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Synthesis and Antibacterial Assay of 9-Substituted Aryl-1,8-dioxo-octahydroxanthenes

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The present report highlights the heterogeneous catalytic activity of nano copper ferrite for the one pot synthesis of 9-substituted aryl-1,8-dioxo-octahydroxanthenes when dimedone reacts with various aromatic aldehydes under solvent free conditions, catalyzed by nano catalyst. The main advantageous of this protocol includes excellent yields, mild reaction conditions and short reaction times.

Keywords: Nano copper ferrite, One-pot synthesis, Solvent free conditions, Xanthenes, Antibacterial assay, Docking.

INTRODUCTION

Xanthene and its derivatives are an important class of organic molecules because they have wide range of biological and pharmaceutical properties such as antibacterial[1], antiviral[2] and these are being utilized as antagonists for paralyzing action of zoxazolamine[3] and in photodynamic therapy[4,5]. Furthermore, these compounds can be used as dyes in laser technologies and pH sensitive fluorescent materials for visualization of bio-molecules[6]. In particular, xanthenediones constitute a key structural motif in a number of natural products[7-9] and have been used as versatile synthons because of the inherent reactivity of inbuilt pyran ring[10]. Synthesis of xanthenediones is a continuing hot topic because these moieties are active pharmaceutical ingredients (API's) and also valuable reactive intermediates for both synthetic and medicinal chemists.

A survey of the literature reveals that various methods have been reported for preparation of xanthene derivatives. The classical method for the synthesis of 9-substituted aryl-1,8-dioxo-octahydroxanthenes involves the condensation of appropriate active methylene compounds with various substituted aromatic aldehydes. For this purpose, two molecules of dimedone (5,5-dimethyl-1,3-cyclohexane) was reacted with various aromatic aldehydes[11] by using different Lewis acid catalysts such as triethylbenzyl ammonium chloride[12], *p*-dodecyl benzenesulfonic acid[13], diammonium hydrogen phosphate[14] under various conditions, sulfonic acid under ultrasonic irradiation[15], ionic liquids[16], Ambedrlyst-1s[17], $NaHSO_4$-SiO_2 or silica chloride[18], phosphomolybdic acid supported on silica gel[19], man-sized MCM-41-SO_3H under ultrasonic irradiation[20], sulfonic acid on silica gel[21], Dowex-50 W ion exchange resin under solvent-free conditions[22], $HClO_4$-SiO_2[23], ZnO and ZnO-acetyl chloride[24] and heteropoly acid supported MCM-41[25].

However, the methods reported, serve their best but still suffer from certain drawbacks such as long reaction times, low yields, use of toxic transition metals as catalysts, use of hazardous organic solvents and tedious workup procedures. Recently, because of the unique properties of nano particles, synthetic chemists focused on nano-catalysts. Therefore, synthesis and characterization of catalysts with lower dimensions have become the most interesting topic of research. Moreover, due to quantum size effects, nanometer-sized particles may exhibit unique properties for a wide range of applications. Keeping the above facts and as a part of our ongoing research, herein we report, first time, use of nano copper ferrite as heterogeneous support for the synthesis of 9-substituted aryl-1,8-dioxo-octahydroxanthenes. This method offers advantageous such as short reaction time, recyclability of the catalyst and easy to work-up procedure.

EXPERIMENTAL

Preparation of nano copper ferrite: The catalyst was synthesized[26] by citrate gel precursor method. Copper(II) nitrate and iron(III) nitrate were taken in stoichiometric proportions and minimum amount of deionized water was added to produce clear cationic solution. Citric acid solution was then prepared in stoichiometric ratio. Aqueous solutions with molar ratio of metal iron solutions were mixed and citric acid was added in equimolar ratio to the above mixed metal iron solution. pH was adjusted to 7 by adding ammonia solution. The aqueous

mixture was heated up to 90 °C to evolve reddish brown gases and became dried gel, which was finally treated at 350 °C for 1 h to observe whether the dry gel burnt out in self propagating manner to form loose powder. The finely powdered particles were calcinated at 600 °C. The powder was then characterized.

