Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/6qpekb81

ANTITUMOR ACTIVITY OF 6-HETERO ARYL-5-HEXENE-2, 4-DIONES AND THEIR METAL COMPLEXES AGAINST DALTON'S ASCITIC LYMPHOMA IN MICE

K.L. Krishnakumar[a*], Manju R[b],

[a*]Department of Chemistry, Karuna College of Pharmacy, Palakkad, Kerala 679533, India
[b]Department of Chemistry, Karuna College of Pharmacy, Palakkad, Kerala-679533, India

***Corresponding Author:** K.L. Krishnakumar
*Professor & Principal, Department of Chemistry, Karuna College of Pharmacy. Palakkad, Kerala-679533. E mail: krishnakumarkl@gmail.com

Abstract
A series of 6-Hetero aryl-5-hexene-2, 4-diones (**1a-c**) and their Cu (II) complexes (1d-**f)** 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. Acute toxicity studies were performed initially in order to determine the safety of titled derivatives and the ED_{50} value was calculated 30 mg/kg. All the derivatives were screened for antitumor activity against Dalton's Ascitic Lymphoma in mice. All the new candidates at a dose of 30mg/kg showed a good antitumor activity against DLA bearing mice when compared to the standard 5-fluoro uracil.

Keywords: ML_2 stoichiometry; IR spectra; ^{1} H NMR; Mass; Complexation; Alanine amino Transferase; Alkaline Phosphatase;Aspartate amino Transferase,

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]. 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 antitumor screening of a series 6-hetero aryl-5-hexene-2,4-diones and their copper complexes using heterocyclic aldehydes likeIndole-3-carboxaldehyde,Pyridine-2-carboxaldehyde and Thiophene 3-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 [6,7]. 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-c**) described in this study were outlined in figure-1.The 1,3-diketones(**1a-c**) were synthesized by the condensation of the aldehydes with acetyl acetone as reported earlier[10].Their Cu complexes **1 (d-f)** 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 membered 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.

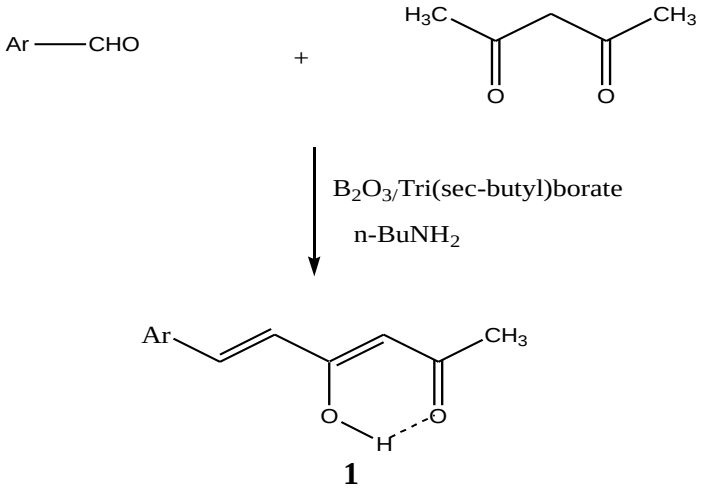
Chemistry

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

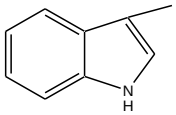
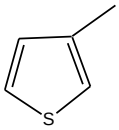
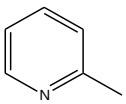
Acetyl acetone (0.075 mol)mixed with boric oxide was suspended in dry ethyl acetate(50 mL) containing tri(sec-butyl) borate(0.1 mol).To this mixture kept at *ca.* 0º C, a solution of heterocyclic aldehyde(0.025 mol) in dry ethyl acetate(15 mL) and n-butyl amine (0.5 mL) were added dropwise for 90 min 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

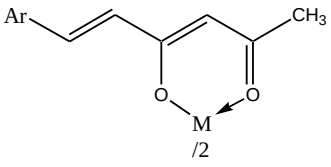


1

Compound	Ar
1a	
1b	
1c	

Synthesis of metal complexes (1d-f)

