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Synthesis and antimicrobial activity of coumarin derivatives metal complexes: An in vitro evaluation

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***ABSTRACT**: Complexes of 3-[{-(3',4'-di methoxy phenyl) }-prop-2-enoyl]-4-hydroxy-6-methyl-2H-chromene-2-one with Cu(II), Ni(II), Fe(II), Co(II) and Mn(II) have been synthesized and characterized using elemental analysis, IR spectra and conductivity measurements. These studies revealed that they are having octahedral geometry of the type $[ML_2(H_2O)_2]$. In vitro antimicrobial 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. The compounds show net enhancement in activity on coordination of metals with ligand but moderate activity as compared to standard drugs.*

***Keywords**: antimicrobial activity, coumarin, metal complexes, structural study*

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Introduction

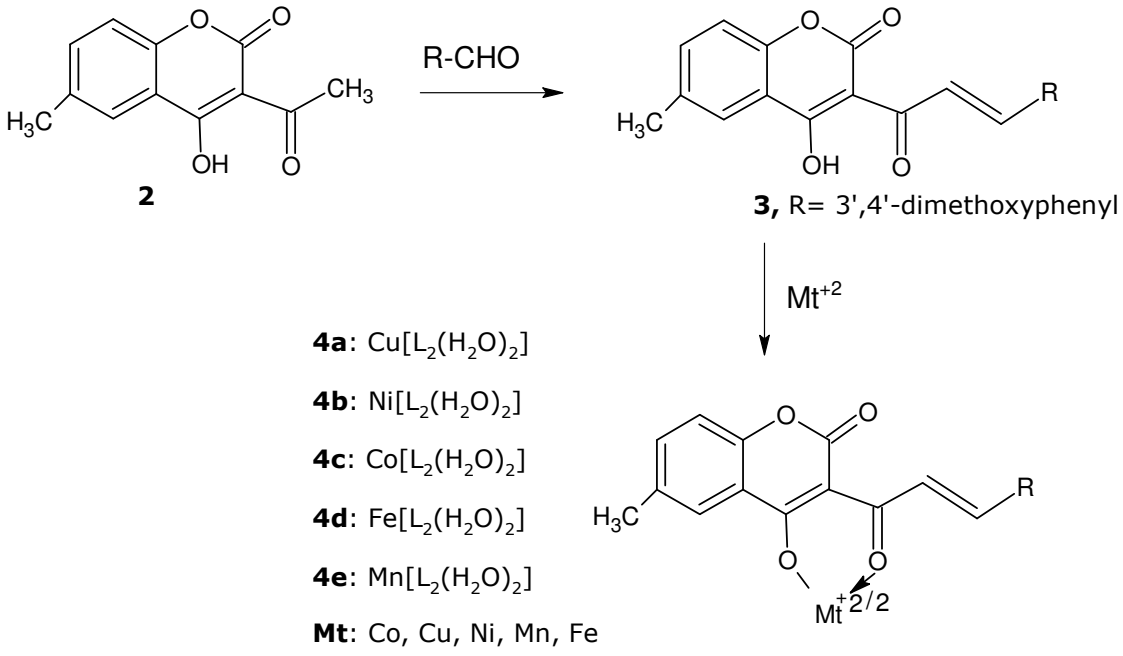
Coumarins contain the parent nucleus of benzo-α-pyrone and occur in plants of the families like Orchidaceae, Leguminaceae [1], Rutaceae, Umbellliferae and Labiatae. Some of the coumarins show distinct physiological photodynamic and bacteriostatic activities [2] and placed for many diverse uses [3]. Their chelating characteristics have long been observed and the bacteriostatic activity seems to be due to chelation.

