



Synthesis, Characterization, Antibacterial, Antifungal, Antioxidant and Anti-inflammatory Actions of Novel β -diketone Complex

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ABSTRACT

β -diketone (β d) is prepared using the name reaction Baker-Venkataraman transformation reaction. In this present work, ester (c') was prepared when 1-(2-Hydroxy-5-methyl-phenyl)-ethanone (a') was treated with 4-Propoxy-benzoic acid (b') at 0°C to 10°C. (c') on Baker-Venkataraman transformation to give β -diketone ligand (L) named as 1-(2-Hydroxy-5-methyl-phenyl)-3-(4-propoxy-phenyl)-propane-1,3-dione. Bidentate ligand (L) treated with Cu (II) nitrate gives Cu (II) complex (CuL). (L) shows tautomerism, this tautomerism phenomenon was studied using FTIR and NMR. Because of enol serve as ligand (L) in the synthesis of (CuL). The prepared (L) can be characterized by HR-MS, elemental analysis, ¹H, ¹³C, DEPT, D₂O exchange, FTIR. The antibacterial, antifungal, antioxidant, anti-inflammatory property of (CuL) is studied.

Keywords: Baker-Venkataraman transformation, β -diketone ligand, Metal complex, Antioxidant activity, Anti-inflammatory activity, Antibacterial activity, Antifungal agent.

INTRODUCTION

Transition elements plays an important role in the formation of chemical complex of co-ordination chemistry. Massive literature is available on the application of complexes. (β d) and it's metal complexes play an important role in co-ordination chemistry¹. As starting material in the formation of new material or in the thin film for optical device applications², radiopharmaceuticals³. (β d)

acts as multipurpose precursor for many reactions of numerous heterocycles⁴ for example flavones⁵, isoxazolyl, pyrazolic⁶, pyrimidines, triazolic⁷. (β d) is used in metal withdrawal by chelating agent⁸, textile⁹, resin¹⁰, sunscreen cosmetics¹¹, HIV-1 avoidance¹². Ln (III) (β d) shows luminescent property¹³. (β d) hydrolase shows enzymatic activities¹⁴. (β d) with different attachments and its relative complexes being Lewis acerbity, volatility, ideal molar heat of sublimation, ideal molar enthalpy of formation, steam



pressures, catalytic property calculated¹⁵. (β d) shows pharmacological property such as antimicrobial, anticancer¹⁶, antibacterial, antifungal, antioxidant¹⁷, anti-inflammatory¹⁸. (β d) is found to be dynamic against anti-influenza¹⁹. (β d) also used in new advanced energy conserving lighting technology i.e. organic light emitting diodes (OLED)²⁰. (β d) show inside similar molecule hydrogen link keto and enol tautomerization²¹. Considering all above properties, in this paper, include (L') as a (β d) and it's complex (CuL'). Synthesized (L') and complex (CuL') are characterized by analytical techniques and they are used to test antibacterial, antifungal, antioxidant and anti-inflammatory properties.

Experimental

All analytical quality chemicals are utilized for the analysis work and they are bought from the authorized chemical merchant. Chemicals are used without additional purification. The standard procedure uses to purify solvents. Super dry distilled pure ethanol used for recrystallization and preparation of complex.

Synthesis of 4-Propoxy-benzoic acid 2-acetyl-4-methyl-phenyl ester (c')

At room temperature, equimolar 1-(2-Hydroxy-5-methyl-phenyl)-ethanone and 4-Propoxy-benzoic acid is dissolved in solvent pyridine and stirred for 10 min. using magnetic stirrer. Then to maintain the temperature of the reaction mixture to 0°C. In this mixture POCl₃ added drop by drop with constant stirring, the temperature of the reaction mixture maintains below 10°C. After complete addition of POCl₃, the mixture blend for 3 hours with the help of magnetic stirrer. The growth of process is tested by TL Chromatography and then the blend is kept whole night at normal temperature. Then the blend is added slowly on small mashed ice and if necessary acidulent using 1M HCl. The output of the reaction is filtered and it is washed using water and the creation is re-purified using ethyl alcohol. The purity of the compound is tested by TLC. The yield of the product (c') is 81%. m. p. 94°C.

