APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2006; **20**: 477–482
Published online 23 June 2006 in Wiley InterScience
(www.interscience.wiley.com) DOI:10.1002/aoc.1089

Antitumour activity of synthetic curcuminoid analogues (1,7-diaryl-1,6-heptadiene-3,5-diones) and their copper complexes

V. D. John and K. Krishnankutty*

Department of Chemistry, University of Calicut, Kerala 673635, India

Received 10 January 2006; Revised 13 February 2006; Accepted 19 April 2006

Four new curcuminoid analogues, 1,7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione, 1a; 1,7-di(2-furyl)-1,6-heptadiene-3,5-dione, 1b; 1,7-di(2-naphthyl)-1,6-heptadiene-3,5-dione, 1c; 1,7-bis(2-chlorophenyl)-1,6-heptadiene-3,5-dione, 1d; and their copper(II) complexes of ML_2 stoichiometry were synthesized and characterized by UV, IR, ^1H NMR, ESR and mass spectral data. The compounds were investigated for their possible cytotoxic and antitumour activities. It was found that copper chelates are remarkably active compared with free curcuminoid analogues. All the compounds were found to be cytotoxic towards Ehrlich ascites carcinoma cells and cultured L929 (lung fibroblast cells). In the case of culture studies, concentrations needed for 50% cell death were around 5 μg/ml for copper complexes and 10 μg/ml for curcuminoid analogues. Copper complex of 1a with hydroxyl group in the phenyl ring was found to be most active towards L929cells (1 μg/ml produced 43.3 ± 1.3% cell death). Compound 1b, which possesses a furyl ring system, was found to show least activity towards increase in life span of tumour-bearing mice (increase in life span 39.31%). Copper chelates of all curcuminoid analogues showed a significant reduction ($p < 0.001$) of solid tumour volume in mice. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: synthetic curcuminoids; copper complexes; IR; NMR; mass spectra; cytotoxicity; antitumour activity

INTRODUCTION

The rhizomes of the traditional Indian medicinal plant turmeric (*Curcuma longa* Linn) have been used for centuries as a colouring agent and spice in many food preparations. Turmeric and its active chemical constituents, curcuminoids, have been reported to possess a number of medicinal uses, particularly in the treatment of inflammation[1,2] arthritis[3,4] and cancer.[5,6]

Structurally curcuminoids are 1,7-diaryl-1,6-heptadiene-3,5-diones (1,7-diarylheptanoids) and are known to form metal complexes similar to other 1,3-diketones (Fig. 1).[7] It has been reported that metal complexation of these α,β-unsaturated 1,3-diketones leads to dramatic changes in their biochemical activities,[8–11] including antitumour activity. As a part of our studies[7,12] on chemical and biochemical properties of synthetic analogues of active chemical compounds of such indigenous valuable green medicines and their metal complexes, the present study reports the synthesis, structural characterization, and cytotoxic and antitumour activities of four new curcuminoid analogues, **1a–d**, and their copper(II) complexes.

*Correspondence to: K. Krishnankutty, Department of Chemistry, University of Calicut, Kerala 673635, India.
E-mail: john_vd@rediffmail.com

MATERIALS AND METHODS

Cells

Ehrlich ascites carcinoma (EAC) cells were obtained from the Cancer Research Institute, Mumbai, India; Dalton's lymphoma ascites (DLA) cells, from the Cancer Institute, Adayar, India and L929 (lung fibroblast cells) from National Facility for Animal Cell and Tissue Culture, Pune, India. EAC and DLA were maintained as ascites tumours in Swiss albino mice. L929 cells were maintained in culture using minimum essential medium (MEM) containing 10% goat serum and antibiotics.