General method of synthesis of 9-substituted aryl-1,8-dioxo-octahydroxanthenes: A mixture of 5,5-dimethyl-1,3-cyclohexanedione (2eq) (**1**), substituted aromatic aldehydes (1 eq) (**2**) and $CuFe_2O_4$ nano particles (15 mol %) were stirred at 120 °C in an oil bath for the time indicated in Table-1. The completion of the reaction was monitored by TLC. After completion of the reaction, the reaction was cooled to room temperature and a solid product was obtained. The product was dissolved in methanol and the catalyst was recovered by magnetization. The crude products were further purified by recrystallization from ethanol. All the synthesized products were characterized by IR, NMR and Mass spectroscopic data and their melting points were compared with authentic samples. The reaction times, percentage of yield and m.p.'s were presented in Table-1. The spectral data of the synthesized compounds (**3a-3j**) are given below.

Spectral data for selected compounds

Compound (3a): 9-Phenyl-3,3,6,6-tetramethyl-1,2,3,4,5,6,7,8-octahydroxanthene-1,8-dione m.p. 204-205 °C. IR (KBr, ν_{max}, cm^{-1}): 2954,1664,1364,1199. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27-7.09 (m, 5H, ArH), 4.74 (s, 1H, C9-H), 2.46 (s, 4H, $2CH_2$), 2.19 (q, *J* = 16.5 Hz, 4H, $2CH_2$), 1.10 (s, 6H, $2CH_3$), 0.98 (s, 6H, $2C_{H3}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.2, 162.3, 143.8, 128.4, 127.6, 115.2, 50.6, 40.7, 32.0, 31.6, 29.3, 27.2. MS (*m/e*): 350 (M^+). Anal. Calcd for $C_{23}H_{26}O_3$: C, 78.83; H, 7.47. Found: C, 78.96; H, 7.40.

Compound (3b): 9-(4-Methylphenyl)-3,3,6,6-tetramethyl-1,2,3,4,5,6,7,8-octahydroxanthene-1,8-dione. m.p. 215-217 °C. IR (KBr, ν_{max}, cm^{-1}): 3030, 2980, 1685, 1660, 1620, 1490, 1365, 1200, 1135, 1000, 850, 840. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.16 (d, *J* = 8.0Hz, 2H, Ar H), 7.01 (d, *J* = 8.0 Hz, 2H, ArH), 4.70 (s, 1H, CH), 2.45 (s, 4H, $2CH_2$), 2.23 (s, 3H, CH_3), 2.19 (q, *J* = 16.3 Hz, 4H, $2CH_2$), 1.09 (s, 6H, $2CH_3$), 0.99 (s, 6H, $2CH_3$).^{13}C NMR (100 MHz, $CDCl_3$): δ 196.4, 162.1, 141.1, 135.7, 128.7, 128.1,115.7, 50.7, 40.8, 32.1, 31.3, 29.2, 27.3, 20.9. MS (*m/e*): 364 (M^+). Anal.Calcd for $C_{24}H_{28}O_3$: C, 79.09; H, 7.74. Found: C, 79.16; H 7.49.

Compound (3c): 9-(4-Hydroxyphenyl)-3,3,6,6-tetramethyl-1,2,3,4,5,6,7,8-octahydroxanthene-1,8-dione. m.p. 220-222 °C. IR (KBr, ν_{max}, cm^{-1}): 3324, 2959, 1652, 1617, 1363. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10 (d, *J* = 8.4 Hz, 2H, Ar H), 6.60 (d, *J* = 8.4 Hz, 2H, Ar H), 5.72 (s, 1H, OH, D_2O exchangeable), 4.67 (s, 1H, CH), 2.45 (s, 4H, $2CH_2$), 2.20 (q, *J* = 16.2 Hz, 4H, $2CH_2$), 1.09 (s, 6H, $2CH_3$), 0.99 (s, 6H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.8, 162.5, 140.7, 135.5, 128.2, 128.0,115.2, 50.6, 40.1, 32.4, 31.2, 29.1, 27.2. MS (*m/e*): 366 (M^+). Anal.Calcd for $C_{23}H_{26}O_4$: C, 75.38; H, 7.15. Found: C, 75.16; H 7.29.

Compound (3g): 9-(4-Bromophenyl)-3,3,6,6-tetramethyl-1,2,3,4,5,6,7,8-octahydroxanthene-1, 8-dione. m.p. 230-232 °C; IR (KBr, ν_{max}, cm^{-1}): 3030, 1624, 1586; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.47 (1H, s, CH),7.25-8.34 (m, 4H, ArH.); ^{13}C NMR ($CDCl_3$): δ_C 37.46,116.64, 118.02, 120.21, 122.39, 124.38, 126.93, 128.91,129.12, 129.88, 131.03, 131.23, 131.58, 143.98, 148.65; MS (*m/e*, %) 437 (20), 281 (100), 252 (40), 75 (15).