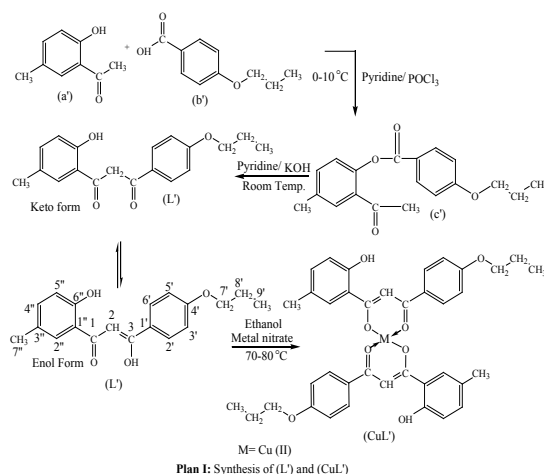
Synthesis of β -diketone (L')

4-Propoxy-benzoic acid 2-acetyl-4-methyl-phenyl ester (c') (3.121 g, 0.01 mol) is dissolved in (15 mL) pyridine and in this mixture pulverized powder KOH (1.122 g, 0.02 mol) is combined. The

mixture is blend for 3 hours and the growth of process checked by TLC. Afterward the accomplishment of the process, the mixture is spread on mashed ice and it is acidify through 1 M conc. HCl. Yellow solid of (L') is obtained. It is filtered by using Buchner funnel. The product is recrystallized using ethanol. The purity of the compound is tested by TLC. The yield of the product is 71%, m. p. 190°C.

Synthesis of metal complex (CuL')

The complex is made in metal to the ligand molar ratio 1:2 respectively. In hot alcoholic solution of (L'), hot alcoholic metal nitrate is combined dropwise. The mixture is blend and reflux for 3-4 hours. As a result precipitation of complex takes place afterward the combination of alc. NH₃. The product is then filtered and washed by using ethyl alcohol and dry. The colour of (CuL') is green. Yield 68%, m. p.: 290°C, elemental analysis: calculated C, 66.51; H, 5.58; O, 18.65; Cu, 9.26; found C, 66.57; H, 5.96; Cu, 9.40.



Characterization

Melting points find out using Thiel's tube, glass capillary method. The elemental analysis is accomplished using FLASH EA 1112 series Thermo Finnigan. FT-NMR using Bruker AV. II., ¹H frequency is 400 MHz and ¹³C frequency is 100MHz with the help of TMS internal std. and CDCl₃ as sol. FTIR reported on FTIR Spectrophotometer make Bruker Optics and model ALPHA-T. The sample is placed on ATR crystal. UV-Visible tested on Perkin Elmer. Conductance is reported on Equip-Tronic conductivity meter. The magnetic susceptibility is tested via Guoy balance. Physical properties of the ligand and complex is studied. The analytical data metal to ligand molar

ratio of the complex is 1:2. Absence of coordinated water molecules in tetra-coordinated metal complex, due to this the probable geometry is tetrahedral.

¹H, ¹³C NMR, HR-Mass statistics of ligand (L')

¹H, ¹³C FT-NMR of the ligand are tested using CDCl₃ solvent with the projected skeleton. ¹H FT-NMR, 400 MHz, CDCl₃ δ: signal of methyl group linked to -CH₂-CH₂-O- group at 1.058 (t, 3H, -CH₃), 1.833 (m, 2H, -CH₂), signal of methyl group linked to benzene ring at 2.327 (s, 3H, -CH₃), 3.984 (t, 2H, -CH₂), Signal of -CH= group linked to carbonyl group at 6.733 (s, 1H, -CH=), signals of aromatic H appeared at 6.878-7.977 (m, 7H, Ar-H), signal of phenolic-OH at 11.955 (s, 1H, Ar-OH), signals of enolic -OH at 15.846 (s, 1H, enolic-OH), ¹³C FT-NMR 100 MHz, CDCl₃ δ: Signal of carbonyl carbon at 194.73 (s, C-1, >C=O), 90.92 (s, C-2, >CH=), 177.76 (s, C-3, >C-OH), 128.04 (s, C-1'), 128.82 (s, C-2'), 114.45 (s, C-3'), 162.84 (s, C-4'), 114.58 (s, C-5'), 128.07 (s, C-6'), 69.76 (t, C-7'), 22.46 (m, C-8'), 10.47 (t, C-9'), 125.60 (s, C-1''), 131.22 (s, C-2''), 119 (s, C-3''), 136.52 (s, C-4''), 118.68 (s, C-5''), 160.16 (s, C-6''), 20.59 (s, C-7''), DEPT 135 use to confirm CH, CH₃ and CH₂ in ligand. Total 10 CH, CH₃ and 2 CH₂ presents in ligand are confirmed by DEPT 135. D₂O Exchange uses to confirm enolic-OH, Phenolic-OH functional group proton. HR-MS: 313.1438 (M+H), 335.1258 (M+Na).

