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SYNTHESIS OF 1, 3 – DIKETOKETONES AS A NOVEL ANTIMICROBIAL, ANTIFUNGAL AND ANTIOXIDANTS AGENTS

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Abstract

An improved method for synthesis of 2,4-diacetyl-cyclohexanone derivatives is reported with enhanced yield in presence of DMSO as solvent. In situ, reaction of aromatic and hetero aromatic aldehydes with β-oxo-ketones undergoing Knoevenagel condensation, Michael addition followed by aldol condensation to give the desired product in excellent yield. displayed prominent mild to moderate antimicrobial and antifungal, antioxidant activities. The 2,4-diacetyl-cyclohexanone derivatives thus obtained have been characterized by ^{1}H NMR, ^{13}CNMR, FT-IR, MASS, HRMS and X-ray single Crystallography spectroscopic methods.

Key words: Antimicrobial activity, antifungal activity, antioxidant activity, aldehyde, β-keto ketones, DMSO, Knoevenagel /Michael/Aldol reaction.^{1}HNMR, ^{13}C NMR.

Introduction:

Substituted cyclohexanones have long been served as potential bioactive compounds. [1] These cyclohexanone moieties are also widely used for synthesis of several types of heterocyclic compounds.[2] Hydrogen bonding is unique type of intra- and inter-molecular interactions,

plays an important role in biological and chemical processes.[3] Multisubstituted cyclohexanone derivatives are highly important for pharmacological activities as well as starting materials for the synthesis of natural products and their derivatives [4], multisubstituted cyclohexanone derivatives have established diverse biological activites for these molecules such antitubercular, antileprous, hypolipidemic, antiphage, anticancer activities.[5] Cyclohexanone derivatives have potent pharmacological activity in the treatment of a broad spectrum of medical conditions [6]. Cyclohexanone derivatives formation and rearrangement of a biogenetic like intermediate [7]. Free radicals are unstable molecules that can damage cells and, tissues. Various free radicals are created in body during digestion of food and also during breathing the polluted air. [8] The cell damage by such free radicals may increase risks for cancer, heart disease, cataracts, diabetes, or related infections. Living organisms in normal natural metabolism produce reactive species such as oxygen and nitrogen based radicals (superoxide e (O^{-2}), hydroxyl radical OH), hydrogen peroxide (H_2O_2), nitric oxide radical (NO), (ROS) [9] and peroxynitrite (ONOO) [10] (RONS) and RONS [11] at low to moderate concentrations

Here we synthesized polycarbonyl derivatives by condensation of aromatic aldehydes with acetyl acetone with excellent yields with X – ray study.

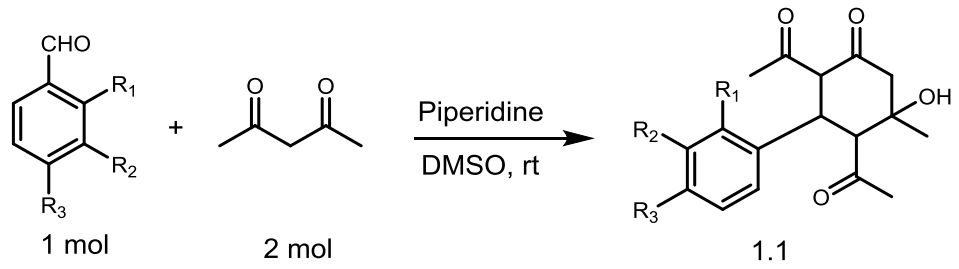
RESULTS AND DISCUSSION

Chemistry.

The general synthetic protocol involves the synthesis of multi substituted cyclohexanone derivatives based on Knoevenagel, Michael, Aldol reactions of aromatic aldehyde with β-oxo ketones. A series of cyclohexanone (**1-12**) derivatives (Scheme 1,) were prepared by base catalyzed condensation of aromatic/heteroaromatic aldehyde with β-oxoketones in DMSO as solvent with excellent yields (75-99%). Use of bases like Et_3N, K_2CO_3, piperidine and Pyrrolidine in DMSO solvent, yield of final product was 50- 99 %. Piperidine in presence of DMSO was found to be best base. For the synthesis of poly carbonyl multi substituted cyclohexanone derivatives reaction was optimized by varying quantity of base (piperidine) . And by using piperidine 0.25 equiv. base in DMSO solvent yield increase 75 to 99 %. The electron withdrawing substituent like Cl, F, Br, NO_2, at ortho position of phenyl ring gave very good yield in DMSO solvent. Keeping in view of new approaches, we studied the effect of solvent on the percentage of yield. The solvent like methanol, ethanol, and PEG gave moderate

yields of cyclohexanone derivatives g. While percentage of yield of product are found to be excellent in DMSO solvent.