The complexes of metallic salts are more potent and less toxic in many cases as compared to the parent drug [4] . These metal complexes are found to be interesting due to their biological applications like antifungal [5], antibacterial [6] and anti tumor [7] activity. Some chalcones derived from coumarin derivatives, possess significant antimicrobial activity [8]. Some 3-acetyl/acetoacetoacetyl-4-hydroxy benzopyran-2-ones have been reported as an anti-HIV agent [9]. Thus it was thought worthwhile to synthesize various novel metal complexes and to evaluate them for antimicrobial activity.

Chemistry

The growing potent literature of recent years reveals that chalcone a very active synthon and coumarin also show activity such as co agulant, bacterial and insecticidal prompted us to synthesize some new chalcone (III) by condensation of 3-acetyl 4-hydroxy-6-methyl- coumarin (2) with different aromatic aldehyde. These metal (II) complexes (4a-e) have been prepared by refluxing metal salt solution and the alcoholic solution of ligand (3) (Scheme 1).

Scheme 1



2

3, R= 3',4'-dimethoxyphenyl

4a: $Cu[L_2(H_2O)_2]$

4b: $Ni[L_2(H_2O)_2]$

4c: $Co[L_2(H_2O)_2]$

4d: $Fe[L_2(H_2O)_2]$

4e: $Mn[L_2(H_2O)_2]$

Mt: Co, Cu, Ni, Mn, Fe

Material and Methods

Chemistry

All the reagents were of AR grade. All the melting points were determined in open capillary tubes and are uncorrected. Infrared spectra (KBr) (νmax, cm^{-1}) were recorded on a Shimadzu 435 –IR Spectrophotometer. The metal and anions are estimated using standard procedure [10] .Elemental analyses are quite comparable with their structure. 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 were determined using Thoshniwal Conductivity Bridge.

3-[{3-(3',4'-Di methoxy phenyl)}-prop-2-enoyl]-4-hydroxy-6 methyl-2H-chromen-2-one

3-[{3-(3',4'-Di methoxy phenyl)}-prop-2-enoyl]-4-hydroxy-6 methyl-2H-chromen-2-one were prepared according to the reported method (3) [11]. A mixture of 3-Acetyl-4- hydroxy-6-methyl-2-benzopyranone (2.52 mg, 0.01M); 3', 4'-di methoxy benzaldehyde (4.15 mL, 0.025 M) and piperidine (1 mL) were added into ethanol (50 mL). The reaction mixture was refluxed on water bath for 4 h, cooled and solid was separated. Then it was crystallized from ethanol, reddish yellow coloured compound was obtained. (m.w. 362 gm, 70%), M.P. 217°C. ^{1}H NMR($CDCl_3$) δ ppm : 2.41 (s, 3H, C<u>H</u>$_3$), 3.85 (s, 2 x 3H, OC<u>H</u>$_3$), 8.21(d, 1H, -C<u>H</u>=CH), 7.97 (d, 1H, -CH=C<u>H</u>), 3.92 (s, 1H, -O<u>H</u>), 6.66 to 7.82 (m, 6H, Ar-<u>H</u>). Elemental analysis; found: C, 68.85%, H, 4.91%,O,26.23% for $C_{21}H_{18}O_6$ required C, 68.80%, H, 4.85%, O, 26.15%. IR bands νmax (KBr cm^-): 1670 (C=O), 1578 (CH=CH), 1031 (C-O-C), 1708 (C=O of δ- lactone ring), 3429 (OH).

Bis [3-[{3-(3', 4'-Di methoxy phenyl)}prop-2-enoyl] 4-hydroxy-6- methyl-2H-chromen-2-one]copper(II)complex [Cu($C_{21}H_{17}O_6$)$_2$(H_2O)$_2$] (4a)

Copper chloride solution (10.0 mL, 0.1 M) 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', 4'-Di methoxy phenyl)} prop-2-enoyl] 4-hydroxy-6-methyl-2H-chromen-2-one (3) (3.62 mg, 0.1 M) 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. (m.w. 827.54 gm 62%). Elemental analysis: found C, 60.90%, H, 4.59%, Cu, 7.67 % for [Cu($C_{21}H_{17}O_6$)$_2$] required C, 60.80%, H, 4.45%, Cu, 7.50%. IR bands νmax (KBr, cm^-): 1619 (C=O), 1587 (CH=CH), 1072 (C-O-C), 1732 (C=O of δ-lactone ring), 590-500 (Cu-O).