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

Pharmacological screening

LD_{50} Determination

The toxicological profile of the synthesized antitumor derivatives were determined by using standard LD_{50} method in mice[11]. Albino mice weighing 20-25 g were divided in 12 groups of 6 mice each. Administration of the tested compounds dissolved in 1% aqueous solution of Tween 80 at doses of 500,750 and 1000 mg/kg (body weight) were given intraperitoneally. The control groups were given vehicle only. The toxic symptoms, mortality rates and postmortem findings in each group were recorded 24 h post administration. LD_{50} of the tested compounds was calculated according to the following formula.

$$LD_{50} = Dm — \frac{\Sigma (Z \times d)}{n}$$

Where *Dm* = Largest dose which killed all animals, *Z* = mean of dead animals between two successive groups, d = constant factor between two successive doses, *n* = number of animals in each groups, Σ= sum of (z x d).

Induction of cancer using DLA cells

Male Swiss albino mice (20-25g) were produced from animal experimental laboratory, and used throughout the study. Dalton's Lymphoma ascites (DLA) cells were supplied by Amala cancer research center, Trissur, Kerala, India. The cells maintained in vivo in Swiss albino mice by intraperitoneally transplantation. While transforming the tumor.Cells to the grouped animal the DLA cells were aspirated from peritoneal cavity of the mice using saline. The cell counts were done and further dilution were made so that total cell should be 1×10^6, this dilution was given intraperitoneally. Allow the tumor grow in the mice for minimum seven days before starting treatments [12].

Treatment Protocol

Swiss Albino mice were divided in to nine group of six each. All the animals in eight groups were injected with DLA cells (1 x 106 cells per mouse) intraperitoneally, and the remaining one group is normal control group.

Group 1 served as the normal control.

Group 2 served as the tumor control. Group 1 and 2 receives normal diet and water.

Group 3 served as the positive control, was treated with injection fluorouracil at 20mg/kg body weight intraperitoneally.

Group 4-9 served as a treatment control group and was administered synthetic derivatives (**1a-f**) in a dose of 30mg/kg dissolved with 0.5ml DMSO administered through intraperitoneally.In this study, drug treatment was given after the 24 hrs of inoculation, once daily for 14 days. On day 14, after the last dose, all mice from each group blood were withdrawn from each mouse by retro orbital puncture or bleeding and the parameters such as hematological, serum enzyme, serum lipid, body weight, life span(%) and cancer cell count were determined.

Evaluation of clinical parameters

The fluid (0.1ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of ice cold normal saline or sterile phosphate buffer solution and 0.1 ml of tryphan blue (0.1 mg/ml) and total numbers of the living cells were counted using heamocytometer. The parameters such as haematological profile, serum enzyme level, lipid profile, change in the body weight and percentage increase of life span were evaluated[13]. In Hodgkin lymphoma, high cholesterol level and low triglyceride level has been reported. Hence we investigated this parameter for our current study.

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 $C_{10}H_{10}O_2S$	45	82	61.83 (61.65)	5.19 (5.42)	16.47 (16.22)		16.51 (16.53)	378 285
1c $C_{11}H_{11}NO_2$	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	
1a $(C_{14}H_{12}NO_2)_2Cu$	60	245	65.17 (65.32)	4.69 (4.46)	12.40 (12.54)	5.43 (5.52)	12.31 (12.16)	388 290
1b $(C_{10}H_9O_2S)_2Cu$	45	≥ 300	53.88 (53.67)	4.03 (4.08)	14.22 (14.32)	14.25 (14.34)	14.12 (13.59)	402 288
1c $(C_{11}H_{10}NO_2)_2Cu$	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, the signal at 15.5 is absent due to the replacement of enolic proton during complexation.The decresed 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. 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-c)

Compound	IR spectral data cm^{-1}						H NMR spectral data Chemical shift (ppm)			
	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	Mass spectral data m/z
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	1700.7	1605.2	1533.52	1421.5	1155.8	782.0	7.5	2.1	7.8	194.25, 179.8, 152,109.01, 137
1c	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 (1d-f)

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	Alek-enyl	
1d	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
1e	1601.3	1556.5	1520.0	1411.4	1019.3	770.60	444.9	5.1	6.8	450.03,397.3 32.9,302.4
1f	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

Antitumor activity

Effect on haematological parameters

The haematological parameters such as RBC, haemoglobin and platelets were decreased and WBC count was significantly increased in the DLA control group when compared to the normal control group (Table-5). Treatment with various synthetic compounds such as 1a, 1b, 1c, 1d, 1e and 1f at dose of 30mg/kg body weight significantly increases the haemoglobin content, RBC, platelets and significantly decreased the WBC count to about normal level. All these results suggest the antitumor nature of the synthetic derivatives. However, the standard 5-fluorouracil at the dose of 20 mg/kg body weight produced a better result in all these parameters.