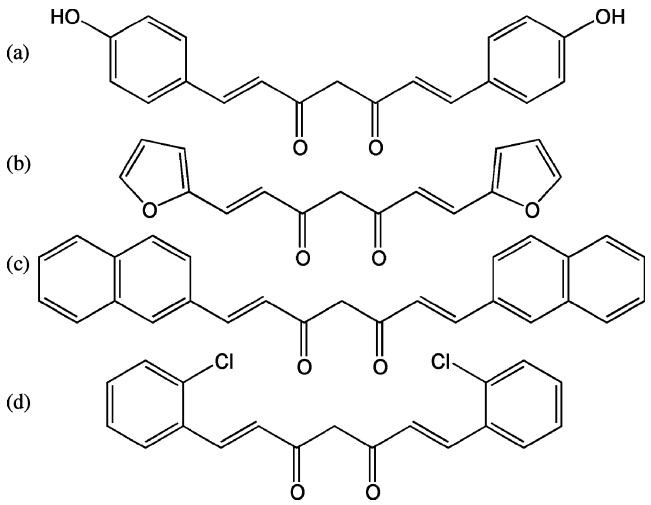


Figure 1. Structure of curcuminoid analogues. (a) (HL^1) 1,7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione. (b) (HL^2) 1,7-di(2-furyl)-1,6-heptadiene-3,5-dione. (c) (HL^3) 1,7-di(2-naphthyl)-1,6-heptadiene-3,5-dione. (d) (HL^4) 1,7-bis(2-chlorophenyl)-1,6-heptadiene-3,5-dione.

Animals

Swiss albino mice were purchased from Veterinary College, Thrissur, Kerala. They were fed with normal mouse chow (Lipton India) and water *ad libitum*.

Synthesis of the 1,7-diaryl-1,6-heptadiene-3,5-diones

The compounds were prepared by the condensation of aldehydes (4-hydroxybenzaldehyde, 2-furfuraldehyde, 2-naphthaldehyde and 2-chlorobenzaldehyde) with acetylacetone–boric oxide complex in ethyl acetate medium in the presence of tributyl borate and *n*-butylamine as reported earlier.[13] The product was purified by column chromatography over silicagel (60–120 mesh) using 2:1 (v/v) chloroform:acetone mixture as the eluant and recrystallized twice from hot benzene to get pure crystalline material.

Preparation of copper complexes

The Cu(II) complexes were prepared as given below. A methanolic solution of copper(II) acetate (25 ml, 0.001 mol) was added with stirring to a solution of diketone (25 ml, 0.002 ml) in methanol and refluxed gently for ~1 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered and recrystallized from hot methanol.

Short-term cytotoxic assay

In vitro cytotoxic studies were carried out using the diketone and the copper(II) complexes dissolved in minimum quantity of DMSO. The tumour cells aspirated from the peritonial cavity of tumour bearing mice were washed with PBS (phosphate-buffered saline). The cell suspension (1×10^6 cells in 0.1 ml) was added to tubes containing various concentrations (1–50 μg/ml) of the compounds and volume was made up to 1 ml using PBS. The mixture was incubated for 3 h at 37 °C and the percentage of dead cells were evaluated by trypan blue dye exclusion method.[14]

Determination of cytotoxicity of compounds in tissue culture

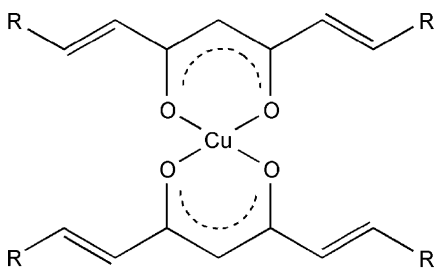
L929 cells were used for tissue culture studies. The cells (5×10^3 cells/well) were plated in a 96-well flat bottom plates and incubated at 37 °C in 5% CO_2 atmosphere. After 24 h incubation various concentrations (1–10 μg/ml) of compounds were added to the wells and incubated for a further period of 48 h. After incubation the cells were stained with crystal violet and cytotoxicity was calculated by measuring optical density at 570 nm after eluting the dye from the cells.

