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SYNTHESIS, SPECTRAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF COPPER(II), COBALT(II) AND ZINC(II) COMPLEXES OF 6-METHOXY-3-FORMYLCHROMONE

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Keywords:

6-Methoxy-4-oxo-4H-chromene-3-carbaldehyde, Schiff base, MIC, Metal complexes, Antimicrobial activity

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ABSTRACT: A new series of Cu(II), Zn(II) and Co(II) complexes have been synthesized from 6-Methoxy-3-phenyliminomethyl-chromen-4-one, a Schiff base derived from 6-Methoxy-4-oxo-4H-chromene-3-carbaldehyde and aniline. The nature of bonding and the structure of the complexes have been deduced from IR, UV, 1H NMR spectroscopy. The biological activity of the ligand and metal complexes have been examined against both gram-positive as well as gram-negative bacteria by the Agar well method using DMSO as solvent and Gentamicin as standard drug. The zone of inhibition values and MIC were measured at 37 ºC for 24 h. Antimicrobial screening tests displayed better results for the metal complexes as compared to the ligand.

INTRODUCTION: Heterocyclic compounds having nitrogen or oxygen atoms attract a great deal of attention towards coordination chemistry. Chromones are pervasive in nature with advantageous effects in the field of medicine. Chromones and its analogues exhibit various pharmacological activities like anti-cancer [1], anti-bacterial [2], anti-oxidant [3], anti-fungal [4], anti-HIV [5], anti-ulcers [6] etc. Schiff bases designed from chromones along with their metal complexes often show varied biological as well as pharmaceutical activities [7, 8]. The complexes of chromone-3-carbaldehyde derivatives have been well studied in the literature.

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Q. Wang *et al.*, have reported synthesis of isonicotinoyl hydrazone from methoxychromone-3-carbaldehyde and also its Ln(III) complexes (Ln = La, Sm) [9]. V. Barve *et al.*, have synthesized and characterized Schiff base derived from 3-formylchromone and their copper(II) complexes [10]. Cu(II) complex of 7-methoxychromone-3-carbaldehyde-benzoyl-hydrazone have been synthesized by G. Qi *et al.*, with general formula $[CuL(HB_2BO)]Cl_2O$ [11].

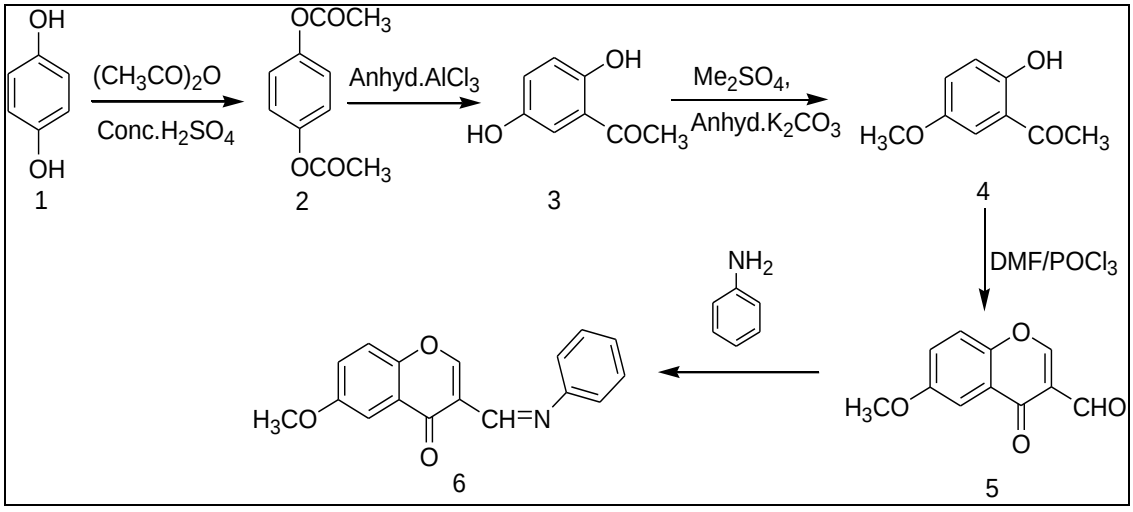
In the present study, we report the synthesis, characterization and the biological activity of transition metal complexes of the Schiff base ligansd derived from 6-Methoxy-4-oxo-4H-chromene-3-carbaldehyde and aniline.