Similarly other metal complexes were prepared. The complexes did not show clear melting point. They charred at temperature above 290^{O}C.

Conductivity

The conductivity of metal complexes was determined by using Thoshniwal Conductivity Bridge. It was dissolved in DMF and conductivity was measured.

Conductivity of the DMF was measured and solution of the complexes in DMF with different concentration was measured.

The molar conductivity was calculated using the formula:

$$\textbf{Molecular conductivity} = \frac{1000 \times K}{C}$$

Where, K=Conductivity of the sol. of the complexes in DMF. C = Concentration of the complexes (10^{-3} M). The conductivity data are in (Table 1) and the data indicate that the complexes are non- electrolyte in nature [12].

IR Spectral analyses

The Infrared spectra of the metal complexes were recorded on Shimadzu 435-IR Spectrophotometer between 4000-400 cm^{-1} (see Table 2).

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 similar position in the IR spectra of metal complexes.

(III) The FT-IR spectra of all the complexes exhibited a broad peak around 3540 - 3320 cm^{-1} a sharp peak in the range 1623-1617 cm^{-1}. These peaks can be assigned to OH stretching and bending vibration, which indicate the presence of coordinated water molecule in the complexes. The peaks at 1623 and 1626 cm^{-1} corresponding to C=O have been shifted to +15-23 cm^{-1} the complexes, which indicate coordination through oxygen atom of the carbonyl group.

(IV) The peak at 1720 cm^{-1} is attributed to δ-lactone ring. In short, most of the bands appeared in the spectra of corresponding ligand are observed at the similar position in the IR spectra of metal complexes.

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

^{1}H NMR Spectral analysis

^{1}H NMR Spectra of the Ni(II) complex was recorded. Unfortunately, however due

to the presence of a metal ion, proton resonance was not effected and one could observe only broad peaks indicating the formation of the complex.

Pharmacology

The antimicrobial activity was assayed by Cup plate agar diffusion method [13] by measuring inhibition zones in mm. *In vitro* antimicrobial activity of all synthesized compounds and standard drugs have been evaluated against four strains of bacteria which include two Gram +ve bacteria such as *Staphylococcus aureus, Bacillus megaterium* and two Gram-ve bacteria such as *Escherichia coli, Proteus vulgaris* and one fungi *Aspergillus nige*r.

The antibacterial activity was compared with standard drugs viz. Amoxycillin, Ampicillin, Ciprofloxacin, Erythromycin and antifungal activity was compared with standard drug viz. Griseofulvin.

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 h old subculture of *S. aureus*, *B. megaterium, P. vulgaris*, and *E. coli* in separate conical flasks at 40-50 ^{O}C and mixed well by gentle shaking. About 25 mL of the contents of the flask were poured and evenly spread in petridish (90 mm in diameter) and allowed to set for two h. 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 37 ^{O}C for 24 h 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. Sterilized Sub rouse dextrose agar medium was inoculated with 72 h old 0.5 mL suspension of fungal spores in a separate flask. About 25 mL of the inoculated medium was evenly spread in a sterilized petridish and allowed to set for 2 h. The cups (10 mm in diameter) were punched in petridish and loaded with 0.04 mL (40 µg/mL) of solution of sample in DMF. The plates were incubated at 30^{O}C for 48 h. 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 petridish one cup was filled up with solvent which acts as control. The zones of inhibition are recorded in Table 3.

Results and Discussion

Antimicrobial activity of the synthesized compounds and standard drugs is given Table 3. From the Table it is clear that the zones of inhibition area is much larger for the metal chelates than the ligand. The increase in antimicrobial activity is due to faster diffusion of metal complexes as a whole through the cell membrane or due to the combined activity of the metal and ligand [14].