Determination of tumour-reducing activity

Groups of Swiss albino mice (six per group) were injected intraperitonially (i.p.) with Ehrlich ascites tumour cells (1×10^6 cells/animal). The animals were injected (i.p.) with test compounds (200 μmol/kg body weight) suspended in gum accasia and the injections were continued for 10 days. The control animals received only the vehicle. The mortality rate of mice were noted in each group and the percentage increase in life span (% ILS) of the treated group was calculated using the formula $\%ILS = 100\ (T - C)/C$, where T is the mean survival time of treated mice and C is that of control expressed in days.

Determination of the effect of compounds on solid tumour development

The effect of various compounds on solid tumour development was studied using Swiss albino mice. Groups of mice (six per group) were injected subcutaneous with DLA cells (10^6 cells in 0.1 ml) on the right-hind limbs. One group was kept as control and other groups were injected (i.p.) with test compounds (200 μmol/kg body weight) and the injections



2

Figure 2. Structure of the copper complexes (**2**) of 1,7-diarylheptanoids.

DOI: 10.1002/aoc

were continued for 10 days. Tumour diameter was measured every third day for one month and tumour volume calculated using the formula, $V = 4/3\pi r_1 r_2^2$ where r_1 and r_2 are the minor and major radius respectively.[14]

Analytical methods

The complexes were analysed for their metal contents by using an AAS (Perkin Elmer 2380). Carbon and hydrogen percentages were determined by micro analysis (Heraueus Elemental analyzer) from CDRI Lucknow, India. The UV spectra of the compounds were recorded on a Shimadzu UV–vis 1601 spectrophotometer. The IR spectra were recorded using KBr discs on 8101 Shimadzu FTIR spectrophometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian 300 NMR spectrometer. The FAB mass spectra were recorded on a Jeol SX-102 mass spectrometer using argon (6 kV,10 mA) as the FAB gas and meta-nitrobenzyl alchohol (NBA) as the matrix from CDRI Lucknow, India and ESR spectra of copper complexes were recorded using Varian E-12 ESR spectrometer at 77 K. The TGs of compounds were recorded using Perkin Elmer Thermal Analyser from STIC, Cochin University.

RESULTS AND DISCUSSION

Structural characterization of the 1,7-diaryl-1,6-heptadiene-3,5-diones

The analytical data of the 1,7-diaryheptanoids (**1a–d**) given in Table 1 agree well with their formulation. Further the FAB mass spectra of compounds show intense molecular ion $(P + 1)^+$ peaks. Peaks due to elimination of O, OH, H_2O and $C_3HO_2^-$ species from the molecular ions are characteristic of the spectra (Table 1). Structure **1** of the compounds is clearly established from a comparison of the observed UV, IR and ^{1}H NMR spectral data (Table 1) with the reported spectral data[7] of related compounds.

Thus the UV spectra of the compounds in methanol show two absorption maxima corresponding to $n \rightarrow \pi^*$ transition (376–408 nm) and $\pi \rightarrow \pi^*$ transition (260–266 nm). The IR spectra of compounds show a strong band at ~1620 cm^{-1} assignable to intramolecularly hydrogen bonded carbonyl function (Table 1) and a broad band in the range 2600–3800 cm^{-1}. The absence of any band assignable to normal or α,β-unsaturated carbonyl group in the region 1640–1740 cm^{-1} of the spectra indicates that these compounds exist entirely in the enolic form. The ^{1}H NMR spectra of all the compounds (Table 1) displayed a one proton singlet in the low field at ~16 ppm and another singlet at $\delta \approx 5.9$–6.9 ppm assignable respectively to the strong intramolecularly hydrogen bonded enolic and methine protons respectively.[7,15] The compound **1a** shows another singlet at $\delta \approx 10.04$ ppm due to the phenolic group. The *trans* oriented alkenyl protons can be identified from the position of their signals ($\delta \approx 7.9$ ppm) and from their observed J values ($J \approx 16$ Hz).