RESULTS AND DISCUSSION:

Chemistry: The Schiff base 6-Methoxy-3-phenyliminomethyl -chromen -4 -one was synthesized by condensation of 7-methoxy-4-oxo-4*H*-benzopyran-3-yl-carboxaldehyde and aniline.

2,5-Dihydroxyacetophenones was the starting material for the synthesis of 5-Methoxy-2-hydroxyacetophenone which was in turn converted to 6- methoxy- 4- oxo- 4*H*- benzopyran- 3- yl-carboxaldehyde through Vilsmeier-Haack formylation in good yields (80-85%). First of all, 2,5-Dihydroxyacetophenone (3) was synthesized from hydroquinone. Hydroquinone (1) was converted to hydroquinone diacetate (2) through Friedel crafts acylation with acetic anhydride using sulphuric acid as catalyst. Dry hydroquinone diacetate was in turn gently heated with anhydrous aluminium chloride to give 2,5-Dihydroxyaceto phenone.

Then methylation of 2,5-Dihydroxyacetophenone (3) was carried out by refluxing in anhydrous conditions with dimethylsulfate and ignited potassium carbonate to give 2-hydroxy-5-methoxyacetophenone (4). Then 2-hydroxy-5-methoxy acetophenone was treated with phosphorus oxychloride in dry N,N-dimethylformamide to produce 7-methoxy-4-oxo-4*H*-benzopyran-3-yl-carboxaldehyde (5). Schiff base (6) is synthesized by condensation of 6-methoxy-4-oxo-4H-benzopyran-3 – yl - carboxalde hyde and aniline as bright yellow colored compound (**Scheme1**).



SCHEME 1: SYSTEMIC PATHWAY FOR SYNTHESIS OF 6-METHOXY-3-PHENYLIMINO-METHYL-CHROMEN-4-ONE

Then the metal complexes of Cu(II), Zn(II) and Co(II) were synthesized from this Schiff base (6) taking it as a ligand. The proposed structure of the metal complexes is given in **Fig. 1**.

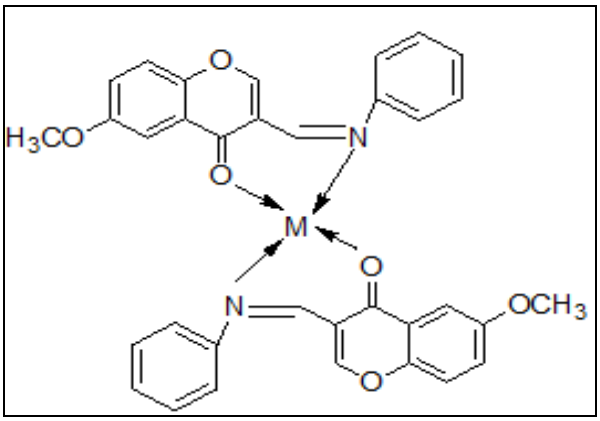


FIG. 1: PROPOSED STRUCTURES OF METAL(II) COMPLEXES OF SCHIFF BASE LIGAND (M = CU, Co, AND Zn)

IR spectra: IR spectral data of the ligands and metal complexes are presented in **Table 2**. Compared to the IR spectrum of the ligands, the frequency of $\nu_{C=N}$ (1597 cm^{-1}) moves to lower energy in the spectra of the complexes, confirming coordination of the azomethine nitrogen to the metal. C=O (cyclic keto group present in the phenyl ring (1645cm^{-1}) moved to a lower frequency in complexes, suggesting coordination via the C=O oxygen [12, 13].

