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In Vitro Evaluation of Certain 6-Hetero Aryl-5-Hexene-2,4-Diones and Their Metal Complexes for Cytotoxic Activity

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Abstract

A series of 6-Hetero aryl-5-hexene-2, 4-diones (**1a-b**) and their Cu (II) complexes (**1c-d)** of ML_2 stoichiometry were synthesized by condensation of heterocyclic aldehydes with acetyl acetone through boric anhydride mediated mechanism. Boric anhydride act by blocking the medial methylene group of acetyl acetone and provides a new pathway other than facile Knoevenagel condensation. All the final structures were assigned on the basis of IR, ^{1}H NMR and mass spectra analysis. The test compounds were studied for short term *in vitro* cytotoxicity using Dalton's Lymphoma Ascites cell lines (DLA). The compound code **1c** and **1d** found to show significant cytotoxic action.

Keywords: ***ML_2 stoichiometry; IR spectra; ^{1} H NMR; Mass; Complexation; Dalton's Lymphoma Ascites Cell lines, Cytotoxicity***

1. Introduction

Curcuminoids (1,7-diaryl-1,6-heptadiene-3,5-diones), extracted from the rhizomes of the Indian medicinal plant turmeric (*Curcuma Longa Linn*), have been reported to possess significant antitumor activities[1]. The medicinal activity of curcumin has been known since ancient times and this molecule has been the object of several investigations in the field of biology, medicine and pharmacology over last decades. It has been revealed that the biological significance, especially medicinal importance of curcuminoids, is enhanced by complex formation with various inorganic species such as metal ions [2, 3]. Extensive literature is available with synthesis and characterization of metal chelates of synthetic curcuminoids. Earlier workers have reported the synthesis and characterization of various synthetic analogues of curcumin and related compound by using vanillin and other aromatic aldehydes [4, 5, 6]. Very few literatures were available with the use of heterocyclic aldehydes for the synthesis of curcumin related compounds. The present paper reported the synthesis, characterization and cytotoxic screening of a series 6-hetero aryl-5-hexene-2,4-diones and their copper complexes using heterocyclic aldehydes like Indole-4-carboxaldehyde and Pyridine- 4-carboxaldehye. Tumor is a mass of tissues which propagate rapidly, spread throughout the body and may ultimately cause death of the host. Chemotherapy is an effective treatment against various types of cancer either singly or in

combination with surgery or radiotherapy [7]. However, curcumin has been shown to exhibit antitumor activity and extremely safe even at high doses. It has not yet been approved as a therapeutic agent. The poor aqueous solubility, relatively low bioavailability, and intense staining colour of curcumin have been highlighted as major drawbacks [8]. This fostered our attempts to synthesize synthetic analogues of curcumin and their Cu (II) metal complexes against cancer as they are less likely show these problems. The presence of phenolic group together with conjugated β-diketone structure is suggested to be responsible for the anti tumor activity of curcumin related compounds. Previously heterocyclic derivatives aroused a considerable attention in the anti tumor activities[9].It was predict that a structural analogue of curcumin in which phenolic group is replaced by heterocyclic ring would display novel molecular templates with interesting biological activities in animal models. So our research proposal was the reaction between acetyl acetone with various heterocyclic aldehydes in a n-butyl amine induced basic medium to obtain synthetic analogues of curcumin in which hetero rings were attached to 1,3-diketone system through a olefinic linkage

The syntheses of 6- Hetero aryl-5-hexene-2, 4-diones **1(a-b**) described in this study were outlined in figure-1. The 1,3-diketones(**1a-b**) were synthesized by the condensation of the aldehydes with acetyl acetone as reported earlier[10,11].Their Cu complexes **1 (c-d)** were prepared by refluxing the obtained ligands with ethanolic solution of copper described in figure -2.The metal cation replaces enolic hydrogen of unsymmetrical *β*-diketone to form six member chelate ring.

2. Experimental

General

The chemicals were supplied by Sigma Aldrich (India). Melting points were determined by open tube capillary method and are uncorrected. The homogenicity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) using the solvent system Chloroform: Acetone (5:1). The spots were evaluated by exposure to UV light. Electronic spectra were recorded in methanol solution (10^{-4}) on a UV-1601 Schimadzu recording spectrophotometer.IR spectra were obtained on a Schimadzu 8101 A FTIR spectrophotometer. NMR on a Varian Mercury Plus 300MHz NMR spectrometer. Mass spectra were recorded on Jeol/Sx-102(FAB) mass spectrometer.