Structural characterization of copper complexes

1,7-Diarylheptanoids form well defined crystalline complexes with Cu^{2+} ions and their elemental analytical and mass spectral data (Table 2) clearly suggest their $[ML_2]$ stoichiometry. The thermograms of the complexes in air showed a single-stage decomposition pattern. The observed weight loss correspond to the elimination of the two ligand moieties in the temperature range 350–400 °C in agreement with the formulation of the complexes.

All the complexes behave as non-electrolytes (specific conductance <15 Ω^{-1} cm^{-1} in DMF) and do not contain the anion of the metal salt used for their preparation. The complexes show normal paramagnetic moment of ~1.75 BM. The spectral data of the complexes are in conformity with structure **2** of the metal complexes (Fig. 2). In the IR spectra of metal chelates, the band due to hydrogen bonded carbonyl function at ~1620 cm^{-1} disappeared and instead a strong band assignable to the stretching of the coordinated carbonyl moiety[7] appeared at ~1590 cm^{-1} (Table 2). Additional bands appear at ~475 and ~420 cm^{-1} assignable to ν(M-O) vibrations.[7] The band due to trans −CH=CH- remains as such in the complex at ~970 cm^{-1}. The replacement of enolic proton of the ligands by metal ion is further confirmed by the disappearence of the signal at δ ~16 ppm in the ^{1}H NMR spectra of the complexes. The methine signals shifted towards the down field of the spectra indicating the decreased electron density around central carbon atom of the pseudo aromatic metal chelate ring system. In the FAB mass spectra of copper chelates, peak due to $[CuL_2]^+$ are prominent. Peaks due to $[CuL]^+$, $[Cu_2L]^+$, L^+ and fragments of L^+ are also present in the mass spectrum of complex with considerable intensity. Fragments containing copper are easily identified because of 3 : 1 natural abundance of ^{63}Cu and ^{65}Cu isotopes.

The ESR spectra of the copper (II) complexes of (**1a**) and (**1c**) were measured at 77K in DMF solution. The observed $g_\parallel$, $g_\perp$, $A_\parallel$ and $A_\perp$ values are given in Table 3.

The g-values are comparable to that reported for copper acetylacetonates[16,17] for which $g_\parallel = 2.264$ and $g_\perp = 2.036$. This suggests extensive delocalization in the chelate ring and significant covalent character for the metal ligand bonds.

Cytotoxicity

The results of short term *invitro* cytotoxicity of the diketones and their copper(II) complexes towards Ehrlich ascites cells (Table 4) indicate that metal chelation enhance the cytotoxicity of compounds considerably.

The results of the cytotoxicity of the 1,7-diarylheptanoids and their copper complexes towards cultured L929 cells given in Table 5 also indicate that the copper chelates are more cytotoxic than the respective 1,7-diarylheptanoids. Compound **1b**, which possesses a furyl ring system (16.8 ± 1.4% cell death at 1 μg/ml concentration), is the least active compound and the copper complex of **1a** with a hydroxyl

DOI: 10.1002/aoc

Table 1. Elemental analysis, UV, IR, ^{1}H NMR and mass spectral data of the 1,7-diarylheptanoids (**1a–d**)

Compound	Elemental analysis found/calcd (%) C	H	UV data (λ_{max}, nm)	IR spectral data (cm^{-1}, ν C–O chelate)	^{1}H NMR spectral data chemical shift (ppm) Enol	Methine	Alkenyl	Aryl	Mass spectral data, *m/z*
1a	73.8	5.1	263	1616	17.2	6.8	6.8	6.8–7.3	308, 290, 215, 189, 161, 147,
(HL^1)	(74.0)	(5.1)	408				8.2		119, 118
1b	83.8	5.9	267	1614	15.6	6.5	6.5	6.6–8.1	257, 221, 191, 179, 163, 147,
(HL^2)	(84.1)	(6.0)	391				7.2		136, 121
1c	85.7	5.1	260	1620	16.0	6.9	6.7	7.5–8.2	377, 249, 223, 195, 181, 120
(HL^3)	(86.2)	(5.3)	386				8.5		
1d	67.1	4.3	266	1620	16.1	5.8	6.6	7.1–7.7	345, 329, 289, 233, 207, 178, 165,
(HL^4)	(66.2)	(4.1)	376				8.0		137, 136, 125