The proof of N and O coordination is demonstrated by bands in the spectra of complexes in the regions 527-545 cm^{-1} and 429-460 cm^{-1} assigned to M-N and M-O modes, respectively [14]. IR data of some important functional groups present in the Schiff base and the metal complexes are presented in **Table 1**.

TABLE 1: CHARACTERISTIC IR STRETCHING BANDS OF LIGAND AND ITS METAL COMPLEXES IN cm^{-1}

Compound	$\nu_{C=O}$	$\nu_{C=N}$	ν_{M-O}	ν_{M-N}
Ligand	1645	1597	-	-
$C_{36}H_{26}O_6N_2CuCl_2$	1618	1470	545	460
$C_{36}H_{26}O_6N_2CoCl_2$	1620	1570	527	460
$C_{36}H_{26}O_6N_2ZnCl_2$	1639	1470	525	429

Electronic spectra: The UV-visible absorption spectra of the Schiff base ligand [L] and its complexes were done in DMF at room temperature. The values of the absorption wavelength and its band assignments are listed in **Table 2**. The absorption of the ligand [L] is characterised by two main absorption bands at 230 and 325 nm. The band appearing at lower energy is attributed to n to π* transition of conjugation between the lone pair of electrons of p orbital of N-atom of azomethine group and π conjugated bond of the benzene ring. The bands appearing at higher energy are attributed to π to π* of the benzene ring and π to π* transition of the azomethine group [15, 16].

The UV-visible absorption spectra of all the complexes show similarities, which indicates similarity in their structures and usually exhibit the characteristic bands of the free ligands with some changes in frequencies as well as in intensities. The absorption bands of the complexes are somewhat shifted to shorter wavelength (Blue shift) upon complexation as compared to those of the free ligand. Such change in the shifts and intensity of the absorption bands indicates the coordination of the ligand to the metal ion.

TABLE 2: UV-VIS SPECTRAL DATA Λ_{max} (nm) OF LIGAND AND ITS METAL COMPLEXES IN cm^{-1}

Compound	λ_{max} (nm)	Band assignment
Ligand	230,	π to π*
	325	n to π*
$C_{36}H_{26}O_6N_2CuCl_2$	219	π to π*
$C_{36}H_{26}O_6N_2CoCl_2$	220	π to π*
$C_{36}H_{26}O_6N_2ZnCl_2$	216	π to π*

Antibacterial activity: All the novel synthesized compounds were evaluated for antibacterial activity against a broad range of pathogenic bacterial strains using agar well method. The antibacterial activity was screened against six pathogenic bacterial strains viz. *Bacillus cereus*, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermis*. All the compounds tested were found to possess good antibacterial activity against all six strains bacteria taken by developing a zone of inhibition in the range of 12-20 mm (**Table 3**).

For further insights on the antibacterial action, minimum inhibitory concentration (MIC) was determined using broth dilution assay (**Table 4**). According to the antibacterial activity results, all the compounds exhibited good activity against all the six pathogenic bacterial strains when compared to the standard drug gentamicin with MIC value in the range 25-200 µg/mL.

TABLE 3: ZONE OF INHIBITION (MM) OF ACTIVE COMPOUNDS AGAINST PATHOGENIC BACTERIAL TEST STRAINS USING AMPICILLIN AS POSITIVE CONTROL

Compound	*B. cereus*	*S. epidermis*	*A. hydrophila*	*S. aureus*	*E. coli*	*P. aeruginosa*
Ligand	11	12	12	12	12	12
Cu	17	19	17	17	20	19
Co	18	18	17	18	18	16
Zn	18	18	16	18	19	20
Gentamicin	30	29	29	31	31	30

Average diameter of well = 8 mm; All the experiments were carried out in triplicate.

TABLE 4: MINIMUM INHIBITORY CONCENTRATION OF ACTIVE COMPOUNDS IN µg/ml AGAINST PATHOGENIC BACTERIAL TEST STRAINS USING GENTAMICIN AS POSITIVE CONTROL

Compound	*B. cereus*	*S. epidermis*	*A. hydrophila*	*S. aureus*	*E. coli*	*P. aeruginosa*
Ligand	200	200	>200	>200	200	>200
Cu	50	50	75	100	50	50
Co	75	75	75	50	50	100
Zn	50	75	100	50	50	50
Gentamicin	0.39	<0.39	1.56	1.5625	1.5625	0.78

All the experiments were carried out in triplicate and the results expressed as average values.

