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SYNTHESIS AND CHARACTERIZATION OF BIOLOGICALLY ACTIVE METAL COMPLEXES OF COUMARIN DERIVATIVES

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ABSTRACT

This paper reports the isolation of ML_2 complexes, where, M = $3d^5$ to $3d^9$ elements and ligand L is 3-[{3-(3'-chloro)}-prop-2-enoyl] -4-hydroxy-6-methyl-2H-chromen-2-one. The metal complexes are synthesized and characterized by elemental analysis, IR spectra and conductivity measurements. All metal complexes are highly stable and non-hygroscopic. *In vitro* anti microbial activity of all synthesized compounds and standard drugs have been evaluated against four strains of bacterial culture and one fungus, which includes two gram +ve bacterial culture and two gram -ve bacterial culture, which show net enhancement in activity on co-ordination of metals with ligand but moderate activity as compare to standard drugs.

Key words: Coumarin, Copper, Iron, Nickel, Cobalt, Manganese

INTRODUCTION

Coumarins contain the parent nucleus of benzo - α - pyrone and occur in plants of the families like Orchidaceae, Leguminaceae[1], Rutaceae, Umbellliferae and Labiatae. Some of the coumarins show distinct physiological photodynamic and bacteriostatic activities[2] and placed for many diverse uses[3]. Their chelating characteristics have long been observed and their bacteriostatic activity seems to be due to chelation. Though few natural 4-hydroxy coumarins have been reported and two antibiotics novobiocin[4] and coumerycin[5] with a 4-hydroxy coumarin structure, have been isolated. As a result, several methods for it's synthesis have been developed and also anti co-agulants like tromexan[6], warfarin[7], cyclimarol[8], marcoumar[9] came into market.

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O - Hydroxycoumarin derivatives have been advantageously employed in the detection and determination of many metals by gravimetric and spectrophotometric methods and can be also applied as metal complexing agents in the group of O-O donor system.

Some chalcones derived from coumarin derivatives, possess significant antimicrobial activity[10]. The growing potent literature of recent years demonstrate that chalcone being a very active synthon and coumarin also show activity such as anti co-agulant, antibacterial and insecticidal, prompted us to synthesize some new chalcone and it's metal complexes bearing 4 - hydroxy - 6 - methyl coumarin moiety.

EXPERIMENTAL

Synthesis of 3-[{3-(3'-chloro phenyl)}-prop-2-enoyl]-4-hydroxy-6 methyl-2H-chromen-2-one[11]

A mixture of 3-acetyl-4-hydroxy-6-methyl coumarin (2.52 g, 0.01M); 3'-chloro benzaldehyde (0.025 M) and piperidine (1 mL) were added into ethanol (50 mL). The reaction mixture was refluxed on water bath for 4 hrs., cooled and solid was separated. Then it was crystallised from suitable solvent, lemon yellow coloured compound was obtained; yield 72%, M. P. 223^{o}C.

Synthesis of bis [3-[{3-(3'-chloro phenyl)}prop-2-enoyl] 4-hydroxy-6- methyl-2H-chromen-2-one]copper(II)complex : $[Cu(C_{19}H_{12}O_4Cl)_2(H_2O)_2]$

Copper chloride solution (10.0 mL, 0.1M) diluted to 50 mL. and excess of ammonium hydroxide was added to get the pH between 10.5-11.0. It was refluxed with excess of alcoholic solution of 3-[{3-(3'-chloro phenyl)}prop-2-enoyl]4-hydroxy-6-methyl-2H-chromen-2-one (a) (0.1M) on a water bath for half an hour when light green precipitates of copper complex were obtained. The precipitates were filtered, washed with distilled water and dried at 100^{o}C. The complex was crystallized from DMF.

Similarly other metal complexes were prepared. The complexes did not show clear melting point. They charred at temperature above 300^o C.