Table 2. Elemental, analytical and spectral data of the Cu(II) complexes of the 1,7-diarylheptanoids

Cu(II) complexes	Molecular formula	Elemental analysis (found/calcd; %) C	H	Cu	IR spectral data (cm^{-1}) νC=O	νM–O	Mass spectral data, *m/z*
1a	$[Cu(L^1)_2$	67.0	4.2	9.1	1588	465	670, 484, 364, 298, 170, 120
	$CuC_{38}H_{30}O_8$	(67.3)	(4.4)	(9.3)		419	
1b	$[Cu(L^2)_2]$	60.9	3.5	10.9	1586	470	637, 576, 460, 289, 136, 120
	$CuC_{30}H_{22}O_8$	(62.7)	(3.8)	(11.0)		419	
1c	$[Cu(L^3)_2]$	78.1	4.8	7.7	1561	479	814, 559, 439, 307, 289, 136, 120
	$CuC_{54}H_{38}O_4$	(79.6)	(4.6)	(7.8)		422	
1d	$[Cu(L^4)_2]$	61.2	3.7	8.1	1599	465	749, 527, 391, 307, 178, 154, 120
	$CuC_{38}H_{26}O_4Cl_4$	(60.6)	(3.4)	(8.4)		420	

Table 3. ESR parameters of some Cu(II) complexes in DMF at 77 K

Cu(II) complex	$g_\|$	$g_\perp$	$A_\| \times 10^{-4}$ cm^{-1}	$A_\perp \times 10^{-4}$ cm^{-1}
(**1a**) HL^1	2.2789	2.0695	167.06	46.1
(**1c**) HL^2	2.2875	2.0694	162.78	45.4

group in the phenyl ring (43.3 ± 1.3% cell death at 1 μg/ml concentration) is the most active compound.

Effect of compounds on ascites tumour reduction

In Table 6 the effects of the compounds on ascites tumour reduction are given. All the compounds when administered (i.p.) could produce significant increase ($p < 0.001$ from normal) in the life span of mice bearing ascites tumour. Copper(II) complexes produced a considerable increase in life span of tumour-bearing mice compared with that of the diketone ligands. The increase in life span (%ILS) of tumour-bearing mice was 64.71, 39.31, 45.73 and 54.91% through the administration of **1a, 1b, 1c** and **1d**, respectively, whereas their respective copper chelates produced 86.13, 54.34, 66.47 and 76.88% increase in life span.

Table 4. Short-term *in vitro* cytotoxicity of compounds towards ehrlich ascites cells

Compounds	Percentage cell death at different concentrations 1 μ g/ml	2.5 μ g/ml	5 μ g/ml	10 μ g/ml	25 μ g/ml	50 μ g/ml
1a (HL^1)	25	32	42	60	75	90
1b (HL^2)	17	24	28	39	55	71
1c (HL^3)	19	26	31	41	57	74
1d (HL^4)	21	30	36	50	62	81
$[Cu(L^1)_2]$	29	38	52	71	100	100
$[Cu(L^2)_2]$	23	30	40	47	63	94
$[Cu(L^3)_2]$	25	31	43	57	78	100
$[Cu(L^4)_2]$	27	34	51	69	94	100