Table-5 Effect of compounds (1a-f) on Hematological parameters

TREATMENT	Total WBC Cells /mlx10^3	Rbc Count Mill/cumm	Hb Gm/dl	PCV %	Platelets Lakhs/cumm
G1	10.20 ±1.58	4.56±0.98	12.80 ±1.52	13.80±2.40	3.55±0.70
G2	15.40 ±2.65^{a**}	2.22±0.38^{a**}	6.85± 0.88^{a**}	32.10±3.69^{a**}	1.28±0.52^{a**}
G3	11.08 ±1.98^{b**}	4.20±0.72^{b**}	11.3 ±1.12^{b**}	18.40±1.33^{b**}	2.60±0.75^{b**}
G4	12.23±2.25^{b**}	3.52±0.46^{b**}	10.05±0.90^{b**}	23.12±2.05^{b**}	2.40±0.78^{b*}
G5	12.40 ±2.32^{b**}	3.42±0.40^{b**}	10.38±1.08^{b**}	22.12±2.25^{b**}	2.26 ±0.74b**
G6	12.72 ±2.68^{b**}	3.20±0.28^{b**}	10.62±1.22^{b**}	20.89±1.92^{b**}	2.35 ±0.68b**
G7	12.66 ±2.75^{b**}	3.40±0.34^{b**}	10.40±1.16**	21.25±2.02^{b**}	2.45 ±0.80^{b**}
G8	12.85 ±2.80^{b**}	3.68±0.46^{b**}	10.72±1.12^{b**}	23.38±2.16^{b**}	2.35 ±0.86^{b**}
G9	12.65 ±2.75^{b**}	3.66±0.46^{b**}	10.74±1.12^{b**}	20.94±1.92^{b**}	2.44 ±0.80^{b**}

G1 – Normal Control, G2 – Cancer Control, G3 – Positive control, G4 – Treatment control (1a), G5 – Treatment control (1b), G6 – Treatment control (1c), G7 – Treatment control (1d), G8 – Treatment control (1e), G9 – Treatment control (1f).

PCV %=Packed cell volume

- All values are expressed as mean ± SEM for 6 animals in each group.
- **a – Values are significantly different from control (G_1) at P < 0.00
- **b – Values are significantly different from cancer control (G_2) at P < 0.001

Effect on biochemical parameters

The inoculation of DLA cells caused significantly increase in the level of total cholesterol, aspartate amino transferase, alanine amino transferase, alkaline phosphatase in the tumor control animals(**G2**), when compared to the normal group. The treatment with various synthetic compounds (**1a-f**) at dose of 30mg/kg body weight reversed these changes towards the normal level which showed in table 6.All

the value was found to be significant .The treatment with standard 5- Fluorouracil also gave similar results

Table-6 Effect of compounds (1a-f) on serum Enzymes and lipid proteins

Treatment	Cholesterol (mg/dl)	TGL (mg /dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
G_1	110.26±4.36	126.42±3.41	40.30 ±1.65	36.66 ±1.30	131.15 ±3.56
G_2	143.90±6.12^{a**}	210.30±7.22^{a**}	92.6±2.80^{a**}	62.25±2.72^{a**}	246.22±5.65^{a**}
G_3	121.24±5.23^{b**}	150.22±4.32^{b**}	58.36 ±1.90^{b**}	44.32±1.68^{b**}	162.30±4.20^{b**}
G_4	127.48±5.05^{b**}	168.55±5.06^{b**}	68.22 ±2.36^{b**}	50.10±1.92^{b**}	170.08±4.28^{b**}
G_5	126.39±5.16^{b**}	164.42±4.32^{b**}	70.12±2.23^{b**}	48.15 ±1.75^{b**}	180.15±4.32^{b**}
G_6	124.15±4.56^{b**}	170.15±4.68^{b**}	64.56±1.92^{b**}	49.06 ±1.22^{b**}	176.30±4.16^{b**}
G_7	123.45±4.92^{b**}	169.37±4.55^{b**}	66.25±2.05^{b**}	51.54 ±1.82^{b**}	174.27±4.22^{b**}
G_8	125.40±4.30^{b**}	162.20±3.98^{b**}	67.26±2.18^{b**}	47.58 ±1.60^{b**}	175.65±4.08^{b**}
G_9	123.40±4.30^{b**}	161.14±4.68^{b**}	63.46±1.62^{b**}	46.52 ±1.50^{b**}	172.60±4.26^{b**}