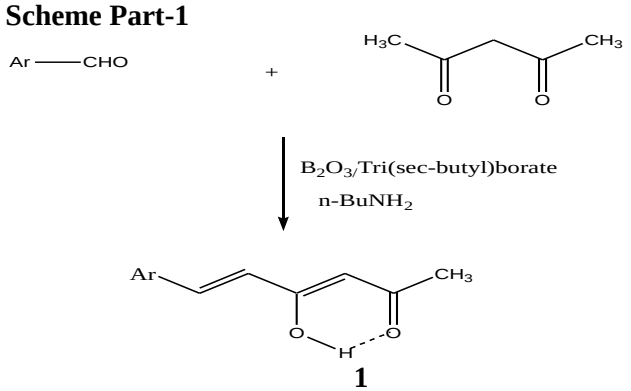
All biochemical investigations were done by using COBAS MIRA PLUS-S Auto analyzer from Roche Switzerland. Haematological tests were carried out in COBAS MICROS OT 18 from Roche. Newly added Hi-Tech instruments MAX MAT used for an auto analyzer for all biochemistry investigations in blood sample.

Chemistry

Synthesis of 6-Hetero aryl-5-hexene-2,4-diones (1a-b)

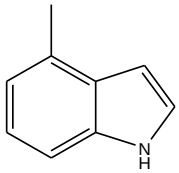
Acetyl acetone (0.075 mol) mixed with boric oxide was suspended in dry ethyl acetate (50 mL) containing tri(sec-butyl) borate (0.1 mol). To this mixture kept at *ca.* 0º C, a solution of heterocyclic aldehyde(0.025 mol) in dry ethyl acetate(15 mL) and n-butyl amine (0.5 mL) were added dropwise for 90 min with constant stirring. The stirring was continued for an additional period of *ca* .2 h and the solution was set aside overnight. The reaction mixture was then stirred for *ca*.1 h with hot ca. (50º C) hydrochloric acid (0.4 M,20 mL) and extracted repeatedly with ethyl acetate. The combined extracts was concentrated in vaccum and purified by column chromatography (silica gel mesh60-120). The yellow band developed in the lower region was recovered by successive elution with 5:1 v/v mixture of chloroform-acetone and the combined eluates on evaporation yielded the 6-Hetero aryl-5-hexene-2, 4-diones. The compounds were recrystallized from hot benzene to get chromatographically (TLC) pure material.

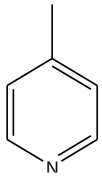
Scheme Part-1



1

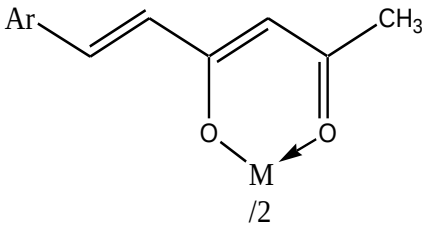
Compound Ar

1a 

1b 

Synthesis of metal complexes (1c-d)

Copper (II) complexes were prepared by the following general method. To a refluxing solution of the diketone (0.002 mol) in methanol (15 mL), an aqueous solution of metal (II) acetate (0.001 mol, 10 mL) was added and the reaction mixture was refluxed for *ca*. 3 h. and cooled to room temperature. The precipitated complex was filtered, washed with water, then with ethanol and dried in vaccum.



M=Cu(II)

2 Cytotoxicity study:

Anti-cancer evaluation using in-vitro [12,13] Dalton's lymphoma ascites method (DLA method). Cell viability was determined by trypan blue exclusion method. Viable cells suspension ($1x10^6$ cells in 0.1ml) was added to tubes containing various concentrations of the test compounds and the suspension. These assay mixtures were incubated for 3 hours at 37°C. Further cell suspension was mixed with 0.1 ml of 1% trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue color of trypan blue while live cells do not take up the dye. The numbers of stained and unstained cells were counted separately.

3. Result and Discussion

Chemistry

The 6-Hetero aryl-5-hexene-2,4-diones formed are crystalline in nature with sharp melting points. Physical and analytical data of unsaturated carbonyl compounds are presented in

Table-1

Table-1 Physical and analytical data of 6-Hetero aryl-5-hexene-2, 4-diones

Compound		Yield (%)	mp(ºC)	Elemental Analysis calculated (found)%					λ max (nm)
				C	H	0	N	S	
1a	$C_{14}H_{13}NO_2$	60	124	73.99 (73.42)	5.77 (5.83)	14.08 (14.02)	6.16 (6.73)		382 276
1b	$C_{11}H_{11}NO_2$	60	60	69.83 (69.65)	5.86 (5.65)	16.91 (16.84)	7.40 (7.86)		388 280

These compounds formed stable metal complexes with Cu^{2+}.Elemental analysis data of all these complexes (Table -2) corresponds to ML_2 stoichiometry.