DOI: 10.1002/aoc

Table 5. Cytotoxicity of compounds towards tissue cultured L929 cells

Compounds	Percentage cell death at different concentrations			
	1 μg/ml	2.5 μg/ml	5 μg/ml	10 μg/ml
1a (HL^1)	30.2 ± 3.0	34.6 ± 2.7	41.3 ± 3.1	51.2 ± 3.3
1b (HL^2)	16.8 ± 3.0	21.3 ± 2.8	29.8 ± 2.2	37.6 ± 3.7
1c (HL^3)	24.1 ± 2.7	29.8 ± 1.9	37.6 ± 1.7	45.6 ± 2.4
1d (HL^4)	28.2 ± 2.1	33.1 ± 2.7	39.8 ± 1.7	49.2 ± 2.7
$[Cu(L^1)_2]$	43.3 ± 3.3	48.7 ± 2.7	58.7 ± 1.7	78.1 ± 3.4
$[Cu(L^2)_2]$	30.5 ± 2.9	38.4 ± 2.4	46.6 ± 2.9	54.2 ± 3.1
$[Cu(L^3)_2]$	36.5 ± 2.9	41.2 ± 2.2	48.9 ± 2.1	60.6 ± 2.7
$[Cu(L^4)_2]$	38.9 ± 2.9	45.9 ± 2.4	50.2 ± 2.8	66.4 ± 3.1

Table 6. Effect of compounds on ascites tumour reduction

Compound	No. of animals with tumour	No. of days survived	Increase in life span (%)
Control	6/6	17.3 ± 1.1	
1a, (HL^1)	6/6	28.5 ± 2.7	64.71*
1b, (HL^2)	6/6	24.1 ± 2.6	39.31*
1c, (HL^3)	6/6	25.2 ± 2.1	45.73*
1d, (HL^4)	6/6	26.8 ± 2.9	54.91*
$[Cu(L^1)_2]$	6/6	32.2 ± 2.1	86.13*
$[Cu(L^2)_2]$	6/6	26.7 ± 2.4	54.34*
$[Cu(L^3)_2]$	6/6	28.8 ± 2.7	66.47*
$[Cu(L^4)_2]$	6/6	30.6 ± 3.1	76.88*

* $p < 0.001$.

Values are means of ±SD of six determinations. Animals were injected (i.p.) with Ehrlich ascites tumour cells (1×10^6 cells/animal) compounds (200 μmol/kg body weight) were injected (i.p.) for 10 days.