Magnetic susceptibility

The magnetic moment of (CuL') is measured at room temperature for one unpaired electron is 2.22 B.M which is somewhat higher than the (1.73B.M) spin only value for unpaired one electron, which can be peculiar magnetic moment and orbit spin coupling nearer to spin only value generally of square pyramidal type.

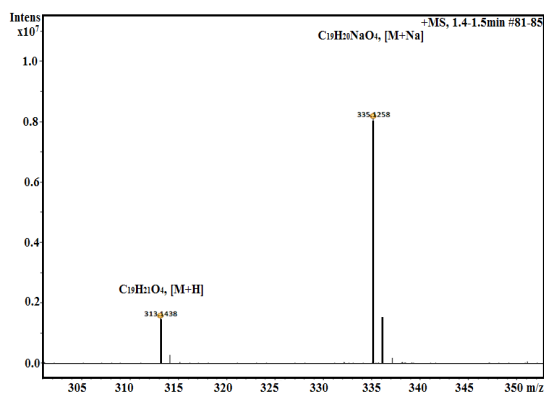


Fig. 1. HR-MS of (L')

Molar conductance

Molar conductivity of (CuL') is measured in DMSO at concentration of 10⁻⁴ M. Molar conductivity of (CuL') is 25.77 Ω⁻¹ cm² mol⁻¹ which indicates that (CuL') is non-electrolyte and covalent in nature.

FT-IR Spectra

The FT-IR spectrum of ligand and its metal complex is recorded and equated. The (L') ligand entirely enolized in ethanol, ν(C=O) of ligand 1624.3 cm⁻¹. ν(C=C) 1582.7 cm⁻¹ and ν(C-O) at 1198.9 cm⁻¹. In metal complex IR frequency of carbonyl ν(C=O) 1608.5 cm⁻¹ which is lower than IR frequency of (L') 1624.3 cm⁻¹²². This lowering frequency of complex shows that (L') coordinated with Cu metal ion. New band at 532 cm⁻¹ due to (M-O) metal to oxygen bond in metal complex. This proves that metal co-ordinates with (L') through oxygen. Absence of peak between 3547-3407 cm⁻¹ confirm that the absence of coordinated water in ligand and in complex²³. The FT-IR spectrum of the (L') and (CuL') are comparable apart from little minor variation in vibration band produced via metal ion.

Electronic absorption Spectra of (CuL')

The UV spectrum of (CuL') taken in DMSO as a solvent. The observed wave numbers (ν) are 26455.03 cm⁻¹, 32786.89 cm⁻¹, 40650.41 cm⁻¹, 45662.10 cm⁻¹, 46296 cm⁻¹. The band range observed between 31000-38000 cm⁻¹ also metal to ligand charge transfer band between 25000-26000 cm⁻¹ obscures the d-d transition shows square pyramidal geometry.