Compound(3i): 9-(4-Nitrophenyl)-3,3,6,6-tetramethyl-1,2,3,4,5,6,7,8-octahydroxanthene-1,8-dione m.p. 219-221 °C. IR (KBr, ν_{max}, cm^{-1}): 2958, 1670, 1650, 1520, 1362, 1206, 870. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (d, *J* = 8.0 Hz, 2H, ArH), 7.46 (d, *J* = 8.0 Hz, 2H, ArH), 4.82 (s, 1H, CH), 2.49 (s, 4H, $2CH_2$), 2.21 (q, *J* = 16.2 Hz, 4H, $2CH_2$), 1.12 (s, 6H, $2CH_3$), 0.99 (s, 6H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.1,162.9, 151.3, 146.6, 129.4, 123.5, 114.5, 50.5, 40.8, 32.3, 32.1, 29.6,29.2, 27.2. MS (*m/e*): 395 (M^+). Anal. Calcd for $C_{23}H_{25}NO_5$: C, 69.85; H, 6.37; N, 3.54. Found: C, 69.96; H 6.49; N, 3.43.

Antibacterial assay: Antibacterial studies were carried out on human pathogenic bacteria. *Salmonella typhi*, *Vibrio cholerae*, *Shigella dysenteriae* and *Enterococcus faecalis*, which were clinical isolates collected at *King George* Hospital, Visakhapatnam, India. *S. typhi*, *V. cholerae*, *S. dysenteriae* and *E. faecalis* are gastrointestinal pathogens. Antibacterial activity was performed by agar well diffusion method and minimum inhibitory concentration (MIC)[27,28]. Agar well diffusion method was performed to determine the inhibitory zones in millimeter (mm). MIC was determined by broth dilution assay and the experiment was conducted between 1-1000 µg/mL of compound concentration. Ciprofloxacin (antibiotic) and DMSO were used as positive control and negative control respectively[29].

Molecular docking studies: X-ray crystal structures of proteins used in docking studies are obtained from Protein Data Bank. Topoisomerase I (PDB ID 1T8I) was used in docking studies. Co-cystalized ligands and water molecules are removed from target protein using Argus lab. Ligands are prepared using Chemoffice (Cambridge). Energy minimization was done using molecular mechanics. The minimized was executed until root mean square value reached smaller than 0.001 Kcal/mol. Such energy minimized ligands and receptor used for docking studies using GEMDOCK (Generic Evolutionary Method for molecular docking) is a generic evolutionary method with an empirical scoring function for the protein-ligand docking, which is a problem of paramount importance in structure-based drug design, combines both continuous and discrete search mechanisms. A population size of 300 with 70 generations and three solutions were used, in docking accuracy setting[30,31].

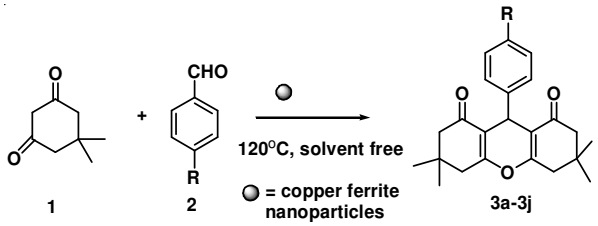
RESULTS AND DISCUSSION

In continuation of our interest in the area of clean synthesis, under solvent-free conditions, for the development of new synthetic methodologies herein, we report a simple, efficient and one-pot reaction of dimedone and aldehydes using nano copper ferrite at 120 °C for the preparation of 9-substituted aryl-1,8-dioxo-octahydroxanthenes (**3a-3j**) in high yields (**Scheme-I**) and the results were presented in Table-1.