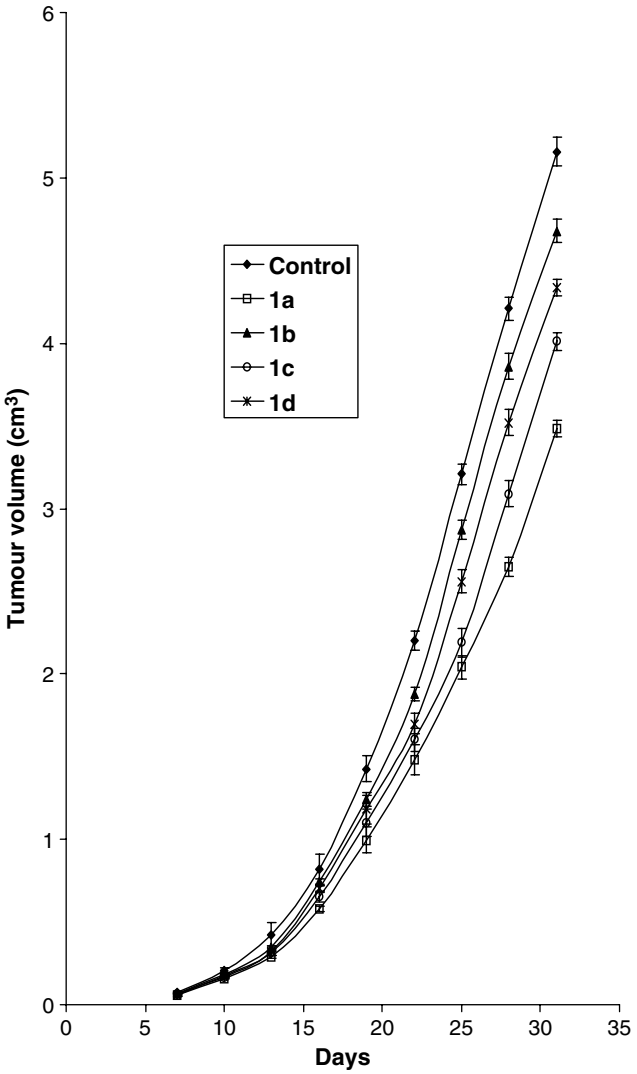


Figure 3. Effect of curcuminoid analogues on solid tumour development.

Effect of compounds on solid tumour development

Reductions of solid tumour volume in mice by the intraperitonial administration of compounds are given in Figs 3 and 4. These figures show that, compared with free curcuminoids, their respective copper(II) complexes are remarkably active in reducing tumour volume. Tumour volumes were respectively 5.160, 3.48, 4.34 and 4.01 cm^3 on day 31 for control, **1a, 1b, 1c** and **1d**. The tumour volumes on day 31 for copper complexes of **1a, 1b, 1c** and **1d** were, respectively, 2.86, 3.87, 3.22 and 3.45 cm^3.

The results clearly reveal that **1a** with hydroxyl groups on the phenyl rings show the maximum activity towards cytotoxicity on Ehrlich ascites cells (Table 4), cytotoxicity on cultured L929 cells (Table 5), percentage increase in life span (Table 6) and reduction of solid tumour volume in mice (Figs 3 and 4). The copper complexes dramatically enhanced the activity as evident from the data. Among the compounds studied **1b** with furyl rings showed least activity. The compound with extended conjugation (**1c**) and the compound with chlorosubstitution on the phenyl ring (**1d**) did not produce much activity. However it has been proved that copper complexation enhanced the activities in all the cases.

Earlier studies have shown that curcumin is an inhibitor of lipid peroxidation,[18] which promotes anticancer properties of the compound. It has also been reported that curcumin–gold complex is an effective antiarthritic agent.[10] The present study suggests that metal complexation significantly increased the cytotoxic.and antitumour activities of compounds. As reported earlier,[9,19,20] the hydroxyl group in the phenyl ring enhances the antitumour activity. This may be the reason for the exceptional activity of **1a** and its copper complex.