Elemental analyses of metal complexes indicates that the metal : ligand (M : L) ratio is 1 : 2 for all the divalent metal ions. The conductivity of metal complexes was determined using Thoshniwal Conductivity Bridge. It was dissolved in DMF and conductivity was measured. Conductivity of the DMF alone was also measured and solution of the complexes in DMF with different concentration was also measured. The molar conductivity was calculated using the formula.

$$\text{Molar conductivity} = \frac{1000 \times K}{C}$$

Where, K = Conductivity of the sol. of the complexes in DMF and C = Concentration of the complexes.

RESULTS AND DISCUSSION

IR spectral analyses of metal complexes

The infrared spectra of the metal complexes were recorded on Shimadzu 435-IR Spectrophotometer between 4000-400 cm^{-1}.

The examination of the IR spectra of all the complexes reveals that –

(i) All the IR spectra have identical bands at their respective positions.

(ii) Most of the bands appeared in the spectra of ligand, are observed at the similiar position in the IR spectra of metal complexes.

(iii) Only the discermible difference in the IR spectra of metal complexes has been appeared. The band between 3200 - 3400 cm^{-1} due to - OH group in the spectra of ligands is less broader in the spectra of all the metal complexes. This might be due to complexation of metal ion. The less broadness might be due to water molecules associated with complex formation.

(iv) In addition, the IR spectra of complexes showed new bands between 590-500 cm^{-1} assigned to metal-ligand vibration (M-O).

Microbiological evaluation of synthesized compounds

Antimicrobial activity

Product	:	Chalcone and metal complexes
Method	:	Cup-plate agar diffusion method[12]

Gram Positive bacteria	:	*Staphylococcus aureus,* *Bacillus megaterium*
Gram Negative Bacteria	:	*Escherichia coli* *Proteus vulgaris*
Fungi	:	*Aspergillus niger*
Concentration	:	40 µg/mL
Solvent	:	N, N- Dimethylformamide
Standard drug	:	Amoxycillin, Ampicillin Ciprofloxacin, Erythromycin Griseofulvin

The antibacterial activity was compared with standard drugs viz. amoxycillin, ampicillin, ciprofloxacin, erythromycin and antifungal activity was compared with standard drug viz. griseofulvin. The zones of inhibition were measured in mm.

Antibacterial activity

The purified products were screened for their antibacterial activity by using cup-plate agar diffusion method. The nutrient agar broth prepared by the usual method, was inoculated aseptically with 0.5 mL of 24 hrs. old subculture of *S. aureus*, *B. megaterium, P. vulgaris* and *E. coli* in separate conical flasks at 40-50ºC and mixed well by gentle shaking. About 25 mL of the contents of the flask were poured and evenly spread in Petri dish (90 mm in diameter) and allowed to set for two hrs. The cups (10 mm in diameter) were formed by the help of borer in agar medium and filled with 0.04 mL (40 µg/mL) solution of sample in DMF.

The plates were incubated at 37ºC for 24 hrs and the control was also maintained with 0.04 mL of DMF in similar manner and the zones of inhibition of the bacterial growth were measured in millimeter and recorded in Table 3.

Antifungal activity

A. niger was employed for testing antifungal activity by cup-plate agar diffusion method. The culture was maintained on Sub rouse dextrose agar slants. Sterillised Sub rouse dextrose agar medium was inoculated with 72 hrs. old 0.5 mL suspension of fungal spores in a separate flask. About 25 mL of the inoculated medium was evenly spreaded in a sterillised petri dish and allowed to set for 2 hrs. The cups (10 mm in diameter) were punched in petri dish and loaded with 0.04 mL (40 μg/mL) of solution of sample in DMF. The plates were incubated at 30°C for 48 hrs. After the completion of incubation period, the zones of inhibition of growth in the form of diameter in mm was measured. Along the test solution in each petri dish, one cup was filled up with solvent, which acts as control. The zones of inhibition are recorded in Table 3.

Most of the compounds inhibit the growth of the above organism, which cause diseases in many plants. Hence, such type of compounds may find use as agricultural and garden bactericides and fungicides.