All values are expressed as mean ± SEM for 6 animals in each group.
TGL- Triglycerides, AST- Aspartate amino Transferase
ALT- Alanine amino Transferase
ALP- Alkaline Phosphatase
**a – Values are significantly different from control (G1) at $P < 0.01$
*b – Values are significantly different from cancer control (G2) at $P < 0.05$
**b – Values are significantly different from cancer control (G2) at $P < 0.01$

Effect on tumor growth

In the DLA tumor control group, the average life span of animal was found to be 50% where as synthetic drug such as 1a, 1b, 1c, 1d, 1e and 1f at dose of 30mg/kg body weight increase the life span to 74%, 72%, 70%, 76%, 74% and 75% respectively(Table-7). These values were significant. However the average life span of 5- Fluro uracil treatment was found to be 90%, indicating its potent antitumor nature. It was also supported by the significant reduction in packed cell volume and viable Tumor cell count in various synthetic compounds at dose of 30 mg/kg treatment when compared to the DLA tumor control.

Table.7 Effect of compounds (1a-f) on the life span, body weight and cancer cell count of tumor induced mice.

Treatment	Number of animals	% ILS Life span	Increase in Body weight grams	Cancer cell count ml X 10^6
G_1	6	>>30 days	2.54±0.56	-
G_2	6	50%	8.12±0.88^{a**}	2.80±0.42^{a**}
G_3	6	90%	3.80±0.48^{b**}	1.36±0.21^{b**}
G_4	6	74%	5.32±0.68^{b**}	1.50±0.25^{b**}
G_5	6	72%	5.40±0.70^{b**}	1.70±0.34^{b**}
G_6	6	70%	5.12±0.55^{b**}	1.62±0.22^{b**}
G_7	6	76%	5.18±0.50^{b**}	1.60±0.24^{b**}
G_8	6	74%	5.23±0.48^{b**}	1.63±0.28^{b**}
G_9	6	75%	5.02±0.60^{b**}	1.48±0.26^{b**}

All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from control (G1) at $P < 0.01$
*b – Values are significantly different from cancer control (G2) at $P < 0.05$
**b – Values are significantly different from cancer control (G2) at $P < 0.01$

4. Conclusion

Three heterocyclic synthetic analogues of curcumin and their Cu 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.

In DLA tumor bearing, a regular rapid increase in Ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in Ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells Treatment with various synthetic compounds such as **1a, 1b, 1c, 1d, 1e** and **1f** at dose of 30mg/kg body weight inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the lifespan of animals. It may be concluded that various synthetic compounds by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DLA bearing mice. Thus various synthetic compounds such as (**1a-f**) at dose of 30mg/kg have antitumor activity against DLA bearing mice. Usually, in cancer chemotherapy the major problems that are being encountered are of myelo suppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or haemoglobin percentage and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions. Treatment with all synthetic compounds such as (**1a-f**) brought back the hemoglobin (Hb) content, RBC and WBC count more or less to normal levels significantly. This clearly indicates that 6-Hetero aryl-5-hexene-2, 4-diones and their Cu complexes possess protective action on the haemopoietic system. It was reported that the presence of tumor in the human body or in the experimental animals is known to affect may function of the liver. The significantly elevated level of total cholesterol and enzyme level in serum of tumor inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversal of these changes towards the normal by various synthetic drugs treatments. In the present study, the biochemical examination of DLA inoculated animals showed marked changes indicating the toxic effect of the tumor. The normalization of these effects observed in the serum treated with various synthetic compounds such as (**1a-f**) supported the potent antitumor and hepatoprotective effect of the derivatives. Based on the close examination on substitution of titled molecular templates, Cu (II) complexes like 1d, 1e and 1f produced a considerable increase in life span of tumor –bearing mice compared to that of diketone ligands (1a, 1b, and 1c).The percentage increase in life span of tumor –bearing mice were 74, 72 and 70% by administration of 1a, 1b and 1c respectively. Whereas their Cu (II) chelates produced 76, 74 and 75% increase in life span.