Table-2 Physical and analytical data of Cu metal chelates of the 6-hetero aryl -5-hexene-2, 4-diones.

Compound	Yield (%)	Mp (ºC)	Elemental Analysis calculated (found)%					λ max (nm)
			C	H	0	N/S	M	
1c $(C_{14}H_{12}NO_2)_2Cu$	60	245	65.17 (65.32)	4.69 (4.46)	12.40 (12.54)	5.43 (5.52)	12.31 (12.16)	388 290
1d $(C_{11}H_{10}NO_2)_2Cu$	60	≥ 300	60.06 (60.14)	4.58 (4.42)	14.55 (14.58)	6.37 (6.34)	14.44 (14.52)	395 292

All the complexes behave as non electrolytes (< 15 Ω^{-1} in DMF) and do not contain the anion of the metal salt used for their preparation. The diketones and their metal complexes were characterized on the basis of their electronic, IR, NMR and mass spectral data.

Electronic spectra

The UV spectra of the compounds in 95% ethanol (10^{-3} M) showed two broad band's at ca. 388 and 285 nm respectively due to n→π* and π→ π* transitions. In the metal complexes the former bond showed a bathochromic shift (5-14 nm) indicating the involvement of dicarbonyl function in complexation [14,15].

Infrared spectra

The IR spectra of the diketones show two prominent band at *ca*.1700 and 1635 cm^{-1} assignable respectively to the chelated acetyl and cinnamoyl ν(C=O) vibrations[16]. The observed position and intensity of these bands indicate that the compound exist in strong intramolecular hydrogen bonding as in structure 1. The occurrence of an intense band in the region 3500-2500 cm^{-1} also support the hydrogen bonding.At least four prominent bands are observed in the region 1590-1400cm^{-1} presumably due to the various ν(C=C) vibrations. A medium intensity band at *ca* 931 cm^{-1} in the spectra of the compounds can be assigned to the trans-CH=CH-absorption. The important IR absorption and their probable assignments are given in Table-3.

In complexes the broad band in the region 3500-2500 cm^{-1} is cleared up which indicate that the chelated proton is replaced by metal ion during complexation. The region above 2000 cm^{-1} in complexes show several medium and weak intensity bands from aromatic and alkenyl ν(C=C) stretching vibrations. The absence of any strong bands in 1800-1650 cm^{-1} region is one of the characteristic feature of metal complexes of 1,3-diketones. But instead two new bands at *ca.* 1635 cm-1 and 1580ncm-1 is due to metal chelated carbonyl groups.The replacement of enolic proton by metal ion is also evident from the absence of broad free ligand band in the region 3200-2700 cm-1 in the spectra of complexes. The appearance of two medium intensity in the region 500-400 cm-1 in the metal complexes due to ν(M-O) vibration is a further evidence for complex formation[17].

^{1}H NMR

The ^{1}H NMR spectra of all the synthesized dicarbonyls show a single proton signal above 15 ppm assignable to the intramolecularly hydrogen bonded enolic proton. Other signals appearing are in the range δ 6.6--6.9 ppm, 7.72--8.34(alkenyl proton) and 7.2 -8.1 due to aromatic and hetero aromatic protons. The integrated intensities of aryl and alkenyl protons agree well with the structure.1

In the H NMR spectra of Cu(II) complexes of 1,3 diketones[18], the signal at 15.5 is absent due to the replacement of enolic proton during complexation. The decreased intensity around the central metal atom of the pseudo aromatic chelate ring system is further confirmed by shift of methane signal towards downfield of spectra. The integrated intensities of the various signals are in conformity with the structure 2. The characteristic chemical shifts of various protons are summarized in table 4

Mass spectra

Mass spectra of all the unsaturated diketones showed intense molecular ion $P^+/(P+1)^+$ peaks in conformity with their formulation .Peaks due to $(Ar\text{-}CH{=}CH\text{-}CO\text{-}CH_2)^+$, $(Ar\text{-}CH{=}CH)^+$, $(Ar\text{-}C{\equiv}C)^+$,$(Ar\text{-}CH{=}CH\text{-}C{\equiv}O)^+$ etc are characteristic of all the spectra[19]. FAB mass spectra of all the complexes showed relatively intense $P^+/(p+1)^+$ peaks in agreement with their ML_2 stoichiometry. The base peak in all the spectra are due to the ligand moiety and peaks due to fragments are sometimes more intense than molecular ion peak. They are easily identified because of the 2:1 natural abundance of ^{63}Cu and ^{65}Cu isotopes. The suggested formulation and structure of complexes clearly in agreement with the observed spectra of complexes. Spectral details of ligands and their complexes were given in table-3 and table -4 respectively.