Scheme1: Synthesis of 2,4-diacetyl-5-hydroxy-5-methyl-3-phenylcyclohexanone derivatives.



97 - 99 %

1) R_1 = H R_2 = H R_3 = H, 2) R_1= H R_2 = H R_3 = Cl, 3) R1 = H R2 = H R3 =Br, 4) R_1 = H R_2 = H R_3 = OMe, 5) R_1= NO_2 R_2 = H R_3 = H, 6) R_1= H R_2 = H R_3 = NO_2, 7) R_1 = F R_2 = H R_3 = H, 8) R_1= Cl R_2 = Cl R_3 = H, 9) R_1 = Br R_2 = H R_3 = H, 10) R_1 = H R_2 = NO_2 R_3 = H. 11) R_1 = H R_2 = OMe R_3 = OMe, 12) R_1 = H R_2 = H R_3 = CH_3

We were prompted to synthesize heteroaromatic aldehyde multisubstituted cyclohexanone derivatives with single crystallographic study fig 1.

X-ray Data Collection, Structure Solution, and Refinement

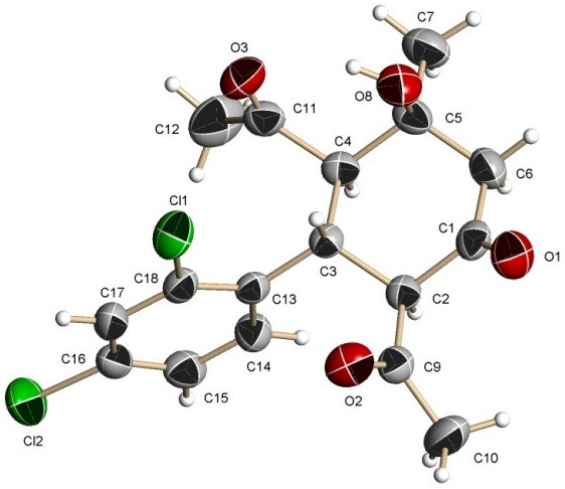


Figure1. Molecular structure of 4-diacetyl-3-(2,3-dichlorophenyl)-5-hydroxy-5- methyl cyclohexanone. Displacement ellipsoids are shown at the 50% probability level.

Table A. Crystal data and structure refinement for m

Identification code	mo_sg_10_6rep_0m	
Empirical formula	C17 H18 Cl2 O4	
Formula weight	357.21	
Temperature	296(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	Pbca	
Unit cell dimensions	a = 9.8971(2) Å	α= 90°.
	b = 17.0611(3) Å	β= 90°.
	c = 20.4295(3) Å	γ = 90°.
Volume	3449.63(11) $Å^3$	
Z	8	
Density (calculated)	1.376 Mg/m^3	
Absorption coefficient	0.393 mm^{-1}	
F(000)	1488	
Crystal size	0.386 x 0.318 x 0.271 mm^3	
Theta range for data collection	2.87 to 25.00°.	
Index ranges	-11<=h<=11, -20<=k<=20, -24<=l<=24	
Reflections collected	51547	
Independent reflections	3034 [R(int) = 0.0904]	
Completeness to theta = 25.00°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3034 / 0 / 213	
Goodness-of-fit on F^2	0.998	
Final R indices [I>2sigma(I)]	R1 = 0.0468, wR2 = 0.0865	
R indices (all data)	R1 = 0.0806, wR2 = 0.1020	
Extinction coefficient	0.00105(19)	
Largest diff. peak and hole	0.174 and -0.213 $e.Å^{-3}$	

Full crystallographic data (excluding structure factors) have been deposited with the Cambridge CrystallographicData Centre as supplementary publication no. **CCDC-658606**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: (þ44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk]

Antimicrobial Evaluation:

An evaluation of the antibacterial activity was done using two Gram-positive, *Bacillus subtilis* (NCIM 2079), *Staphylococcus aureus* (NCIM 2063), and one Gram negative *Escherichia coli (NCIM 2931)* and antifungal *Candida albicans* (NCIM 2091) species, which were collected by Indian Drugs Research Association, Pune (India) from National Chemical Laboratory (NCL) Pune and was assessed for the synthesized cyclohexanone derivatives compounds by well diffusion method.

Table N01. Antimicrobial activity and their MIC value.