The anti microbial activities of tested compounds against different strains of bacteria and fungi are shown in Table 3. From Table 3, it can be concluded that all the compounds have displayed maximum activity against *P. vulgaris*. The compounds 1 and 4 are highly active against *E. coli*. The compounds 1, 4 and 5 showed very good activity against *B. megaterium*, while compounds 3 and 4 showed good activity against *S. aureus*. From the data of antifungal activity, it is observed that almost all the compounds are highly active against *A. niger* except compound 3, which exhibits moderate activity. Pertaining to metal ion, the toxicity of metal complexes has following trend –

$$Mn > Cu > Fe > Ni > Co$$

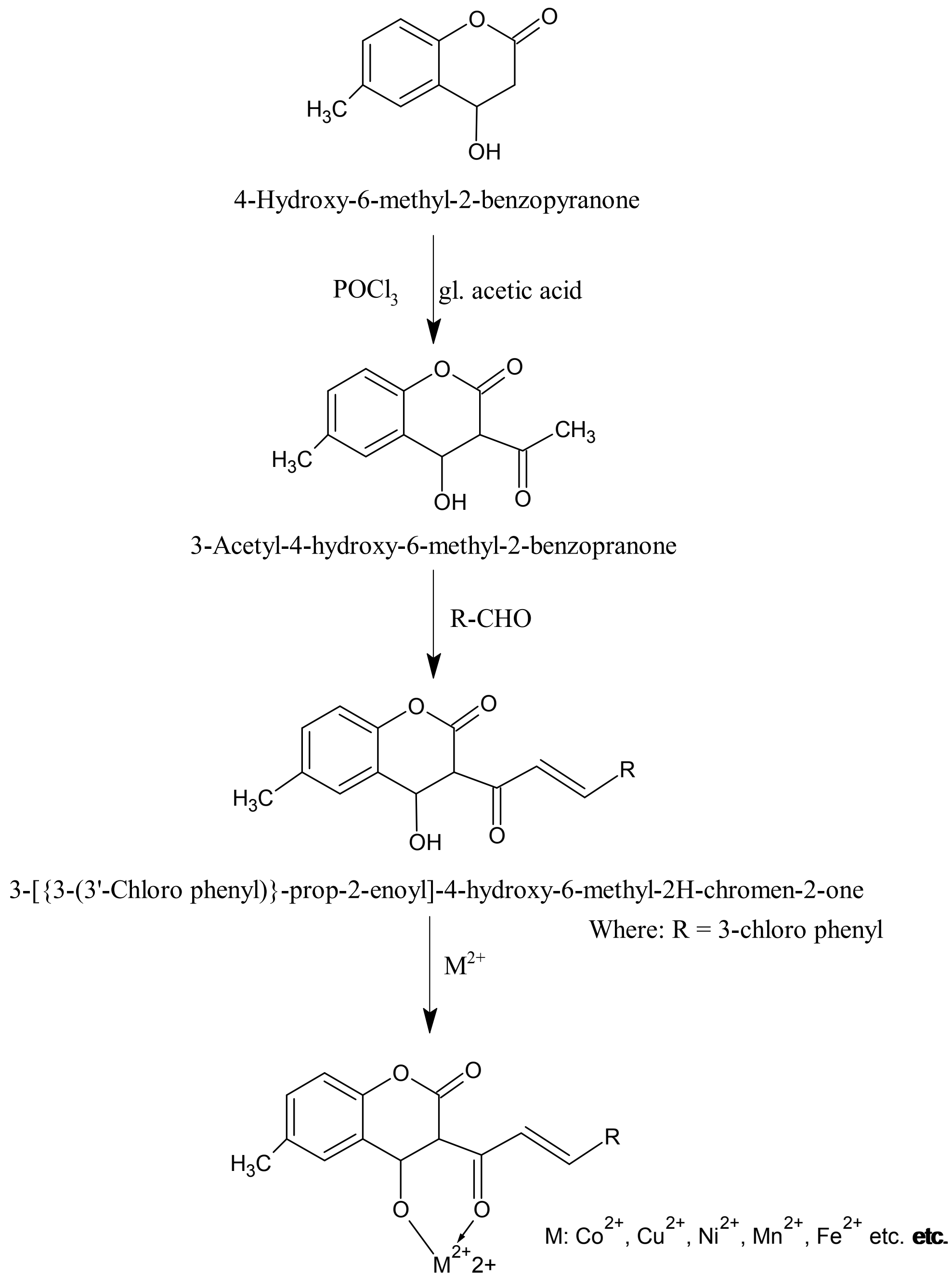
As compared to standard drug, ciprofloxacin, the compounds are less active, while other drugs have parallel activity.