Thermogravimetric investigation of metal [Cu(L')₂] complex

Thermal decomposition of selected metal complex was carried out at a heat amount of 10°C min⁻¹ using N₂ environment between the thermal scale 32.64 to 1000°C in N₂ environment. The thermogravimetric spectrum of [Cu(L')₂] expresses no mass fall between the temperature range 32.64°C to 200°C which clearly shows the absence of lattice and coordinated water molecules in [Cu(L')₂]. The (CuL') gives disintegration from thermo scale 200°C to 400°C, by obs. 61.83%, cal. 62.78% mass fall due to elimination of the (C₂₇H₂₆O₅) part of (L'), the endothermic process (TDTA) peak at 290°C, which assigned to melting point [melting point determined by capillary tube system in static air was (290°C)]. The subsequent disintegration phase starts after 450°C

to 540°C by mass fall of obs. 25.95%, cal. 25.69% resembles to the disintegration of the link ($C_{11}H_{12}O_2$) portion of the (L). An exothermic peak near thermo scale from 508°C in the DTA seen for this phase. On further heating up to 938.81°C, the remaining weight corresponds to that of copper oxide.

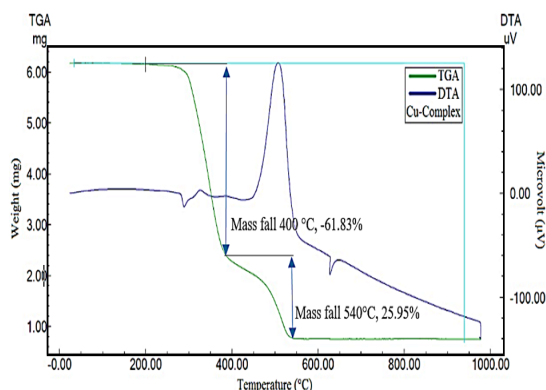


Fig. 2. DTA/TGA Curves of $[Cu(L)_2]$

Antimicrobial Activity

Antimicrobial activity of (L) and (CuL) was determined against *Gram-negative Escherichia coli* and another *Gram-positive Bacillus subtilis* class by well diffusion method. Antifungal activity was determined against *Aspergillus niger* by well diffusion

method. The samples were prepared by dissolving them into DMSO at concentration of 10 mg/ml.

Antibacterial Property

(L) does not show any antibacterial against *E. coli* and *B. subtilis*. The positive control is antibiotic streptomycin and shows region of reserve of 6 mm and 4mm in contradiction of *E. coli* and *B. subtilis* respectively. Negative control contains DMSO does not show any antibacterial activity. (CuL) does not show any antibacterial in contradiction of *E. coli* and *B. subtilis*. The positive control is antibiotic Chloramphenicol and shows region of reserve of 7mm and 9mm in contradiction of *E. coli* and *B. subtilis* respectively. Negative control contains DMSO does not show any antibacterial activity.

Antifungal Property

(L) does not show any antifungal activity against *A. niger*. Positive control for antifungal activity is clotrimazole and shows zone of inhibition of 7mm. Negative control contains DMSO does not show any antimicrobial activity. (CuL) does not show any antifungal activity against *A. niger*. Positive control for antifungal activity is clotrimazole and shows zone of inhibition of 7mm. Negative control contains DMSO does not express antimicrobial activity.

Table 1: antimicrobial action of ligand and its metal complex

S. N.	Compounds	Antibacterial activity Zone of reserve (mm)				Antifungal activity Zone of reserve (mm)	
		<i>E. coli</i>		<i>B. subtilis</i>		<i>A. niger</i>	
		+ve Control	-ve Control	+ve Control	-ve Control	+ve Control	-ve Control
1	(L)	-	-	-	-	-	-
2	Streptomycin	6	-	4	-	-	-
3	Clotrimazole	-	-	-	-	7	-
4	(CuL)	-	-	-	-	-	-
5	Chloramphenicol	7	-	9	-	-	-
6	Clotrimazole	-	-	-	-	7	-

Table 2: Percent inhibition and IC_{50} values

Compounds	% inhibition at Concentration(μ g/ml)					IC_{50} μ g/ml
	200	400	600	800	1000	
(CuL)	22.00	32.11	44.67	59.78	68.33	644.74
Ascorbic acid	52.55	65.41	76.32	86.95	95.25	126.84

Antioxidant activity

Antioxidant activity of (CuL) checked against the standard as ascorbic acid.

For synthesized chemical compounds, the IC_{50} value 644.74 μ g/ml is greater than standard

ascorbic acid value 126.84 μ g/ml. Thus, the synthesized (CuL) complex shows antioxidant activity.