Experimental: Melting Points were determined by Buchi M-560 and are uncorrected. The IR spectra of all the compounds have been analysed on Perkin Elmer model 2000 FT - IR spectrophotometer by making KBr discs for solid samples. The ^{1}HNMR spectra have been recorded using Bruker Avance 400 NMR spectrometer taking TMS as internal. The chemical shift values are on δ scale and coupling constant values (J) are in Hz. The ESI-MS spectra were recorded on LCQ advantage ESI-MS (Thermal-Finning Inc.). Analytical TLC were performed on pre coated Merck silica gel 60 F_{254} plates with fluorescence indicator, the spots were visualised by irradiation with UV light. Column chromatography was carried out using silica gel (100-200 mesh). UV data was recorded in methanol on shimadzu, UV-250 II PC UV-VIS recording spectrophotometer. All other chemicals were purchased either from Merck, Spectrochem, Sigma-Aldrich without further purification.

Synthesis of 2,5- Dihydroxyacetophenone (3): To 5g of hydroquinone (1), 13ml of acetic anhydride and 2-3 ml of conc. H_2SO_4 was added. The reaction mixture was poured over ice to obtain white coloured precipitates (7g) of hydroquinone diacetate (2). Then dry hydroquinone diacetate was gently heated with $AlCl_3$ to 100-110 °C for 30min, then temperature was raised to 160-165 °C and maintained for about 4 hours. The progress of this reaction was observed by TLC (PE:EA). Afterwards 300 g of crushed ice and 10 ml of conc. HCl was added in order to decompose excess of $AlCl_3$. The mixture was filtered and recrystallised with 95% ethanol to give 4 g of pure 2,5-Dihydroxy acetophenone as green needles. Yield 80%, mp - 205-206 °C ^{1}H (400, $CDCl_3$). δ 2.57 (s, 3H, -$COCH_3$), 6.79 (d, J = 8.1, 1H, H-3), 6.98 (d, J = 8.1, 1H, H-4), 7.17 (s, 1H, H-6), 9.17 (brs, 1H, OH-5), 11.31 (brs, 1H, OH-2).

Synthesis of 2-hydroxy-5-methoxyacetophenone (4): A mixture of 15g (0.098mol) of dihydroxy acetophenone, 15 ml of dimethylsulfate (0.148 mol) and 300 ml of acetone were refluxed with 25g of anhydrous K_2CO_3 for approximately 4-5 hours continuously to obtain 2-hydroxy-5-methoxy derivative. The progress of the reaction was observed by TLC (PE:EA::70:30). The mixture was filtered and the filterate was distilled to give the product and purified by Column chromatography. Yield 82 %, Color - White needles, mp - 45-46 °C. ^{1}H (400, $CDCl_3$): δ 2.60 (3H, s, -$COCH_3$), 3.80 (3H, s, -OCH_3), 6.91 (lH, d, J_o = 9.0 Hz, H-3), 7.09 (lH, dd, J_m = 3.0, J_o =9.0 Hz, H-4), 7.16 (lH, d, J_o = 3.1Hz, H-6) and 11.85 (lH, s, OH).

Synthesis of 6-methoxy-4-oxo-4 H-benzopyran-3-yl-carboxaldehyde: To a stirred solution of 2-hydroxy-5-methoxyacetophenone (0.04mol) in 16 ml anhydrous DMF, 16 ml of $POCl_3$ was added at 55-60 °C and resulting mixture was stirred for 13 hrs continuously and then poured on crushed ice (100g). The reaction progress was observed with TLC (PE:EA::70:30). Then the product gets filtered. It was washed with water. The crude product obtained was recrystallized with ethanol. Yield 90%, Color - Reddish brown, Mp - 164-166 °C. ^{1}H (400, $CDCl_3$). δ 3. 8554(s, 3H, -OCH_3), 7.2655-7.2350 (m, 1H, J_o = 9.12 Hz, H-7), 7.4154-7.3925 (d, 1H, J_o = 9.16 Hz, H-8), 7.5758-7.5681 (d, 1H, J_m = 3.08 Hz, H-5), 8.4632 (s, 1H, H-2), 10.3829 (s, 1H, H-3).