Tabel 1 : Elemental and metal analysis of metal (II) complexes

S.No.	Molecular Formula	Mole. Weight	% of Carbon		% of Hydrogen		% of Chlorine		% of Metal		Conductivity
			Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	
1	$Cu[C_{19}H_{12}O_4Cl]_2(H_2O)_2$	776.54	58.72	58.60	3.60	3.50	9.14	9.00	8.18	8.00	7.60
2	$Ni[C_{19}H_{12}O_4Cl]_2(H_2O)_2$	771.71	59.08	59.00	3.62	3.50	9.20	9.10	7.60	7.40	8.70
3	$Co[C_{19}H_{12}O_4Cl]_2(H_2O)_2$	771.93	59.07	59.00	3.62	3.50	9.19	9.00	7.63	7.50	9.70
4	$Fe[C_{19}H_{12}O_4Cl]_2(H_2O)_2$	770.85	59.15	59.00	4.15	4.00	9.21	9.10	7.24	7.10	10.30
5	$Mn[C_{19}H_{12}O_4Cl]_2(H_2O)_2$	758.93	60.08	59.90	3.68	3.50	9.35	9.10	7.23	7.10	11.00

Tabel 2. IR spectral data of metal (II) complexes

S. No.	Metal complexes	Frequencies (cm^{-1})						
		Alkane	Aromatic	Ketone	Alkene	M-O Band	Ether	Halogen
		-CH	-CH	-C=O	CH=CH		C-O-C	C-Cl
1	$Cu[C_{19}H_{12}O_4Cl]_2(H_2O)_2$	2928	1511	1650	1609	590-500	1112	781
		2834	1249	1710				
		1443	832					
		1383						
2	$Ni[C_{19}H_{12}O_4Cl]_2(H_2O)_2$	2920	1562	1697	1612	590-500	1130	785
		2858	821	1715				
		1456	1222					
		1384						
3	$Co[C_{19}H_{12}O_4Cl]_2(H_2O)_2$	1922	1554	1689	1584	590-500	1138	782
		2853	1222	1715				
		1460	833					
		1375						
4	$Fe[C_{19}H_{12}O_4Cl]_2(H_2O)_2$	2920	1584	1669	1608	590-500	1137	778
		2850	1223	1710				
		1418	819					
		1376						
5	$Mn[C_{19}H_{12}O_4Cl]_2(H_2O)_2$	2933	1562	161	1602	590-500	1072	780
		2858	1227	1732				
		1464	821					
		1383						



Bis-[{3-(3'-chlorophenyl)}-prop-2-enoyl]-4-hydroxy-6-methyl-2H-chromen-2-one) M(II) complexes

Scheme

Table 3.Microbiological evaluation of synthesized compounds

Organism	Ligand	Compounds					Standard drugs				
	1	**2**	**3**	**4**	**5**	**6**	**Ampicillin**	**Amoxycillin**	**Ciprofloxacin**	**Erythromycin**	**Griseofulvin**
	Zone of inhibition (mm)										
E. coli	16	21	17	16	19	17	16	17	26	22	0
P. vulgaris	18	20	19	19	20	22	24	21	28	18	0
B. mega	17	19	16	18	19	20	20	22	23	10	0
S. aureus	14	16	17	19	18	16	25	29	24	22	0
A. niger	19	20	24	15	17	21	0	0	0	0	21

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