Initially, a blank reaction using benzaldehyde and dimedone (mole rate 1:2) at 120 °C without nano copper ferrite was performed in order to establish the real effectiveness of the catalyst and the results showed that desired product was not formed even after 12 h of heating. We then focused to optimize catalyst loading percentage. In order to evaluate the most appropriate

TABLE-1
SYNTHESIS OF 9-ARYL-1,8-DIOXO-OCTAHYDROXANTHENES USING NANO COPPER FERRITE

Product	R	Time (min)	Yield (%)	m.p. (°C)	Lit. m.p. (°C)
3a	-H	5	95	202-204	202-204[23]
3b	$-CH_3$	15	85	215-217	217-218[23]
3c	$-CH(CH_3)_2$	25	88	238-239	236-239[32]
3d	-OH	30	90	243-245	246-248[12]
3e	$-OCH_3$	30	80	230-232	242-244[23]
3f	$-NMe_2$	15	90	224-226	226-228[23]
3g	$-NEt_2$	17	88	228-230	–
3h	-F	14	91	223-225	224-226[33]
3i	-Br	12	92	230-232	234-236[33]
3j	$-NO_2$	10	95	219-221	226-228[23]

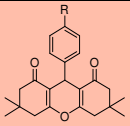


R: **a** = H; **b** = $-CH_3$; **c** = $-CH(CH_3)_2$; **d** = -OH; **e** = -OMe; **f** = $-NMe_2$; **g** = $-NEt_2$; **h** = -Br; **i** = -F; **j** = $-NO_2$

Scheme-I: Synthesis of 9-substituted aryl-1,8-Dioxo-octahydroxanthenes employing reusable Nano copper ferrite as catalyst

catalyst percentage, a model reaction using benzaldehyde and dimedone (mole ratio1:2) was carried out using 0, 5, 10, 15 and 20 mol % of Nano copper ferrite at different temperatures under solvent-free conditions (Table-2). It was found that 15 mol % of Nano copper ferrite showed high yield in lesser reaction time at 120 °C (Table-2).

TABLE- 2
OPTIMIZED CONDITIONS FOR CATALYST LOADING AT120 °C

Product	Catalyst (mol %)	Yield	Time (min)
	0	0	12 (h)
	5	92	8
	10	93	6
	15	95	5
	20	94	7

After completion of the reaction, the catalyst was recovered by magnetization and washed with diethyl ether and the recovered catalyst was reused for few more cycles. During washing with the solvent, it was clearly evident that there was no leaching of catalyst and was confirmed by performing the reaction with the filtrate. The leaching of metal after three cycles was found to be 0.17 %. From our investigations, we observe that nano catalyst shows excellent to good reactivity with promising yields even for the next three cycles in the same reaction. Since, there was no observable loss in the yield percentage; further reusability of nano catalyst was not needed. The results are listed in Table-3.

TABLE- 3
REUSABILITY OF NANO CATALYST

S. No.	Catalyst recovery (%)	Yield (%)
1	–	95
2	97	89
3	86	82
4	80	78

Under the optimized conditions, aromatic aldehyde (**2a-2j**) containing electron donating as well as electron withdrawing groups with different substitution patterns were effectively cyclized to give 9-aryl substituted 1,8-dioxo-octahydroxanthenes (Table-1).

From Table-1, it is observed that the presence of electron withdrawing groups on benzaldehyde (compounds **3f** and **3j**) require less reaction times and produce high yields than electron donating groups on benzaldehyde (compounds **3b**, **3c**, **3e**, **3f** and **3g**). The presence of electron withdrawing group accelerates the formation of carbocation at the carbonyl carbon of benzaldehyde. Interestingly, the basic molecule itself, without any substitution, reacts in presence of catalyst leading to the formation of the product in good yield (90 %). The formation of xanthene moiety is confirmed by spectroscopic methods. In IR spectra stretching frequencies between 1670-1624 cm^{-1} correspond to the carbonyl group of xanthenes. In ^1H NMR spectra, chemical shift at δ 4.82-4.67 corresponds to the -CH proton of tertiary carbon which confirms the formation of target molecule.

Antimicrobial activity: To explore the bioactive lead molecules, the above synthesized compounds were evaluated for their antibacterial activity on human pathogens *viz.*, *S. typhi*, *V. cholerae*, *S. dysentriae*, *E. faecalis* at Department of Organic Chemistry, Microbial Laboratories, A.U, adopting well diffusion method and the results are tabulated in Table-4.