Synthesis of the 6- Methoxy- 3-phenyliminomethyl- chromen- 4- one (L): The Schiff base ligand is prepared by condensation of 6- methoxy- 4- oxo- 4 H- benzopyran- 3- yl-carboxaldehyde (1.74 g, 0.01 M) and aniline (1.36 g, 0.01 M) in absolute ethanol (30 mL), and adding traces of glacial acetic acid to the mixture was refluxed for about 2 h with continuous stirring, then the yellow color compound was separated out. The compound is collected by filtration, washed with distilled water, recrystallized from hot ethanol and dried in a vacuum desiccator. The melting point of the Schiff base ligand is 145-148 ^{o}C, Yield 80%. Color - Yellow. ^{1}H (400, $CDCl_3$). δ 3.629 (s, 3H, -OCH_3), 7.210-7.229 (d, 1H, J_o = 8.16 Hz, H-8), 7.357-7.416 (m, 1H, J_o = 8.87 Hz, J_m =3.68 Hz, H-7), 7.786-7.795 (d, 1H, J_m = 2.99 Hz, H-5), 7.758 (s, 5H, H-2'to H-6')7.947 (s, 1H, H-2), 7.973 (s, 1H, -CH=N).

General procedure for the synthesis of Metal Complexes: The Schiff base ligand (0.01 M) is dissolved in hot solution of methanol and then hot methanolic solution of corresponding anhydrous salts (0.01M) MX_2 [where M = Cu(II), Co(II) and Zn(II) and X= chloride] were mixed together and refluxed with constant stirring for approximately 3-4 h. On cooling colored solids were precipitated

out. The products were filtered, washed with cold methanol, petroleum ether and dried in air and desiccator over anhydrous $CaCl_2$. The samples were stored in an airtight sample vial. All the compounds synthesized were colored and found stable when exposed to air and moisture.

Antimicrobial activity:

Bacteria: All the strains of bacteria *Aeromonas hydrophila*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermis* were procured from Institute of Microbial Technology, Chandigarh, India.

Materials: Mueller-Hinton agar and Nutrient broth were procured from HiMedia, Mumbai, India. Gentamicin, iodonitrotetrazolium chloride (INT) and DMSO used in the assay were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., USA.

Antibacterial activity assay: Agar well test method was used to evaluate the inhibitory potency of pathogenic bacterial growth by the synthesized compounds [17]. The minimal inhibitory concentration (MIC) was assessed by the broth microdilution method [18, 19]. The synthesized compounds were weighed (10 mg) and dissolved in DMSO to prepare the stock solutions of 10 mg/mL. The serial dilution from 2000 to 10 µg/mL was made in a 96-well plate. Fifty µL of a bacterial suspension, obtained from a 24 h culture (~106 cfu/mL) was added to each well. The plates were incubated at 37 °C for 24 h. Gentamicin was taken as a standard drug.

CONCLUSION: 6- Methoxy- 4- oxo- 4*H*-benzo pyran-3-yl-carboxaldehyde and metal complexes have been synthesized and characterized on the basis of analytical and spectral data. All of the new synthesized compounds were screened for their antibacterial activity against six pathogenic bacterial strains.

All tested compounds have shown good activity against all the tested strains with zone of inhibition in the range of 12-20 mm and MIC value in the range 25-200 µg/mL when compared with standard drug gentamicin. Biological activities of the ligand and their metal complexes have shown that the activity of the metal complexes is higher than the ligand. The presence of azomethine group in Schiff base ligand and coordination with metals enhance the antimicrobial activities.

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