Sample No	**Antimicrobial activity**			**Antifungal**
Organism	*B. subtilis*	*S. aureus*	*E.Coli*	*C albicans*
1	250 PPM	500 PPM	125 ppm	NA
2	250PPM	500PPM	250 ppm	ACTIVE
3	125 PPM	NA	250ppm	NA
4	125PPM	250PPM	100ppm	NA
Ampicillin	50 PPM	100PPM	100 PPM	100PPM

Antioxidant Activity:

Determination of reducing power (FRAP assay):

Total antioxidant power was measured by [Benzie IFF & Strain JJ (1996)] Ferric Reducing antioxidant Power (FRAP) assay with slight modification. Reaction mixture containing 0.2 mL of sample, 0.8 mL of acetate buffer (pH 3.6, 300 mM) and 3 mL of FRAP reagent acetate buffer (pH 3.6, 300 mM), 10 mM of TPTZ in 40 mM HCL and 20 mM Iron(III) chloride in 10:1:1

proportion was incubated at room temperature for 30 minutes. Absorbance was measured at 593nnm. Standard calibration curve was obtained in the concentration range of 2-10 μg mL^{-1} of ascorbic acid equivalent (r^2= 0.982)

Free radical scavenging:

DPPH radical scavenging assay

The method previously reported by *Gyamfi et al*, 1999[[i] was used for the determination of scavenging activity of DPPH radical in the extract solution. A portion of 60 μM DPPH was prepared in methanol containing standard ascorbic acid 250μM. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 minutes. The absorbance was measured spectrophotometricaly at 515 nm, where Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + sample extract or standard. Standard calibration curve was obtained in the concentration range of 2-10 μM mL^{-1} of Ascorbic acid equivalent (r^2= 0.996)

ABTS radical scavenging activity

The scavenging activity of plant extract against ABTS radical was determined by following the method described by *Re et al.,* 1999.[[ii] The ABTS radical cation was pregenerated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and incubating for 12–16 h in the dark at room temperature until the reaction was complete and the absorbance was stable. The absorbance of the ABTS solution was equilibrated to 0.70 (± 0.02) by diluting with water at room temperature, then 200 μg/mL BHT added as a standard 1 mL was mixed with 10 μl of the test sample (0.05–10 mg/mL) and the absorbance was measured at 734 nm after 5 min, where Abs control is the absorbance of ABTS radical + methanol; Abs sample is the absorbance of ABTS radical + sample extract or standard. Standard calibration curve was obtained in the concentration range of 2-10 μg mL^{-1} of BHT equivalent (r^2= 0.976).

