibacterial activity of these metal complexes can be explained based on the chelation theory [29].

Table 4: Results of antimicrobial activity of synthesized compounds

Compounds	Zone of Inhibition in mm					
	Gm +ve bacteria		Gm -ve bacteria		Antifungal activity	
	S. Aureus	*S. Pyogenes*	*E. Coli*	*S. Typhi*	*C. Albicans*	*T. Rubrum*
Ligand (HL)	9	8	8	10	7	9
$[Co(HL)_2]$	8	7	10	10	**8**	**11**
$[Ni(HL)_2\,(H_2O)_2]$	9	11	11	12	**9**	**10**
$[Cu(HL)_2]$	14	16	8	10	16	16
$[Zn(HL)_2]$	9	12	10	11	11	12
Cefotaxime	-	-	**25**	**20**	**–**	-
Azithromycin	**26**	**24**	-	-	**–**	-
Clotrimazole	-	-	-	-	**14**	**15**

When a metal ion is chelated with a ligand, its polarity will be reduced to a greater extent due to the overlap of ligand orbital and the partial sharing of the positive charge of the metal ion with donor groups. Furthermore, the chelation process increases the delocalization of the π-electrons over the whole chelate ring, which results in an increase in the lipophilicity of the metal complexes. Consequently, the metal complexes can easily penetrate into the lipid membranes and block the metal binding sites of enzymes of the microorganisms. These metal complexes also affect the respiration process of the cell and thus block the synthesis of proteins, which restrict further growth of the organism. The results of antimicrobial activity of ligand and metal complexes are shown in factors. They are the chelate effect, nature of coordinated ligand, total charge of complex, nature of the ion neutralizing the ionic complex and nuclearity of the metal center in the complex. The increased activity of the metal complex than the free ligand can also be explained on the basis of chelation theory.

4. CONCLUSION

In the present work, Fe(III), Co(II), Ni(II) ,Cu(II),and Zn(II) complexes were prepared from ((12E)-4-methyl-N-((4-oxo-4H-chromone-3-yl)methylene)-1,2,3,-thia - diazole-5-carbohydrazide. The Schiff base was characterized using various spectral techniques. IR spectra revealed coordination of Schiff base ligand with metal ion through azomethine nitrogen, carbonyl oxygen of chromone moiety and carbonyl oxygen of hydrazide moiety. The structural elucidation studies by various spectral techniques (IR, and ^{1}H NMR) suggested the nature of ligand is bidentate in Fe(II) and Ni(II) metal complexes due to coordination of two water molecule and tridentate ligands in Cu(II), Co(II), and Zn(II) metal complexes with ONO donor sites and geometry of the metal complexes are octahedral. Thermogravimetric analysis studies demonstrate the stability of complexes as well as provided the number of coordinated water molecules. Antimicrobial studies suggest that Schiff base and its complexes play a vital role in developing a new class of antibiotics.

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