Xanthene derivatives (**3a-3j**) showed significant antibacterial activity on human pathogens. Zone of inhibitions were observed between 6-15 mm. Among the series of compounds, compound **3f** showed highest zone of inhibition (15 mm) and low MIC (1 µg/mL) on *Vibrio cholerae*. Compound **3f** (N,N-diethyl) showed good activity on both gram positive and gram negative bacteria. The pathogens *S. typhi*, *S. dysenteria*, *E. faecalis* showed resistance to synthesized xanthenes analogues compared to *V. cholerae*. Some of the analogues showed comparable activity with ciprofloxacin. Further molecular docking studies performed on X ray crystal structure of topoisomerase, which is one of the target sites for cytotoxic activity. The results were depicted in Table-5. *In silico* studies were correlated with *in vitro* studies. The binding energies or dock energies of Xanthene derivatives (**3a-3j**) found to be between 119.8-138.5 Kcal/mol. Binding energy is inversely proportional to binding affinity. Compound **3f** binds with high affinity than other synthesized compounds and its binding interactions with glutamic acid[494], threonine[501], lysine[493] residues of topoisomerase

TABLE-4
ANTI-MICROBIAL ACTIVITIES OF 9-ARYL-1,8-DIOXO-OCTAHYDROXANTHENES DERIVATIVES AGAINST VARIOUS HUMAN PATHOGENS

S.No	Compound	*S. typhi*	*V. Cholerae*	*S. dysenteriae*	*E. faecalis*
1	**3a**	9/100	8/100	11/100	9/1000
2	**3b**	9/1000	9/1000	10/>1000	9/>1000
3	**3c**	10/100	10/100	09/100	9/1000
4	**3d**	9/10	10/100	11/100	8/100
5	**3e**	10/100	11/100	10/1000	10/>1000
6	**3f**	14/10	15/1	14/10	13/100
7	**3g**	10/1000	11/1000	11/1000	6/1000
8	**3h**	10/100	10/100	9/100	10/1000
9	**3i**	9/1000	10/1000	8/100	ND
10	**3j**	13/10	10/100	10/100	10/100
11	Antibiotic	17/10	18/1	16/10	15/10

Ciprofloxacin for bacteria; * 50 µg compound per well; ND: Not Determined

TABLE-5
MOLECULAR DOCKING STUDIES OF XANTHENE DERIVATIVES ON TOPOISOMERASE I

Compound	Binding energy (-Kcal/mol)	Interacted amino acids
3a	-123.5	LYS-493 GLU-494 ARG-508
3b	-128.8	ARG-488 TYR-537 LYS-532
3c	-127.3	LEU-602 GLN-599 LYS-603
3d	-119.8	ARG-590 THR-591 THR-718
3e	-133.1	ARG-375 ASN-419 GLU-418
3f	-138.5	GLU-494 THR-501 LYS-493
3g	-129.0	LYS-532 TYR-537 LYS-532
3j	-128.1	PHE-529 GLY-531 ARG-488
3h	-138.0	LEU-487 GLY-531 TYR-537
3i	-131.9	LYS-734 PRO-739 VAL-738

are presented in Fig. 1. Compound **3f** can be a promising cytotoxic agent for therapy of microbial infections.

Conclusion

The $CuFe_2O_4$ nanoparticle catalyst plays a crucial role in the success of the reaction. In the absence of the $CuFe_2O_4$ nano particles the reaction of 1,3-cyclohexanediones (**1**) and benzaldehyde derivative (**2**) was performed and no product was obtained, where as in the presence of catalyst, product was formed with a short reaction and the yields are promising. The crucial role of $CuFe_2O_4$ nanoparticles as a good catalyst

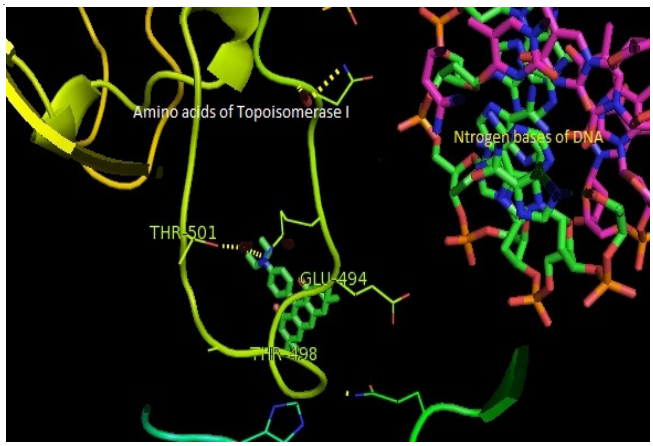


Fig. 1. Molecular docking of synthesized xanthone (**3f**) on topoisomerase **I**

was obviously revealed. Further the microbial investigations confirm that the N,N- dimethyl analogue (**3f**) is a promising lead molecule.

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