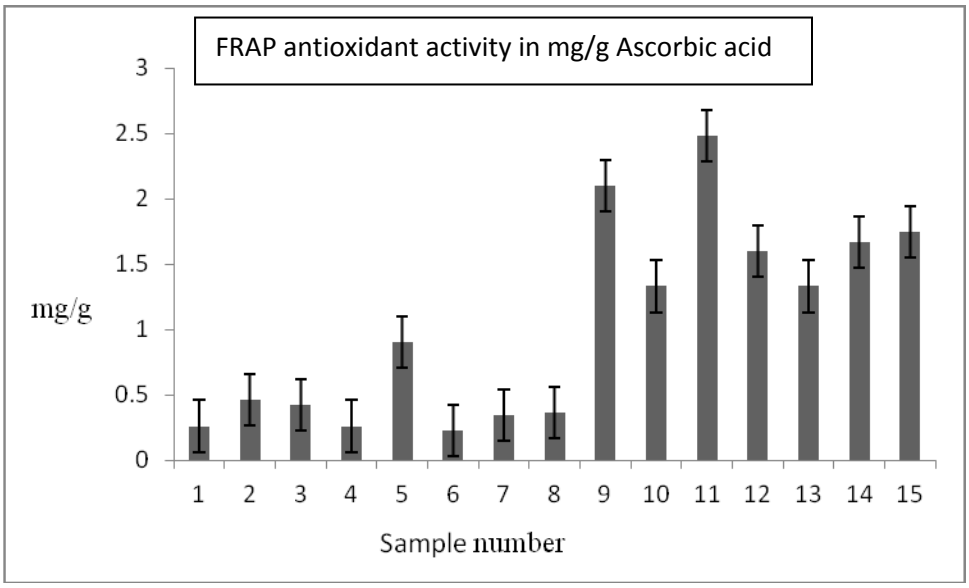
Result and discussion

FRAP mg/g Ascorbic acid equivalent	ABTS	DPPH

	% Inhibition	% Inhibition
0.26±0.04	18.64±4.41	14.28±4.65
0.46±0.03	31.63±8.28	27.97±8.72
0.42±0.02	53.38±10.59	50.89±11.16
0.26±0.02	40.39±11.46	37.20±12.08
0.90±0.11	11.58±3.28	6.84±3.46
0.23±0.01	13.84±2.49	9.22±2.62
0.34±0.06	35.59±4.79	32.14±5.05
0.36±0.10	28.81±3.21	48.51±8.84
2.10±0.11	60.45±4.98	58.33±5.25
1.33±0.14	42.65±7.38	16.66±9.42
2.48±0.30	66.94±7.85	57.14±4.07
1.60±0.20	57.06±8.14	49.40±6.93

Table no.2 Antioxidant activities (FRAP, ABTS and DPPH) of organic compounds. The given values are in triplicates

Figure No. 1 Total antioxidant activity of organic compounds by using FRAPS ferric reducing activity. The values taken are in triplicate basis.



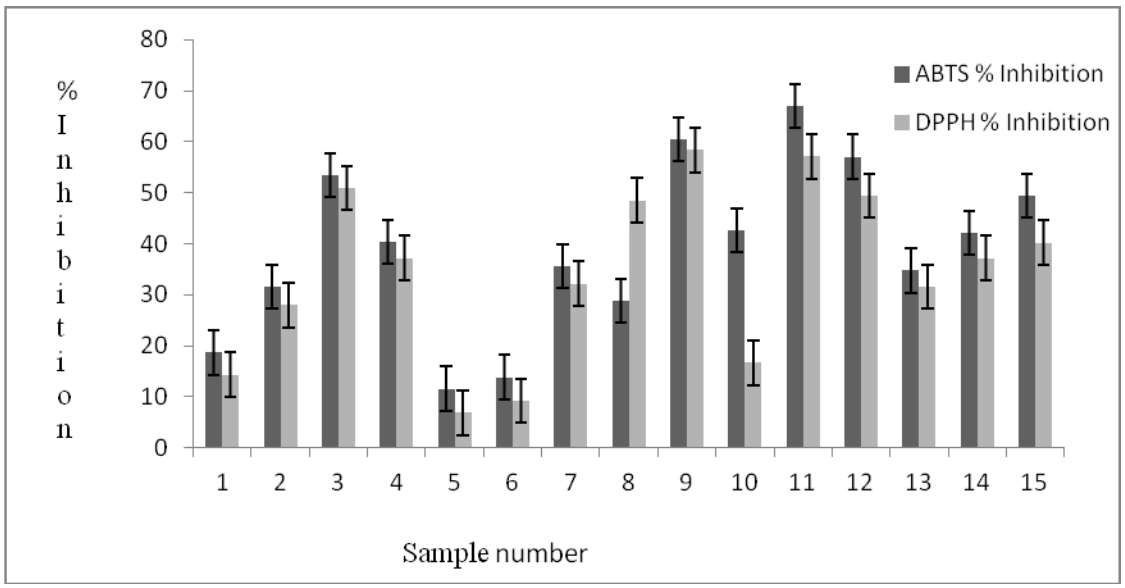


Figure No. 2 ABTS and DPPH of radical scavenging activity of organic samples. The given values are in triplicates

Sample number 11 has shown highest total antioxidant activity FRAP and DPPH scavenging activity because the presence of methoxy group on the aromatic nucleus. Sample number 11 has also showed higher effect of ABTS scavenging activity. It may be due to the presence of electron donating group on the phenyl ring like Curcuminoids and tetrahydrocurcuminoids containing 1,3-diketone moiety with Methoxy, hydroxy functional group show high antioxidant property,

The capacity of the compound to reduce the oxidative stress is helpful. Thus, these compounds can also act as a good antioxidant agents.

Conclusion:

We have elaborated the old methodology for preparation of multisubstituted cyclohexanone derivatives via Knoevenagel, Michael and Aldol reactions. The compounds are obtained in excellent yields. The compound show moderate antimicrobial antifungal property as well as antioxidant activity. The application of these compounds in the designing of new biologically important molecules is underway.