DOI: 10.1002/aoc

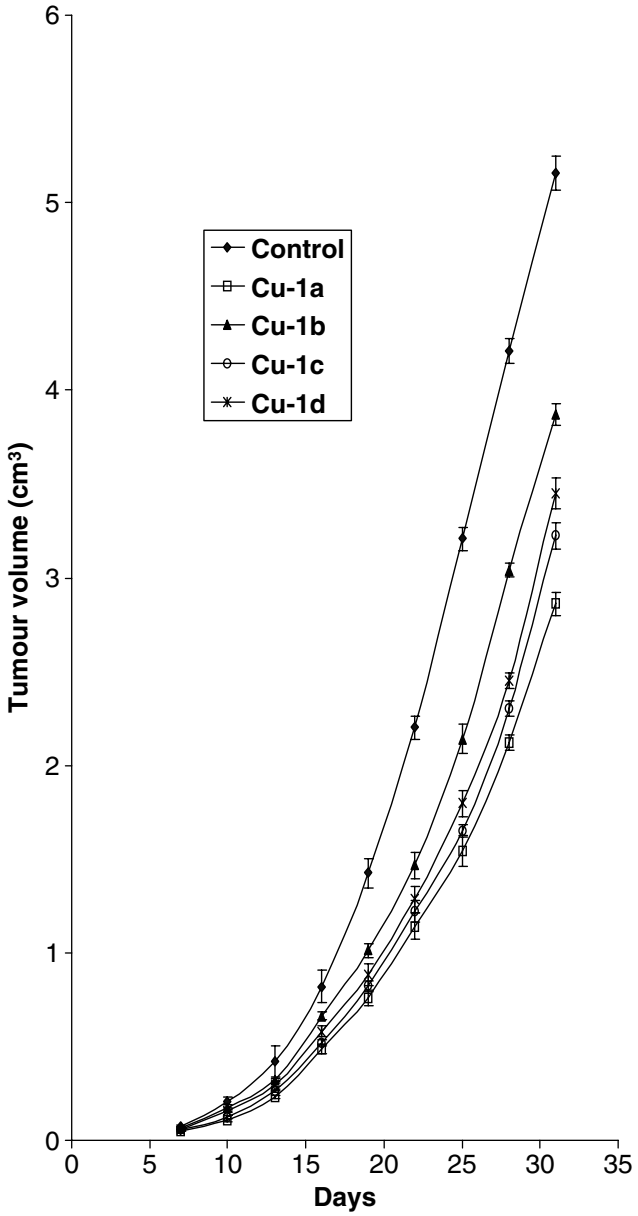


Figure 4. Effect of copper complexes on solid tumour development.

Acknowledgement

The authors would like to thank Dr Girija Kuttan and the Director of Amala Cancer Research Centre, Thrissur, India for the help in antitumour studies.

REFERENCES

1. Ammon HPT, Wahl MA. *Planta. Med.* 1991; **57**: 1.
2. Srimal RC, Dhawan BN. *J.Phar. Pharmac.* 1973; **25**: 447.
3. Chandra D, Gupta SS. *Indian J. Med. Res.* 1972; **60**: 138.
4. Deodhar SD, Sethi R, Srimal RC. *Indian. J. Med. Res.* 1980; **71**: 632.
5. Azuine MA, Bhide SV. *Nutr. Cancer.* 1992; **17**(1): 77.
6. Nagabhushan M, Bhide SV. *J. Am. Coll. Nutr.* 1992; **11**: 192.
7. Krishnankutty K, Venugopalan P. *Synth. React. Inorg. Met-org. Chem.* 1998; **28**(8): 1313.
8. Krishnankutty K, John VD, Kuttan G. *J. Exp. Clin. Cancer Res.* 2002; **21**(2): 219.
9. Krishnankutty K, John VD. *Synth. React. Inorg. Met.-Org.Chem.* 2003; **33**(2): 343.
10. Sharma KK, Chandra S, Basu DK. *Inorg. Chim. Acta* 1987; **135**(1): 47.
11. Clare MJ, Hydes PC. Metal complexes as anticancer drugs. In *Metal Ions in Biological Systems*, Sigel H (ed.). Marcel Decker: New York, 1979; 1–62.
12. Krishnankutty K, Venugopalan P. *J. Indian. Chem. Soc.* 1997; **74**: 562.
13. Pabon HJJ. *Rec. Trav. Chim.* 1964; **83**: 237.
14. Kuttan G, Vasudevan DM, Kuttan R. *Cancer Lett.* 1988; **41**: 307.
15. Lintveclt RL, Holtz HFJ. *Am. Chem. Soc.* 1966; **88**: 2713.
16. Maki AH, McGarvey BR. *J. Chem. Phys.* 1958; **29**: 31.
17. Gersman HR, Swalen JD. *J. Chem. Phys.* 1962; **36**(12): 3221.
18. Sreejayan N, Rao MNA. *Int. J. Pharm.* 1993; **100**: 93.
19. Ruby JA, Kuttan G, Kuttan R, Satyanarayana K, Rao MNA. *J. Clin. Biochem. Nutr.* 1994; **17**: 73.
20. Ruby JA, Kuttan G, Dinesh Babu KV, Rajasekharan KN, Kuttan R. *Int. J. Pharm.* 1996; **131**: 1.

DOI: 10.1002/aoc