Table.3 IR, ^{1}H NMR and Mass spectral data of the 6-hetero aryl -5-hexene 2,4-diones (1a-b)

Compound	IR spectral data cm^{-1}						H NMR spectral data Chemical shift (ppm)			Mass spectral data m/z
	C=O acetyl	C=O cinnamoyl	C-C Phenyl	asym C-C-C Chelate ring	β C-H Chelate ring	ϒ C-H Chelate ring	aryl	methyl	Alekenyl	
1a	1668	1635.5	1568.6	1518.8	1074.2	753.93	8.1	2.3	7.54	227,184, 170.9,143, 129.8,117,
1b	1665	1634.0	1598.2	1471.2	1184.5	785.51	7.27	2.1	8.4	189.08, 174.06 147.1,133,105

Table-4 IR, ^{1}H NMR and Mass spectral data of CU(II) complexes of 6-hetero aryl -5-hexene 2,4-diones (1c-d)

Compound	IR spectral data cm^{-1}							H NMR spectral data Chemical shift (ppm)		Mass spectral data m/z
	C=O acetyl	C=O cinnamoyl	C-C Phenyl	asym C-C-C Chelate ring	β C-H Chelate ring	ϒ C-H Chelate ring	M-O	methine	Alk-enyl	
1c	1635.2	1569.7	1516.5	1444.6	1086.4	754.75	419.8	5.3	6.85	516,360.5, 248.2,179 146.1,101.1
1d	1634.0	1580.2	1526.3	1458.9	1193.4	805.56	480	5.2	7.4	440,393.4 311.4,161.1, 136.8

Cytotoxic evaluation

It was found that at the concentration of 20µg/ml, compound 1c & 1d showing mild cytotoxic activity. At the concentration 50µg/ml all the compounds are showing significant cytotoxic action where the compound code 1c showing highest % of cell death (60.4±2.7). Out of different concentration 10, 20, 50, 100 & 200 µg/ml compound code 1c predominate with cytotoxic action with a cell death % of (95.9±0.5) at 200 µg/mL. Out of the other three derivatives compound code 1d predominate with a % of cell death 95.2±0.07 at a concentration of 200 µg/ml. The results obtained after screening is given in table-5.

Table-5 Cytotoxic action

Drug concentration (µg/mL)	% Cell Death			
	1a	1b	1c	1d
10	0	0	19.2±2.6	6.36±2.13
20	3.8±0	0	27.1±1.6	15.9±1.08
50	8.5±0.8	2.91±0	60.4±2.7	35.1±2.01
100	14±0.7	5.6±0	87.9±1.7	56.6±0.37
200	18±0.3	7.1±0.2	95.9±0.5	95.2±0.07

4. Conclusion

Two heterocyclic synthetic analogues of curcumin and their Cu(II) metal complexes were obtained in this research as a result of experiments. Analytical and spectral data of all ligands was in conformity with the expected structure. Analytical and spectral studies also prove monobasic bidentate coordination of Cu (II) with diketones as their enolic proton is replaced by metal cation.

Dalton's lymphomas are effectively used as interesting model for cancer research here, because it was found useful in preclinical system for evaluating new or known drug in the treatment of various cancers. It is a transplantable T cell lymphoma of spontaneous origin in the thymus of murine host. The cells were counted and further diluted so that the total cells would be 1×10^{6} cells in 0.1 ml. It is a good model system well characterized and reproducible. This study aims at % cancer cell death when treated with synthesized ligands at different concentrations. This was evaluated with the help of colour change that can induced by trypan blue dye. It was surprise to see all the synthesized compounds have significant cytotoxic action. It was also observed that, when these Indole and Pyridine ligands subjected to metal complexation with Cu, they found to show 3 fold increase in cytotoxic potential.

Plasma copper concentration increases in neoplastic and autoimmune diseases as an immune-mediated physiological response to these disease states. Treatment with copper complexes is a therapeutic support of this increase in plasma copper and the attendant distribution of copper to affected tissues to enable de-novo synthesis of copper-dependent enzymes required to bring about remission by re-establishing normal tissue function. So these Cu(II) complexes (1c-d) were considered as the successful outcome of present study. Further suitable derivatization of such compounds on other heteroaromatic nucleus and complexation with metals like Ni(II), Co(II) and Al(III) based up on our report hope to get more selective anticancer agents. Further future work can be done on the synthesized compounds to screen possible pharmacological actions. It will help to find out a lead compound with least side effect.

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