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EXPERIMENTAL:

IR absorption spectra were scanned on Perkin Elmer Spectrum, BX II FTIR spectrometer using potassium bromide (KBr) pellets and the wave numbers were given in cm^{-1}. All the ^{1}H NMR spectra were recorded on a Bruker DPX300 model Spectrometer in $CDCl_3$ using tetramethyl silane as an internal standard, chemical shifts are reported in δ units and the coupling constants (J) are reported in hertz. TLC was performed on the silica gel sheets (Silica Gel 60 GF254) and visualized in UV light (254 nm). Column chromatography was performed using silica gel (100–200 mesh) eluting with ethyl acetate and petroleum ether solvent.

Spectroscopic data for compounds 1 – 5 compounds.

Acetyl acetone 6.07 ml (59.0 mmol), aldehyde 3.0 ml (29.5 mmol) in DMSO 5.0 ml, piperidine 0.583 ml (5.9 mmol) was added, and the reaction mixture was magnetically stirred at ambient temperature. The stirring continued for 5 h the progress of the reaction checked by TLC, after completion of the reaction, the reaction mixture acidified with dil. HCl, quenched into ice cold water, washed with water to afford the pure solid product. Yield: 87 %).

1) Synthesis of 2, 4-diacetyl-5-hydroxy-5-methyl-3-(2-nitrophenyl) cyclohexanone.

White solid, mp. 154℃, ($C_{17}H_{19}NO6$), FTIR, ύ cm^{-1}1518 and 1346 CO1699, 2984, 2923, 2868. 3518, 735. (^{1}H NMR 300 MHz) δ 1.831(3H, s), δ1.700, (3H, s) δ 1.263(3H, s) δ 4.732 (1H, d J =9.3), δ 3.201(1H, s), δ 3.418 (1Hs), δ 2.617(1H, d) J = 18.6 .δ 2.531 (1H, d .J = 18.3Hz). (^{13}CNMR 300 MHz), 214.3, 196.8, 183.0, 149.4, 137.4, 133.1, 130.1, 128.3, 124.8, 89.3, 61.8, 44.61, 39.8, 34.2, 27.8, 25.4.

2) Synthesis of 2, 4-diacetyl-3-(2,3-dichlorophenyl)-5-hydroxy-5-methylcyclohexanone.

Color, white solid, m.p = 178 °C, M.F. = $C_{17}H_{18}Cl_2O_4$ (1H NMR CDCl3 300 MHz) 16.2 (s, 1H), 7.39 (s, 1H), 7.2 (d, J = 7.2Hz), 7.1(d, 1H, J = 8.1 Hz), 4.5 (1H, d, J = 9 Hz), 3.72 (s, 1H), 2.88 (s, 1H), 2.13 (d, 1H, J = 19.5), 1.83 (s, 3H), 1.62 (s, 3H), 1.25 (s, 3H). (^{13}C NMR $CDCl_3$ 75 Hz) 214.1, 203.0, 180.9, 141.9, 139.8, 133.5, 128.4, 128.0, 109.5, 69.4, 61.2, 44.2, 34.2, 33.9, 27.6, 25.8.

3) Synthesis of 2,4-diacetyl-3-(4-bromophenyl)-5-hydroxy-5-methylcyclohexanone

Color white, m.p. 158 °C(11) $C_{17}H_{19}BrO_4$, (^{1}H NMR $CDCl_3$, 300 MHz), 7206 (d, 2H, J = 8.2 Hz) 7.06 (d,2 H, J = 8.2 Hz 3.625 (1H, d, J =12.6), 3.839 (1H t J = 12.2/12.4), 3.215, 1H, d, J =12), 2.44 (1H J =2.4), 2.47 1H d, J = 14.6 2.538, (d, 1H J = 14), (^{13}C NMR $CDCl_3$ 75 Hz), 203.0, 202.7, 136.1, 129.0, 128.9, 133.3, 128.9, 129.0, 53.1, 61.1, 30.3, 67.4, 28.0, 73.2, 33.7, 214.1, 44.6.

4) 2,4-diacetyl-5-hydroxy-3-(3,4-dimethoxyphenyl) -5-methylcyclohexanone; Color (pale yellow), melting point 135 ˚C, $C_{17}H_{20}O_4$: FTIR, cm^{-1}3460.41, 1691.63 1720.56,2970.48, 2908.75, 2837.38, 3014.84, (^{1}H NMR $CDCl_3$, 300 Hz), 6.813 (s 1H), 6.676 (d, 1 H, J = 1.8 Hz), 6.795(d, 1H, J = 1.8 Hz) 3.758(d , 1H J= 12.3), 3.941(s, 1H),3.256 (d, 1H J=11.4), 3.996 (d, 1H J=2.4),2.542 (d, 1H J=2.4), 2.62 (d, 1H, J=14.1).

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