The antimicrobial activity of tested compounds against different strains of bacteria and fungi is shown in Table 3. From Table 3 it can be concluded that all the compounds have displayed maximum activity against *P. vulgaris*. The compound 4b is highly active against *E. coli*. The compounds 4b and 4e also showed very good activity against *B. megaterium*, while compounds 4a and 4c showed good activity against *S. aureus*. From the data of anti fungal activity it is observed that almost all the compounds are highly active against *A. niger* except compound 3, which exhibits moderate activity.

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

The substitution of phenyl ring by $-OCH_3$ have much more effect on the bactericidal and fungicidal activity of complex.

It has been observed that Cu (II) complex have much toxicity. This is expected because the copper salts are mostly used as fungicides.

Such increased activity of the metal complexes can be explained on the basis of Overtone's concept [15] and Tweedy's chelation theory [16].

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

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Table 1. Elemental and metal analysis of metal (II) complexes

Sr.	Molecular formula	Molecular Weight	% OF CARBON		% OF HYDROGEN		% OF METAL		CONDUCTIVITY
			Calcd.	Found	Calcd.	Found	Calcd.	Found	
4a	$Cu[C_{21}H_{17}O_6]_2(H_2O)_2$	827.54	60.90	60.8	4.59	4.5	7.67	7.5	7.7
4b	$Ni[C_{21}H_{17}O_6]_2(H_2O)_2$	822.71	61.26	61.1	4.61	4.5	7.13	7.08	9.3
4c	$Co[C_{21}H_{17}O_6]_2(H_2O)_2$	822.93	61.24	61.0	4.61	4.4	7.16	7.0	9.8
4d	$Fe[C_{21}H_{17}O_6]_2(H_2O)_2$	821.85	61.32	61.2	5.11	5.0	6.79	6.7	11.1
4e	$Mn[C_{21}H_{17}O_6]_2(H_2O)_2$	818.93	61.54	61.3	4.64	4.5	6.70	6.5	10.9

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

Sr.	Metal Complexes	Frequencies Cm^{-1}					
		Alkane	**Aromatic**	**Ketone**	**Alkene**	**M-O**	**Ether**
		$-CH_3$	**-CH**	**-C=O**	**CH=CH**	**Band**	**C-O-C**
4a	$Cu[C_{21}H_{17}O_6]_2(H_2O)_2$	2928	1511				
		2834	1249	1650	1609	590-500	1112
		1442	832	1710			
		1383					
4b	$Ni[C_{21}H_{17}O_6]_2(H_2O)_2$	2920	1562				
		2858	1296	1697	1612	590-500	1130
		1465	821	1715			
		1384					
4c	$Co[C_{21}H_{17}O_6]_2(H_2O)_2$	2922	1554				
		2853	1222	1689	1584	590-500	1138
		1460	833	1715			
		1375					
4d	$Fe[C_{21}H_{17}O_6]_2(H_2O)_2$	2920	1584				
		2850	1223	1669	1608	590-500	1137
		1418	819	1710			
		1376					
4e	$Mn[C_{21}H_{17}O_6]_2(H_2O)_2$	2933	1562				
		2858	1227	1610	1602	590-500	1072
		1464	821	1732			
		1383					

Table 3. Microbiological evaluation of compounds

Organism	Compounds						Standard drugs				
	3	**4a**	**4b**	**4c**	**4d**	**4e**	**Ampicillin**	**Amoxycillin**	**Ciprofloxacin**	**Erythromycin**	**Griseofulvin**
E. coli	18	22	24	18	19	23	16	17	26	22	0
P. vulgaris	19	21	27	19	20	22	24	21	28	18	0
B. mega	17	19	20	23	21	25	20	22	23	10	0
S. aureus	17	23	19	22	18	21	25	29	24	22	0
A. niger	19	20	24	18	22	21	0	0	0	0	21

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