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 (1d,1e, and 1f) 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.

5. Acknowledgement

Authors acknowledge the help of, SAIF IIT, Mumbai for carrying out the spectral analysis

6. References

1) John VD, Krishnankutty K. Antitumour studies of aluminium complexes of synthetic curcuminoids. Main Group Metal Chemistry. 2010 Jun;33(3):157-66.

2) Rao TS, Basu N, Siddiqui HH. Anti-inflammatory activity of curcumin analogues. Indian journal of medical research. 2013 Apr 1;137(4).
3) Sorenson JR. Copper complexes for therapy of cancer and autoimmune diseases. InCopper and Zinc in Inflammatory and Degenerative Diseases 1998 (pp. 113-124). Dordrecht: Springer Netherlands.
4) Ummathur MB, Krishnan A, Ukken MP. Metal complexes of unsaturated polycarbonyl compounds derived from benzoyl acetone and aromatic aldehydes
5) Paul M, Krishnankutty K. Synthesis and Characterisation of Co (II), Ni (lI) and Cu (II) Complexes of Some 6-Aryl-5-bexene-2, 4-diones. Asian Journal of Chemistry. 2002 Apr 1;14(2):949.
6) Yao X, Gu X, Jin S, Shi K, Gao X, Wang Q, Zhao J, Zhang H, Lai X. Anticancer and anti-inflammatory effect of diosmin against dalton ascitic lymphoma induced leukemia. Journal of Oleo Science. 2021;70(5):665-73.
7) Shakeri A, Panahi Y, Johnston TP, Sahebkar A. Biological properties of metal complexes of curcumin. BioFactors. 2019 May;45(3):304-17.
8) Preetha A, Sherin GT , Ajaikumar BK, Chitra S,Harikumar BK,Bokyung S,Sheeja TT,Krishna M, Indira KP,Rajasekharan KN, Bharath BA(2008) Biochem. Pharmacol.76:1590-1611
9) Cheng MS, Yang YH,Wang H,Liao N,Wang J,ChenH(2009)Eur.J.med.Chem45:1808-1812
10) Balaji SN, Ahsan MJ, Jadav SS, Trivedi V. Molecular modelling, synthesis, and antimalarial potentials of curcumin analogues containing heterocyclic ring. Arabian Journal of Chemistry. 2019 Dec 1;12(8):2492-50.
11) Kazakova O, Lipkovska N, Barvinchenko V. Keto-enol tautomerism of curcumin in the preparation of nanobiocomposites with fumed silica. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2022 Sep 5;277:121287.
12) Manam VK, Subbaiah M. Antitumor Efficacy of Silver Nanoparticles Biosynthe-sized from Marine Red Seaweed Halymenia porphyroides Boergesen on Dalton's Lymphoma Ascites in Mice. International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN). 2021 Dec 1;14(6):5683-90.
13) Balakumar P, Alqahtani T, Alqahtani A, Lakshmiraj RS, Singh G, Rupeshkumar M,Thangathirupathi A, Sundram K. A unifying perspective in blunting the limited oral bioavailability of curcumin: a succinct look. Current drug metabolism. 2022 Sep 1;23(11):897-904.
14) Rodrigues FC, Kumar NA, Thakur G. The potency of heterocyclic curcumin analogues: An evidence-based review. Pharmacological Research. 2021 Apr1;166:105489.
15) Omoregie HO, Eseola AO, Akong RA. Mixed ligand complexes of copper (II) with benzoyltrifluoroacetone, 1, 10-phenanthroline and 2, 2′-bipyridine: structure, spectroscopic and antimicrobial properties. Journal of Molecular Structure. 2022 Feb 15;1250:131826.
16) Bellamy LJ. Alkanes. InThe Infrared Spectra of Complex Molecules: Volume Two Advances in Infrared Group Frequencies 1980 (pp. 1-23). Dordrecht: Springer Netherlands.
17) Payton F, Sandusky P, Alworth WL. NMR study of the solution structure of curcumin. Journal of natural products. 2007 Feb 23;70(2):143-6.