Anti-inflammatory activity

The anti-inflammatory activity of compound (CuL) was determined by HRBC membrane

stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of (2% dextrose, 0.8% sodium citrate, 0.05% citric acid & 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cells were washed with iso-saline (0.85%, pH 7.2) & 10% v/v suspension was made with iso-saline. The assay mixture contained the drug (at various concentration as 100-500 μ g). 1 mL phosphate buffer (0.15 M, pH 7.4), 2 mL of hypo-saline (0.36%) and 0.5 mL of HRBC suspension. Diclofenac was used as the reference drug. Instead of sample, same volume of distilled water was used as control. All the assay mixtures were incubated at 37°C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using colorimeter at 560 nm. The percentage of HRBC membrane stabilization or protection was calculated using the following formula:

$$\% \text{ Protection against hemolysis} = 100 - (\text{O.D. of test sample} / \text{O.D. of control}) \times 100$$

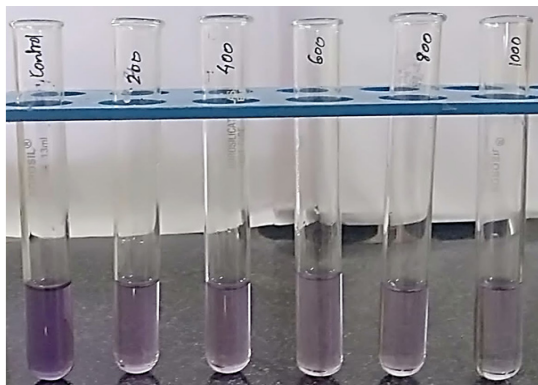


Fig. 3. Antioxidant activity of (CuL') at various concentration

RESULT AND DISCUSSION

The concentration range from 100 μ g/mL to 500 μ g/mL protects the human erythrocyte membranes against lysis induced by hypotonic solution. At concentration of 500 μ g/mL, the sample was inhibited 25.94% of RBC haemolysis as compared with 33.94% produced by Diclofenac at 500 μ g/mL. The results obtained demonstrated that sample can significantly and dose dependently inhibit HRBC haemolysis.

Table 3: Absorbance and percent HRBC membrane protection

Sr. No.	Concentration	Absorbance at 560nm		% Protection	
		Diclofenac	Sample	Diclofenac	Sample
1	0 (Control)	0.925	0.925	0.00	0.00
2	100	0.731	0.813	20.97	12.10
3	200	0.703	0.781	24.00	15.56
4	300	0.678	0.749	26.70	19.02
5	400	0.645	0.716	30.27	22.59
6	500	0.611	0.685	33.94	25.94

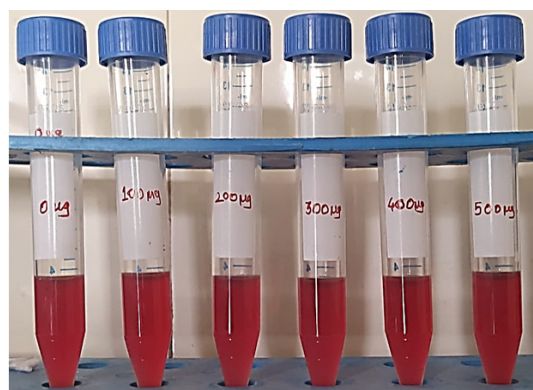


Fig. 4. Dose dependent anti-inflammatory activity of (CuL')

CONCLUSION

(L') prepared via Baker-Venkataraman transformation of (C'). Its transition metal complex (CuL') is created and defined by analytical statistics, spectroscopic statistics and physical statistics. The (L') perform bidentate ligand and approach to d-block metal like Cu (II). The (CuL') has another representation formula [Cu(L')₂] where (L') means 1,3- β -diketone ligand and its metal complex is studied for antioxidant, anti-inflammatory, antibacterial, antifungal agent. The biotic action statistics gives (L') and (CuL') inhibit antibacterial, antifungal movement contrary to different bacteriological and fungiform species. (CuL') shows antioxidant and anti-inflammatory action.

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Conflicts of Interest

The authors declare no conflict of interest.

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